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Cardiac-specific overexpression of *SCN5A* gene leads to shorter P wave duration and PR interval in transgenic mice

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Abstract

The Cardiac sodium channel gene *SCN5A* plays a critical role in cardiac electrophysiology and its mutations, either gain- or loss-of-functions, are associated with lethal arrhythmias. In this study, we investigated the effect of overexpression of *SCN5A* on the cardiac phenotype in a transgenic mouse model (TG-WT L10). Compared to NTG mice, heart rate, QRS duration, and QT intervals remained unchanged in TG-WT mice. Moreover, no spontaneous ventricular arrhythmias were detected in TG-WT hearts. Despite these results, a mild, irregular cardiac phenotype was observed in TG-WT mice. The P wave and PR interval were significantly shorter in TG-WT compared with NTG mice (P, 8.8 ± 0.8 ms vs. 12.6 ± 0.9 ms; PR, 12.5 ± 2 ms vs. 33.5 ± 0.7 ms). Furthermore, spontaneous premature atrial contractions were often detected in TG-WT mice. These results suggest that the expression level of the *SCN5A* gene is a determinant for the length of the P wave duration and PR interval on electrocardiograms (ECG).

Keywords: Cardiac sodium channel gene *SCN5A*; Long QT syndrome (LQTS); Ventricular arrhythmias or tachycardia (VT); Transgenic mice; Electrocardiogram (ECG); P wave and PR interval; Sodium current

The cardiac voltage-sensitive sodium channel $Na_v1.5$ is responsible for rapid influx of sodium ions that initiates and propagates the cardiac action potential in the heart [1–6]. The large peak sodium influx is responsible for excitability and conduction in the myocardium and special conduction tissues. It is encoded by the *SCN5A* gene which consists of 28 exons spanning over 80 kb of DNA on chromosome 3p21 [7]. To date, hundreds of mutations have been identified in *SCN5A* and linked to a number of heart diseases including long QT syndrome (LQTS), Brugada syndrome (BrS), cardiac conduction disease, sudden infant death, idiopathic ventricular fibrillation, and congenital sick sinus syndrome [1,4,5,8,9]. Three *SCN5A* mutations have been studied *in vivo* in transgenic mice, including transgenic overexpression of the N1325S mutation associated with LQTS in the heart (TG-NS/LQT3) [3], knockin of the ΔKPQ associated with LQTS [10], and knockin of the 1795insD mutation associated with bradycardia, conduction disease, LQTS, and BrS [11]. These mice recapitulate the major clinical features that correspond to human patients.

Knockout mice for *SCN5A* have also been generated. Homozygous mice died at embryonic day 10.5 [12]. Heterozygous mice showed prolongation of the P wave and the PR interval on the ECG, which was indicative of slow conduction [13,14]. However, the effect of overexpression of the *SCN5A* gene on the electrophysiology of the heart has not been explored. To further investigate the physiological contribution of $Na_v1.5$ to cardiac function, we

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created a transgenic mouse model with overexpression of wild type human *SCN5A* gene in the mouse heart (TG-WT). The phenotypes of the transgenic mice were compared to non-transgenic littermate control mice (NTG). We demonstrated that the P wave duration and PR interval on the ECG in TG-WT mice were significantly shorter than in NTG mice. TG-WT mice also showed a high frequency of spontaneous premature atrial contractions (PACs). No other cardiac abnormalities were identified. Our results provide the first assessment of the effects of increased $Na_v1.5$ expression on cardiac function *in vivo*.

Materials and methods

Generation of transgenic mice and genotyping. The transgenic mice with selective cardiac expression of human wild type *SCN5A* gene was generated by placing *SCN5A* under the control of the mouse myosin heavy chain-α promoter (TG-WT). The transgenic construct was similar to what we used to generate TG-NS/LQT3 L3 and L12 except that the wild type human *SCN5A* cDNA was used [3,15]. Quantitative Southern blot analysis was performed to estimate the copy number of the transgene [3,15]. The mice were genotyped by multiplex PCR which simultaneously amplified the mouse endogenous gene *AGGF1* (Pe10: 5′-GAG CTCACCTCCGCCTCGAT-3′ and Pe11: 5′-CCTCCTTACTTAGTGCT GGAC-3′, 784 bp) and human *SCN5A* transgene (Pmcn-3nd: 5′-CCT CT GACAGAGAAGCAGGCACTTT-3′ and Pscn5a-5nd: 5′-CTGAACC GGAAGATGGTC TTGCCTTT-3′, 523 bp) [3,15,16]. The amplification of mouse *AGGF1* served as an indicator for the successful PCR to avoid mis-genotyping.

Quantification of the transcript of human SCN5A transgene in TG-WT mouse hearts. Transcript of human *SCN5A* transgene was quantified by quantitative reverse-transcript PCR (RT-PCR) [17]. RT-PCR primers were 5′-CATGCTCATCATGTGCACCAT-3′ and 5′-GGTGCGTAAGG CTGAGACATT-3′. This pair of primers amplified a 259 bp PCR fragment from both the mouse and human *SCN5A* genes. The internal loading control was *18S* rRNA with amplicon size of 274 bp by primers 5′-CGTC TGCCCTATCAACTTT-3′ and 5′-CGGCTGCTGGCACCAGACTT-3′ as described previously. In brief, 5 μg of total RNA from a cardiac ventricular tissue sample was pre-treated with 5 μg of DNase I to remove contaminated genomic DNA (37 °C for 30 min), followed by 90 °C for 5 min to inactivate DNase I. No genomic DNA (transgene) contamination in the pre-treated RNA was confirmed by PCR without RT. Quantitative PCR was performed using cDNA template reverse transcribed with random hexamer. Various numbers of PCR cycles were empirically determined to ensure that PCR amplification was in the logarithm range (18–25 cycles for *18S* rRNA; 25–31 cycles for *SCN5A*). The relative amount of *SCN5A* transcripts was expressed as a ratio of band densities of *SCN5A* to *18S* multiplied by 1000.

Protein expression of human SCN5A transgene in TG-WT mouse hearts. Western blot analysis was carried out to estimate the protein expression of human *SCN5A* transgene in TG-WT mouse hearts. Total protein was extracted from the isolated hearts using lysis buffer containing 20 mM Tris–HCl (pH 8.0), 5 M NaCl, 1 mM EDTA and 0.5% NP-40. The protein concentration was measured using the Bio-Rad protein assay kit, BSA was used as a standard, and all samples were normalized to the same concentration of 5 or 10 μg/μl. 30–75 μg of protein extract was separated by SDS–PAGE gels, and transferred to polyvinylidene difluoride membranes (Immun-blot PVDF Membrane, Bio-Rad). The membrane was blocked in 5% nonfat dried milk in PBST (phosphate-buffered saline (PBS), 0.05% (v/v) Tween 20 (Sigma), pH 7.4), and then probed with an anti-human $Na_v1.5$ antibody [6], followed by a horseradish peroxidase-conjugated secondary antibody. The protein signal was visualized using the ECL Western blotting Detection kit and Hyperfilm ECL (Amersham Biosciences). The member was re-probed using an anti-GAPDH antibody (Chemicon), which serves as loading control.

Electrophysiology. All experiments were conducted in accordance with the guidelines of the Cleveland Clinic Foundation Institutional Review Board on Animal Subjects and conformed to NIH guidelines. Details that describe the isolation procedure have been previously reported [18,19]. Hearts were harvested from age-matched (6–8 months) mice and enzymatically digested via the retrograde, Langendorff perfusion manner. Enzymatic digestion (Type II, 209 U/mg, Worthington) was conducted using a Ca^{2+}-free Tyrode's buffer solution for 28 min. Ventricular and atrial appendages were separately removed and placed in separate incubation buffers (final Ca^{2+} concentration of 1.8 mM). Gentle titration of each was performed and cells were collected by gravity sedimentation. Prior to experimentation, cells were re-suspended in recording bath solution.

Recordings were conducted using a MultiClamp 700A amplifier interfaced to a Pentium computer equipped with Digidata 1322A and pClamp 9 software (Axon Instruments) [3,19]. Only single, quiescent, and rod-shaped myocytes were chosen. Recording electrodes were fabricated from borosilicate glass (FHC) with 1.8–2.3 MΩ resistance. Signals were low-passed filtered at 1 kHz and digitized at 10 kHz and current recordings were not corrected for leak. All recordings were made at room temperature and the recording bath and pipette solutions have been previously reported [3,19].

Telemetric electrocardiographic recording. ECG from non-transgenic and TG-WT L10 mice were recorded and observed from freely moving mice by the Telemetry system (DSI international) as described [3,18]. Male transgenic mice and age-matched littermates were used for this study (6–8 months).

Langendorff perfusion of the mouse heart. The mouse was heparinized with 0.15 ml heparin (1000 U/ml) and anesthetized with 0.65 mg/g, i.p. avertin (20 mg/ml, Aldrich) before sacrifice. The heart was then quickly excised, cannulated, and studied as described previously [20].

Statistical analysis. All values are expressed as means ± SEM. The paired *t*-test was used to evaluate the ECG parameters and electrophysiological recording data. ANOVA was used to compare the expression of *SCN5A* and heart rate. $P < 0.05$ was set for statistical significance.

Results

Development of transgenic mice with cardiac overexpression of wild type human SCN5A

Two transgenic lines with overexpression of the wild type human *SCN5A* gene were developed and designated as TG-WT L5 and L10, respectively (Fig. 1A and B). By quantitative Southern blot analysis, we estimated that TG-WT L5 had 1–2 copies of transgene and L10 had approximately 10 copies of transgene (Fig. 1B). Two previously generated mouse models with overexpression of the LQTS-causing mutation N1325S in *SCN5A* were included in the present study as controls. One model is the transgenic line with 1–2 copies of transgene (TG-NS/LQT3 L3) and the other is the line with 10 copies of transgene (TG-NS/LQT3 L12). The latter has been characterized in detail and found to display characteristic clinical features of LQTS: prolonged QTc and development of spontaneous VT/VF, as well as syncope and sudden death [3,19,20]. As shown in Fig. 1B, TG-WT L5 had a comparable copy number of transgene with TG-NS/LQT3 L3 and TG-WT L10 had a similar copy number to TG-NS/LQT3 L12. As the major goal of this study is to determine the effect of overexpression of *SCN5A* on cardiac function, the results in the present study focused only on TG-WT L10

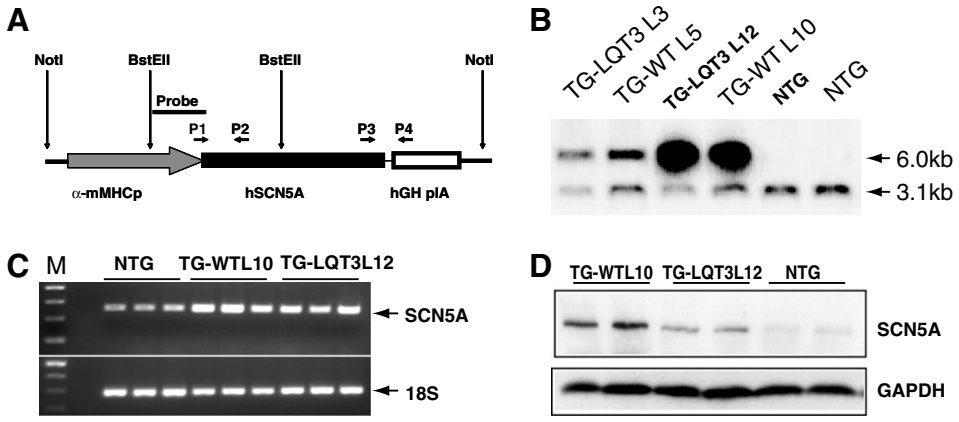

Fig. 1. Generation and characterization of the transgenic mouse model. (A) Transgenic construct for engineering of transgene, human wild type *SCN5A*, into the mouse genome. The *Not*I fragment consists of the mouse myosin heavy chain-α promoter (α-*mMHCp*), transgene *SCN5A*, and the human growth hormone poly(A) signal (*hGH plA*). Probe (1.7 kb), a portion of α-*mMHCp*, was used as a marker for Southern blot analysis. PCR primer pairs P1–P2 and P3–P4 were used to identify transgenic mice during breeding. (B) Southern blot analysis. Mouse genomic DNA was digested with *Bst*EII and hybridized with probe [see above]. The 6.0- and 3.1-kb fragments represent the transgene *SCN5A*, and the endogenous mouse myosin heavy chain-α gene, respectively. Two transgenic lines, TG-WT L5 and L10, were generated. The following genotypes are listed: *TGM(NS31)L3*: lanes 1 and 3; *TGM(NS31)L12*: lanes 4 and 6; and *non-transgenic mice*: lanes 2 and 5. (C) Quantitative RT-PCR shows the amount of total *SCN5A* transcripts in ventricular tissues of TG-WT L10, TG-LQT3 L12, and NTG mice. 18S, 18S rRNA as internal control. (D) Western blot analysis was performed using heart tissue protein extracts from TG-WT L10, TGM(NS31)L12 and NTG mice. A polyclonal anti-SCN5A antiserum was raised against a peptide located in the less-conserved N-terminus of SCN5A. The same filter was probed with a monoclonal anti-GAPDH antibody (Sigma–Aldrich) in order to calibrate the amount of protein extracts loaded in each lane.

mice, the line with the higher copy number of the transgene. The amount of the total *SCN5A* transcript in ventricular tissues of TG-WT L10 and TG-LQT3 L12 was comparable (1.3 ± 0.045 vs. 1.1 ± 0.033, $P > 0.05$), and both were significantly increased compared to NTG hearts (0.15 ± 0.003, $P < 0.05$) as shown by quantitative RT-PCR (Fig. 1C). The protein expression level of $Na_v1.5$ in both TG-WT L10 and TG-LQT3 L12 hearts was also markedly increased compared to that in NTG mice as shown by Western blot analysis (Fig. 1D). These results indicate that the expression of the *SCN5A* gene is markedly increased in TG-WT L10 mice at both the mRNA and protein levels.

Phenotype of TG-WT mice

Mouse ECG was recorded from conscious and free-moving TG-WT L10 and NTG mice by a Telemetry monitoring system (5 mice per group). Representative ECG traces are shown in Fig. 2A for NTG mice and in Fig. 2B for TG-WT L10 mice. The major parameters on ECG were measured and are shown in Fig. 2C–G. The TG-WT L10 mice showed a shorter PR interval than NTG mice (Fig. 2D). The P wave duration in the TG-WT mice was also shorter than that in the NTG mice (Fig. 2E). No differences in the RR interval (heart rate), QT interval corrected for the heart rate (QTc), and the QRS interval were detected between the TG-WT L10 and NTG mice (Fig. 2C, F, and G).

Recently, we reported spontaneous ventricular arrhythmias (VT) in telemetrically monitored TG-NS/LQT3 L12 mice [3] and in isolated Langendorff-perfused hearts from this line of mice [20]. However, the Telemetry system did not detect VT in the TG-WT L10 mice and NTG mice. No VT was detected in isolated Langendorff-perfused hearts either. Similar to the NTG mice, no sudden death was observed in TG-WT L10 mice. However, frequent spontaneous premature atrial contractions (PACs) were detected in TG-WT L10 mice. In contrast, no PACs were observed in NTG mice (Fig. 3). It is interesting to note that generation of PACs was associated with successive reduction of the PR interval with each beat close to the PAC (Fig. 3A).

Electrophysiology of isolated cardiomyocytes from TG-WT mice

Sodium current (I_{Na}) was elicited with a 60 ms ramp protocol from a holding potential of −80 to 0 mV (Fig. 3). Peak I_{Na} was expressed as pA/pF in which the cell capacitance was measured by calculating the area under the capacitive transient elicited by a −10 mV pulse. Sodium current densities for TG-WT and NTG ventricular cells were 14.5 ± 1.2 ($n = 19$) and 16.6 ± 2.3 pA/pF ($n = 10$; $P > 0.05$), respectively, and for atrial cells the densities were 6.4 ± 1.1 ($n = 6$) and 7.4 ± 0.8 pA/pF ($n = 8$; $P > 0.05$), respectively. For ventricular cells, $I_{Na,L}$ was estimated quantitatively by fitting the declining phase of I_{Na} with a biexponential function, $I/I_{max} = A_{1x}(1-e(-t/\tau_1))+A_{2x}(1-e(-t/\tau_2))$ and plotting the slow time component, τ_2; for TG-WT and NTG cells the values were 1.8 ± 0.1 and 1.8 ± 0.3 ms, $P > 0.05$. Action potentials for both ventricular and atrial cells were elicited with a 1000–1500 pA pulse of 25–50 ms duration at a cycle length of 0.5 s. Zero holding current was applied. The time to 90% repolarization (APD_{90}) and cell capacitance (see Fig. 3) were measured.

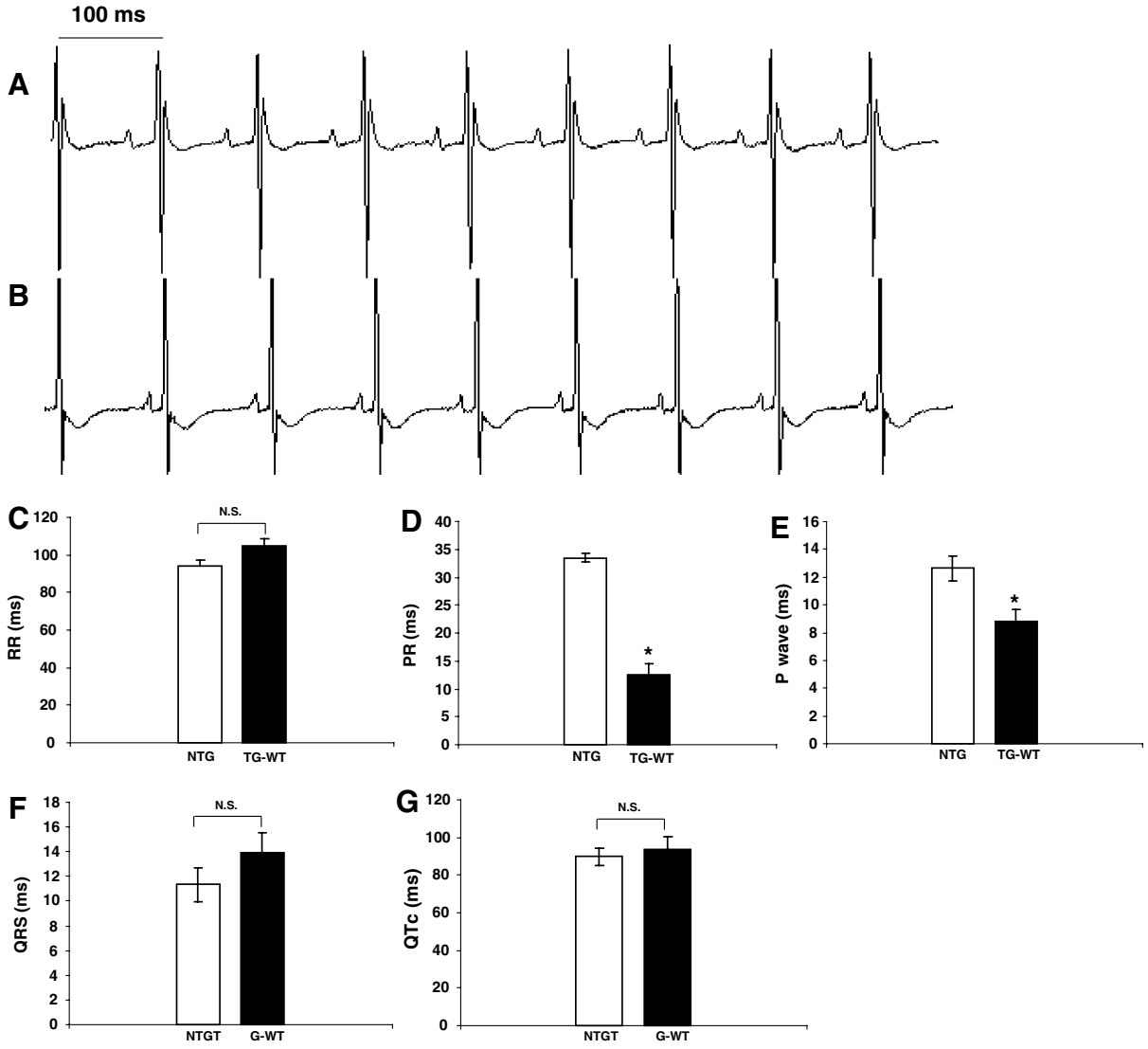

Fig. 2. Representative ECG traces from NTG (A) and TG-WT mice (B). The various ECG parameters were measured and analyzed (C–G). The P wave duration and PR interval in TG-WT mice were significantly shorter than those of NTG mice ($n = 5$, $P = 0.017$ and 0.00086, respectively). No differences were observed in RR, QTC, and QRS duration ($P > 0.05$). NS, no statistical significance.

APD_{90} values for TG-WT and NTG ventricular cells were 68.4 ± 5.0 ($n = 9$) and 69.5 ± 5.3 ms ($n = 11$, $P > 0.05$), respectively, and for atrial cells the values were 27.4 ± 3.2 ($n = 6$) and 27.5 ± 1.7 ms ($n = 6$, $P > 0.05$), respectively, Fig. 4.

Discussion

Both the knockout and transgenic expression techniques are useful systems for exploring the physiological functions of a gene. Previous studies using knockout mice revealed that reduction of *SCN5A* expression by 50% resulted in prolongation of the PR interval [13,14]. In this study, we created transgenic TG-WT L10 mice that overexpress the human cardiac sodium channel gene *SCN5A*. Baseline ECG recordings by a Telemetry system revealed a shortened PR interval and P wave duration in TG-WT L10 mice compared to their littermate controls. As the AV node forms much of the PR segment on the ECG, these results suggest that the expression level of the *SCN5A* gene may alter AV nodal conduction and, as such, decrease the PR interval on the ECG.

Despite these unexpected findings on the P wave and PR interval, we observed no abnormalities in the electrophysiology of the ventricles. The QRS duration and QTc were normal in TG-WT and comparable to NTG mice. More importantly, no ventricular arrhythmias were detected in TG-WT mice. The sodium current densities and APDs from TG-WT ventricular cardiomyocytes were nearly identical to that from NTG cells. Equally important were the similarities in sodium current densities and APDs found in atrial cells. This lends support to our earlier finding that overexpression of *SCN5A* did not significantly increase the

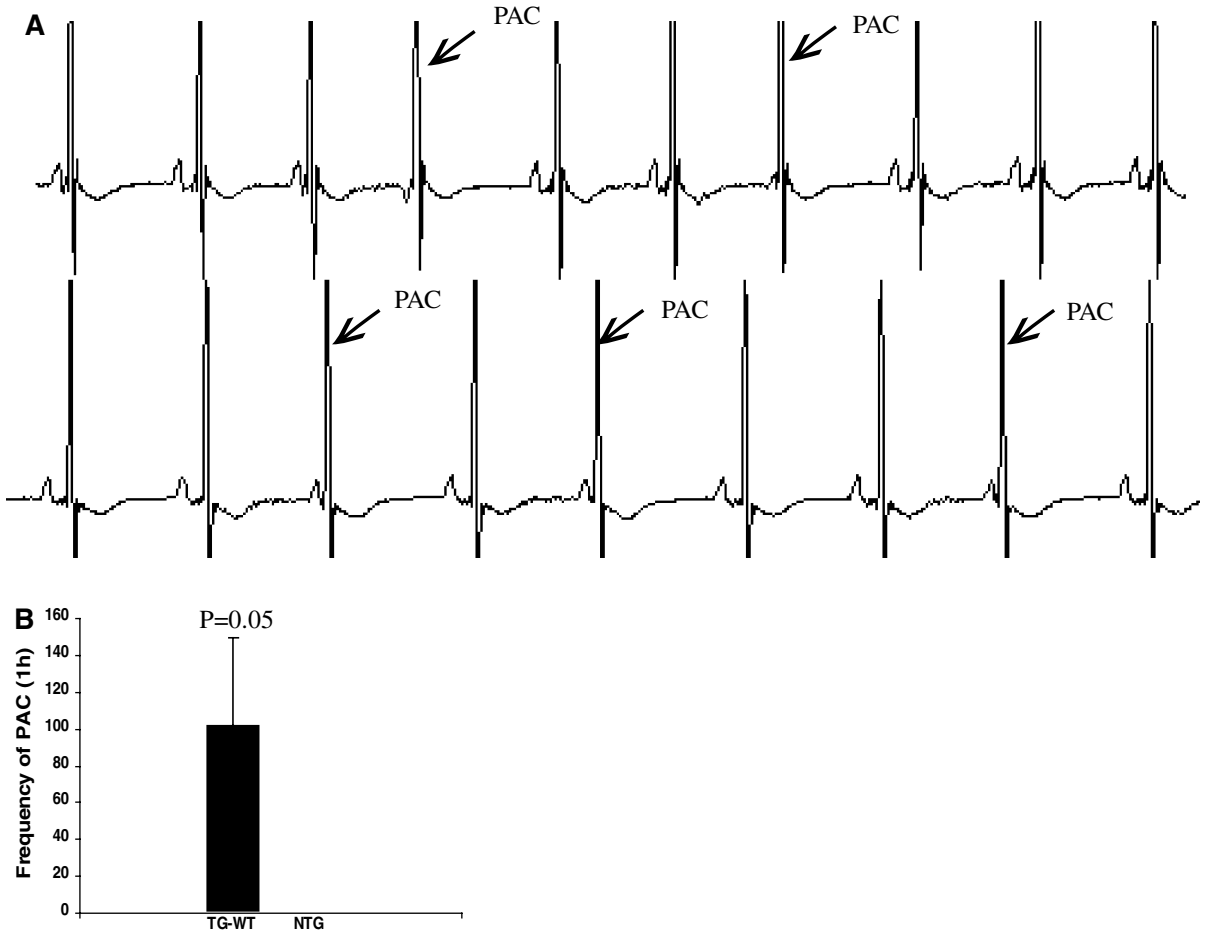

Fig. 3. Detection of premature atrial contractions (PACs) by a telemetry ECG recording system from TG-WT mice. (A) ECG traces showing PACs. A PAC is defined as an ectopic beat with abnormally shaped or absent P-wave before the QRS complex, but without any effect on QRS complexes. PACs are indicated by arrows. (B) The frequency of PACs in TG-WT and control NTG mice was analyzed from ECGs as the number of PACs per hour.

surface density of the sodium channels in ventricular [3] and atrial myocytes. Thus, it would appear that the risks associated with overexpression of *SCN5A* is minimal and the abnormalities that were previously reported in TG-NS/LQT3 L12 mice [3] were directly associated to the N1325S mutation.

Similar to TG-WT L10 mice, TG-NS/LQT3 L12 mice also exhibited the short PR interval phenomenon [3]. This suggests that PR shortening may be a consequence of the overexpression of *SCN5A* alone and, not necessarily the direct effect of the N1325S mutation. Furthermore, the high incidence of arrhythmias, mortalities, and electrophysiological abnormalities that have been reported in TG-NS/LQT3 L12 mice, whereas no such cardiac abnormalities have been detected in TG-WT L10 mice, is also evidence for the fact that the cardiac dysfunctions are a direct consequence of the N1325S mutation and not from *SCN5A* gene overexpression. Such phenotypical comparison between TG-WT L10 and TG-NS/LQT3 L12 strongly validates our TG-NS/LQT3 mice as a suitable model for LQT3.

Overexpression of mutation R302Q in the *PRKAG2* gene in the mouse heart also exhibited a short PR interval [21]. In addition, these mice showed an increased QRS duration and an increased incidence of supraventricular arrhythmias, the features of which are associated with Wolff-Parkinson-White syndrome (WPW) [21]. The *PRKAG2* gene encodes the γ-2 subunit of AMP-activated protein kinase. Although the transgenic model serves as a suitable model for WPW, no clear molecular mechanism by which overexpression of R302Q causes PR interval shortening and/or induction of supraventricular arrhythmias was provided. Considering the similarities in the observations for PR intervals between our TG-WT L10 and the *PRKAG2* mice, it is likely that the *PRKAG2* defect may affect the function and/or expression of the *SCN5A* gene, resulting in WPW.

The molecular mechanism for the P-wave and PR interval shortening in TG-WT L10 mice has not been clearly defined within the scope of this study. We speculate that expression of the cardiac sodium channel in the conductive tissues (i.e., SA node and AV node) may be increased such that conduction of electrical pulses in these tissues is facilitated. Such a scenario would lead to accelerations in atrial depolarization and conduction, resulting in a shorter P wave duration and PR interval. The same mechanisms may also explain the development of PACs in TG-WT L10 mice which presumably originate near or at the AV

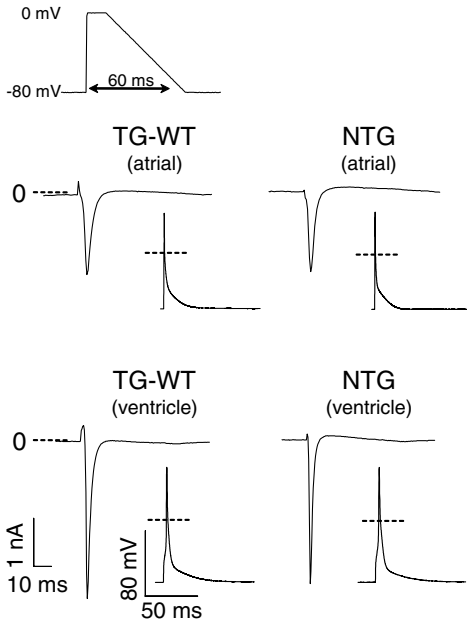

Fig. 4. Sodium currents and action potentials from atrial and ventricular myocytes. Sodium currents from TG-WT and NTG single atrial and ventricular myocytes were generated using the ramp step. Representative sodium current traces for each cell group are shown with the corresponding action potential tracing. Cell capacitance for ventricular cells was estimated as 224.0 ± 22.9 (TG-WT) and 220.2 ± 14.5 pF (NTG), $P > 0.05$, whereas for atrial cells the values were 194.9 ± 17.9 (TG-WT) and 144.9 ± 27.6 (NTG), $P < 0.05$. Sodium current densities and APD_{90} values are listed in the Results section.

node as AV junctional rhythms. Historically and clinically, individuals with short PR intervals and PACs range from asymptomatic to a variety of complex arrhythmias, and our TG-WT L10 mice are at the lower risk range.

In conclusion, cardiac overexpression of the cardiac sodium channel gene *SCN5A* causes shortening of the P wave duration and PR interval.

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