Overlap Syndrome of Cardiac Sodium Channel Disease in Mice Carrying the Equivalent Mutation of Human SCN5A-1795insD

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Background—Patients carrying the cardiac sodium channel (SCN5A) mutation 1795insD show sudden nocturnal death and signs of multiple arrhythmia syndromes including bradycardia, conduction delay, QT prolongation, and right precordial ST-elevation. We investigated the electrophysiological characteristics of a transgenic model of the murine equivalent mutation 1798insD.

Methods and Results—On 24-hour continuous telemetry and surface ECG recordings, Scn5a1798insD/H11001 heterozygous mice showed significantly lower heart rates, more bradycardic episodes (pauses ≥500 ms), and increased PQ interval, QRS duration, and QTc interval compared with wild-type mice. The sodium channel blocker flecainide induced marked sinus bradycardia and/or sinus arrest in the majority of Scn5a1798insD/H11001 mice, but not in wild-type mice. Epicardial mapping using a multielectrode grid on excised, Langendorff-perfused hearts showed preferential conduction slowing in the right ventricle of Scn5a1798insD/H11001 hearts. On whole-cell patch-clamp analysis, ventricular myocytes isolated from Scn5a1798insD/H11001 hearts displayed action potential prolongation, a 39% reduction in peak sodium current density and a similar reduction in action potential upstroke velocity. Scn5a1798insD/H11001 myocytes displayed a slower time course of sodium current decay without significant differences in voltage-dependence of activation and steady-state inactivation, slow inactivation, or recovery from inactivation. Furthermore, Scn5a1798insD/H11001 myocytes showed a larger tetrodotoxin-sensitive persistent inward current compared with wild-type myocytes.

Conclusions—Mice carrying the murine equivalent of the SCN5A-1795insD mutation display bradycardia, right ventricular conduction slowing, and QT prolongation, similar to the human phenotype. These results demonstrate that the presence of a single SCN5A mutation is indeed sufficient to cause an overlap syndrome of cardiac sodium channel disease. (Circulation. 2006;114:2584-2594.)

Key Words: conduction death, sudden electrophysiology genetics ion channels long-QT syndrome sodium

Mutations in the gene encoding the cardiac sodium channel (SCN5A) have been implicated in multiple arrhythmia syndromes, each characterized by distinct ECG and clinical features, including the long-QT syndrome type 3 (LQT3), Brugada syndrome, conduction disease, and sinus node dysfunction.1-6 Mutations causing LQT3 are considered to disrupt fast inactivation of the sodium current, allowing for a persistent inward current during the action potential (AP) plateau phase (gain of function mutations), whereas mutations causing Brugada syndrome and conduction disease are considered to reduce the total amount of available sodium current (loss of function mutations).2,4

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In contrast to this classic distinction, more recent clinical reports and studies on biophysical properties of mutant
sodium channels indicate that these disorders display more overlap than previously understood. In 1999, we described a C-terminal SCN5A gene mutation (1795insD) in a large Dutch family with ECG features of bradyarrhythmia, conduction disease, LQT3, and Brugada syndrome.7,8 Other SCN5A mutations leading to what have become known as “overlap syndromes of cardiac sodium channelopathy” have since been described.9–12 In a study by Priori et al,13 administration of flecainide to 13 LQT3 patients (all SCN5A mutation carriers) led to ST-segment elevation, the hallmark ECG feature of Brugada syndrome. Furthermore, a review of the biophysical properties associated with SCN5A mutations identified in LQT3 patients revealed that a significant number of mutations also exhibited defects typical of Brugada syndrome—causing mutations, such as decreased current density and negative shift in voltage-dependence of inactivation.4

In patients with cardiac sodium channel disease, one cannot rule out the co-inheritance of other genetic variation besides the one in SCN5A as a cause of the multiple arrhythmia manifestations. Furthermore, mutated sodium channels investigated with heterologous expression systems may behave fundamentally differently from those in the native myocyte, and their biophysical characteristics may vary depending on the expression system used. In fact, different kinetic properties have been previously described for the 1795insD mutation when studied in HEK293 cells compared with Xenopus oocytes.7,14 To characterize the biophysical properties of this mutation in the context of the native cardiomyocyte environment as well as its effects in vivo, we have generated transgenic mice carrying the mouse equivalent (1798insD) of the human SCN5A-1795insD mutation. Similar to patients carrying this mutation, heterozygous Scn5a1798insD/+ mice showed bradycardia, conduction slowing, and QT prolongation. In addition, Scn5a1798insD/+ mice displayed right ventricular (RV) conduction slowing, as is described for Brugada syndrome patients. The mutation caused a drastic reduction in peak sodium current density, a delayed time course of fast inactivation, and a small persistent sodium current, explaining the observed multiple phenotypes.

Methods

Generation of Scn5a1798insD/+ Mice
Heterozygous Scn5a-1798insD (Scn5a1798insD/+) mice were generated by Cre/loxP-mediated gene targeting (see online Data Supplement for details, genotyping protocol, and mRNA expression analysis). All experiments were performed on adult (3 to 5 months old) Scn5a1798insD/ mice (strain FVB/N, Charles River Laboratories, Inc, Wilmington, Mass) and their wild-type littermates as control, and were in accordance with governmental and institutional guidelines for animal use in research.

Western Blot
Membrane protein fractions were isolated from mouse whole heart tissue and loaded on SDS-PAGE gel for blotting. Membranes were incubated with primary antibodies anti-Na1.5 (ASC-005, Alomone Labs, Jerusalem, Israel) and anti-calnexin (Calbiochem 208880, EMD Biosciences, Darmstadt, Germany) as a control for protein loading. Incubation was followed by detection using the enzymatic chemiluminescence method (see online Data Supplement for details).

ECG Measurements
For telemetric ECG recordings, a radiotelemetry transmitter (model EA-F20, Data Sciences International, St. Paul, Minn) was surgically inserted into the peritoneal cavity, with 1 subcutaneous lead placed at the right shoulder and a second subcutaneous lead placed at the lower left thorax. Before measurement, mice were allowed to recover for 1 week, followed by 1 week of measurement. The analog ECG signal was converted to digital output and stored on a computer using custom-made signal-monitoring software. Tracings were analyzed manually for heart rate and bradyarrhythmic episodes from 3 randomly selected episodes of 24 hours. Surface ECGs were recorded under isoflurane inhalation anesthesia (0.8 to 1.0% volume) using subcutaneous 23-gauge needle electrodes attached to each limb. Lead I was analyzed manually for heart rate, PQ, QRS, and QT duration. QT intervals were corrected for heart rate using the formula (RR in ms):

\[
QT_C = \frac{QT}{RR^{1/2}} \cdot 100
\]

which may be regarded as the mouse equivalent of Bazett’s formula.15 In a subset of mice, the sodium channel blocker flecainide acetate was administered intraperitoneally (20 mg/kg body weight), and ECGs were continuously measured before and 30 minutes after injection.

Epidermal Mapping Experiments
Mice were anesthetized by an intraperitoneal injection of pentobarbital, after which the heart was excised, cannulated, mounted on a Langendorff perfusion set-up, and perfused at 37°C with a solution containing (in mmol/L) 128 NaCl, 4.7 KCl, 1.45 CaCl₂, 0.6 MgCl₂, 27 NaHCO₃, 0.4 NaH₂PO₄, and 11 glucose (pH maintained at 7.4 by equilibration with a mixture of 95% O₂ and 5% CO₂). Ventricular extracellular epicardial electrograms were recorded from the RV and left ventricle (LV) using a multielectrode (247 unipolar 19×13 electrode grid, interelectrode spacing 300 μm) during sinus rhythm, ventricular pacing at a basic cycle length of 120 ms, and twice the diastolic stimulus current threshold from the center of the electrode. The effective refractory period was determined by reducing the coupling interval of a premature stimulus (after 16 stimuli at basic cycle length 120 ms) in steps of 5 ms until activation of the ventricle failed. Electrograms were acquired using a custom-built 256-channel data acquisition system. Activation maps were constructed from local activation times determined by the time between the stimulus artifact and the maximal negative dV/dt as measured from the unipolar electrograms using custom software. Ventricular activation time was determined as the difference between the first and last moment of activation measured under the recording electrode grid. Maximal conduction velocities in both longitudinal and transverse directions were measured from RV and LV activation maps.

AP and Sodium Current Measurements

Cell Isolation
For the isolation of ventricular myocytes, excised hearts were first perfused for 5 minutes in a Langendorff system (37°C) with normal Tyrode’s solution containing (in mmol/L) 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5.5 glucose, 5 HEPES; pH 7.4 (NaOH). Next, the heart was perfused for 8 minutes with a similar solution in which the calcium concentration was lowered to 1 μmol/L, after which the enzyme Liberase Blendzyme type 4 (0.05 mg/mL; Roche Diagnostics, GmbH, Mannheim, Germany) and trypsin (1 μL/mL of 2.5% solution; Roche Diagnostics, Indianapolis, Ind) were added to the low-calcium solution for 10 minutes. Digested tissue was gently triturated in the low-calcium enzyme solution and isolated cells were washed twice in the low-calcium solution and twice in normal-calcium Tyrode’s solution, both supplemented with BSA (50 mg/mL). Quiescent, rod-shaped cells with clear cross-striations and smooth surface were selected for measurements.
Data Acquisition

Patch pipettes were used to record membrane potentials and currents in the perforated or ruptured whole-cell configuration of the patch-clamp technique (1 to 3 MΩ borosilicate glass). Signals for sodium current were low-pass filtered with a cut-off frequency of 5 kHz and digitized at 5 to 10 kHz; AP measurements were filtered and digitized at 10 and 40 kHz, respectively. Series resistance was compensated by ≈75%. Custom-made software was used for voltage control, data acquisition, and analysis.

AP Measurements

APs were recorded with the perforated patch-clamp technique (Axopatch 200B Clamp amplifier, Axon Instruments, Molecular Devices Corp, Sunnyvale, Calif) using the Tyrode’s solution previously described (37°C); pipette solution contained (in mmol/L) 125 K-glucanate, 20 KCl, 5 NaCl, 2.2 amphotericin-B, 10 HEPES; pH 7.2 (NaOH). APs were elicited at 1 to 8 Hz by 3-ms current pulses through the patch pipette. To reduce variability in the moment of AP upstroke, stimulus amplitude was chosen such that the AP upstroke originated just before the end of the stimulus. The maximal AP upstroke velocity (dV/dtmax) was determined from the first derivative of the stimulus pulse was subtracted. Moreover, we analyzed resting membrane potential, maximal AP amplitude, plateau amplitude defined as the pulse was subtracted. Moreover, we analyzed resting membrane potential, maximal AP amplitude, plateau amplitude defined as the parameter from 10 consecutive APs were averaged. Potentials were corrected off-line for their estimated liquid junction potential.

Sodium Current Properties

Activation, inactivation, recovery from inactivation and slow inactivation parameters of sodium current were determined at room temperature using conventional voltage clamp protocols, with a holding potential of −120 mV and a cycle time of 5 seconds. Current density was calculated by dividing whole-cell current amplitude by cell capacitance (Cm). Data for voltage-dependence of activation and inactivation were fitted with a Boltzmann function,

\[ y = \left(1 + \exp \left[ -\frac{V - V_{1/2}}{k} \right] \right)^{-1}, \]

where \( V_{1/2} \) is the half-maximal voltage and \( k \) is the slope factor. Data for the recovery from inactivation was fitted by a 2-exponential function,

\[ y = y_0 + A_1 \left(1 - \exp \left[-\frac{t}{\tau_1}\right]\right) + A_2 \left(1 - \exp \left[-\frac{t}{\tau_2}\right]\right), \]

where \( A_1 \) and \( A_2 \) are fractions of fast and slow inactivating components and \( \tau_1 \) and \( \tau_2 \) are the time constants of the fast and slow inactivating components, respectively. Because the current decay could not be reliably fit to a 2-exponential function, the time course of inactivation was instead determined by analyzing the time required for 50% of current decay to occur (\( t_{50\%} \)). Development of slow inactivation was analyzed by fitting the data with a monoexponential function:

\[ \frac{I}{I_{\text{max}}} = A e^{-t/\tau}, \]

The bath solution contained (in mmol/L) 7.0 NaCl, 133 CsCl, 1.8 CaCl2, 1.2 MgCl2, 11.0 glucose, 5.0 HEPES, and 5 μmol/L nifedipine; pH 7.4 (CsOH). Pipettes were filled with (in mmol/L) 3.0 NaCl, 133 CsCl, 2.0 MgCl2, 2.0 Na2ATP, 2.0 TEACl, 10 EGTA, 5.0 HEPES; pH 7.3 (CsOH). To investigate sodium current characteristics at 37°C and physiological sodium concentrations, dV/dtmax was measured using alternating voltage- and current-clamp with a custom-made amplifier.16 Using the dV/dtmax as a measure of sodium channel availability, steady-state inactivation, recovery from inactivation, and slow inactivation were analyzed using protocols as indicated. Cells were superfused with the Tyrode’s solution described previously; the pipette solution contained (in mmol/L) 125 K-glucanate, 20 KCl, 1 MgCl2, 5 NaCl, 10 BAPTA, 5 MgATP, 10 HEPES; pH 7.2 (NaOH).

Persistent sodium current was measured at 37°C as 30 μmol/L tetrodotoxin-sensitive current during a descending voltage ramp protocol. The bath solution contained (in mmol/L) 130 NaCl, 10 CsCl, 1.8 CaCl2, 1.2 MgCl2, 11.0 glucose, 5.0 HEPES, and 5 μmol/L nifedipine; pH 7.4 (CsOH). The pipette solution was identical to that used for the sodium current measurements described previously.

Statistical Analysis

Values are shown as mean±SEM, unless otherwise indicated. Unpaired Student t tests and ANOVA (general linear model with repeated measures, SPSS) were used to analyze differences between two groups. Categorical data were analyzed with the Fisher exact test, where appropriate. The level of statistical significance was set to \( P<0.05 \).

The authors had full access to the data and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
Results

Mice Characteristics
Heterozygous Scn5a1798insD/H11001 offspring were born at a Mendelian frequency, whereas homozygotes were not viable (see online Data Supplement). No sudden death was observed in either wild-type or Scn5a1798insD/H11001 mice (age 5 months).

Scn5a mRNA and Protein Expression
In Scn5a1798insD/H11001 mice, 1798insD Scn5a mRNA accounted for half of total Scn5a mRNA (see online Data Supplement). Western blot analysis showed no appreciable differences in Scn5a protein expression levels between hearts from Scn5a1798insD/H11001 (mean ratio Scn5a/calnexin 3.9±1.3, n=3) and wild-type mice (3.5±0.3, n=3; P=NS) (Figure 1).

Electrocardiographic Measurements
Figure 2A depicts representative examples of continuous telemetry recordings. Scn5a1798insD/H11001 mice showed more sinus pauses of longer duration and significantly lower heart rates compared with wild-type mice (Figure 2B). No ventricular arrhythmias were observed in either wild-type or Scn5a1798insD/H11001 mice. On surface ECG recordings, no significant differences in RR interval were observed between wild-type (138.3±4.3 ms) and Scn5a1798insD/H11001 (133.3±3.1 ms; P=NS) mice. However, significant prolongation of PQ interval (35.4±0.8 versus 31.9±0.6 ms; both n=11; P<0.0001), QRS duration (9.9±0.2 versus 8.0±0.2 ms; P<0.001), and QTc interval (44.4±1.1 versus 40.3±1.0 ms; P<0.05) was observed in Scn5a1798insD/H11001 mice compared with wild-type mice (Figure 3C). On administration of the sodium channel blocker flecainide, a significantly larger prolongation of the PQ interval was observed in Scn5a1798insD/H11001 mice (21.5±2.2 ms increase, n=6) compared with wild-type mice (13.1±0.8 ms, n=6; P<0.01), in addition to a significantly greater lengthening of the QRS duration in Scn5a1798insD/H11001 (3.5±0.6 ms increase) versus wild-type mice (1.9±0.3 ms; P<0.05) (Figure 3B and 3D). Furthermore, flecainide induced extreme sinus bradycardia and/or sinus arrest in 4 of 6 Scn5a1798insD/H11001 mice, compared with none of the 6 wild-type mice (Figure 3B). On repeated ECG measurements 24 hours after flecainide administration, ECG parameters for both groups had returned to values similar to those at baseline prior to flecainide administration (data not shown).
Epicardial Mapping Experiments

Figure 4C shows typical examples of activation maps from the LV and RV for a wild-type mouse and an Scn5a<sup>1798insD/H11001</sup> mouse. When stimulated from the center of the electrode grid, crowding of the isochrones (indicating slowing of conduction) was seen predominantly in the RV of Scn5a<sup>1798insD/H11001</sup> hearts. The total activation time during basic stimulation from the center of the electrode was significantly increased in the RV hearts from Scn5a<sup>1798insD/H11001</sup> mice compared with wild-type mice, but not in the LV; during sinus rhythm no significant differences were observed (Figure 4D). In addition, transverse conduction velocity in the RV was reduced in Scn5a<sup>1798insD/H11001</sup> mice compared with wild-type mice; a concomitant increase in anisotropic ratio (ratio of longitudinal and transverse conduction velocity) was found. The effective refractory period was significantly increased in the RV (but not LV) of hearts from Scn5a<sup>1798insD/H11001</sup> mice compared with wild-type mice.

AP Characteristics

Figure 5A shows representative APs measured at 2 Hz, displaying a considerably longer AP and a lower dV/dt<sub>max</sub> (inset) in the Scn5a<sup>1798insD/H11001</sup> myocyte compared with the wild-type myocyte. Figure 5B summarizes AP characteristics of wild-type and Scn5a<sup>1798insD/H11001</sup> cells at a frequency of 2 Hz. On average, dV/dt<sub>max</sub> was significantly smaller in Scn5a<sup>1798insD/H11001</sup> compared with wild-type myocytes (257.9±19.8 V/s versus 371.1±39.4 V/s, P<0.05, left panel). No significant differences in resting membrane potential, maximal AP amplitude, APD<sub>20</sub>, APD<sub>50</sub>, or plateau level were observed (middle panel). Scn5a<sup>1798insD/H11001</sup> cells showed a considerably longer APD<sub>90</sub> at 2 Hz (157.6±17.7 ms, Figure 4) versus wild-type cells (105.4±7.2 ms, Figure 2588 Circulation December 12, 2006).
Prolongation of AP duration in Scn5a1798insD/ mice was evident at frequencies <4 Hz (Figure 5C, left panel), whereas the decreased dV/dtmax was present at all frequencies measured (Figure 5C, right panel). At a stimulus frequency of 1 Hz, early afterdepolarizations were observed in 3 of 10 Scn5a1798insD/ cells (example shown in Figure 5D), but never in wild-type cells (P=0.09, Fisher exact test).

Figure 5. AP measurements in isolated myocytes. A, Representative examples of APs and upstroke velocity (dV/dtmax, inset) measured from wild-type and Scn5a1798insD/ myocytes at 2 Hz. B, Averaged data at 2 Hz for dV/dtmax, resting membrane potential (RMP), maximal AP amplitude (APA), plateau amplitude (Pla) defined as the potential difference between RMP and potential 20 ms after the upstroke, and AP duration (APD) at 20, 50, and 90% repolarization (APD20, APD50, and APD90, respectively; P<0.05). C, Averaged data for APD90 and dV/dtmax at various stimulation frequencies from wild-type and Scn5a1798insD/ myocytes (P<0.05). D, Example of an early afterdepolarization (EAD) observed in an Scn5a1798insD/ myocyte (1 Hz) with incidence of EADs in wild-type and Scn5a1798insD/ myocytes (inset).
Figure 6. Sodium current properties assessed with conventional voltage clamp (protocols shown as insets). A, Examples of sodium current traces recorded from wild-type (left) and Scn5a^1798insD/H11001 (right) myocytes. B, Average current-voltage relationships for wild-type and Scn5a^1798insD/H11001 sodium current (*P<0.05). C, Voltage-dependence of activation; solid lines represent Boltzmann fits with $V_{1/2} = -46.1\pm2.0$ mV and $k = 4.3\pm0.3$ mV for wild-type ($n=7$) and $V_{1/2} = -44.6\pm1.2$ mV and $k = 4.6\pm0.5$ mV for Scn5a^1798insD/H11001 ($n=11, P=NS$). D, Steady-state voltage-dependence of inactivation with Boltzmann fits in solid lines; wild-type ($n=6$) $V_{1/2} = -84.2\pm5.0$ mV and $k = -6.2\pm0.6$ mV, Scn5a^1798insD/H11001 ($n=11$) $V_{1/2} = -86.5\pm3.3$ mV and $k = -6.1\pm0.3$ mV ($P=NS$). E, Recovery from inactivation: wild-type ($n=6$) $\tau = 5.02\pm0.6$ ms and $\tau = 43.4\pm4.8$ ms, Scn5a^1798insD/H11001 ($n=6$) $\tau = 4.98\pm1.0$ ms and $\tau = 43.6\pm3.6$ ms ($P=NS$). F, Time course of current inactivation defined as time required for 50% of current decay to occur ($t_{50\%}$) (*P<0.05). G, Development of slow inactivation.
Sodium Current Properties and Persistent Inward Current

Typical sodium currents measured in whole-cell voltage clamp mode at room temperature from wild-type and Scn5a\(^{1798\text{insD}}\) ventricular myocytes are displayed in Figure 6A. On average, peak current density in Scn5a\(^{1798\text{insD}}\) myocytes was significantly reduced by 39% (41.5 ± 7.5 pA/pF, n = 12) compared with wild-type myocytes (67.5 ± 11.3 pA/pF, n = 9, P < 0.05) (Figure 6B). Half-activation voltages (V\(_{1/2}\)) and slope factors (k) for activation and steady-state inactivation, as well as fast and slow time constants (\(\tau_f\) and \(\tau_s\), respectively) for recovery from inactivation, were not significantly different between wild-type and Scn5a\(^{1798\text{insD}}\) (Figures 6C through 6E). In addition, no significant differences in amplitude of fast (A\(_f\)) and slow (A\(_s\)) recovery from inactivation were observed between wild-type and Scn5a\(^{1798\text{insD}}\) (data not shown). The time required for 50% of current decay to occur (t\(_{50}\)) was significantly prolonged at a voltage range of −45 to −25 mV in Scn5a\(^{1798\text{insD}}\) myocytes compared with wild-type myocytes (Figure 6F). Development of slow inactivation was not found to be different between wild-type mice and Scn5a\(^{1798\text{insD}}\) mice (Figure 6G).

To test whether additional differences in kinetic properties were present when studied at 37°C and physiological sodium concentrations, additional experiments were performed using alternating voltage- and current-clamp protocols as shown in Figure 7. In Scn5a\(^{1798\text{insD}}\) myocytes, the upstroke velocity (dV/dt\(_{\text{max}}\)) of the elicited AP, a direct measure of sodium channel availability, was reduced by 31% at −120 mV (297.1 ± 38.4 V/s, n = 6) compared with wild-type myocytes (430.0 ± 40.1 V/s, n = 8, P < 0.05) (Figure 7A). No significant differences were observed in V\(_{1/2}\) and k for voltage-dependence of steady-state inactivation, \(\tau_f\) and \(\tau_s\), for recovery from inactivation, or amplitude (A\(_f\)) and time constant (\(\tau_s\)) for development of slow inactivation (Figures 7B through 7D). Also, no significant differences in A\(_f\) and A\(_s\) for recovery from inactivation were observed between wild-type mice and Scn5a\(^{1798\text{insD}}\) mice (data not shown).

Figure 8A shows representative current traces in response to a descending voltage ramp protocol (inset) in the absence and presence of 30 μmol/L tetrodotoxin. The persistent sodium current is measured as the tetrodotoxin-sensitive current (Figure 8B). In Scn5a\(^{1798\text{insD}}\) myocytes, the current density of persistent sodium current was observed to be significantly larger at a voltage range of −90 to −10 mV compared with wild-type (Figure 8C).

Discussion

Phenotype of Scn5a\(^{1798\text{insD}}\) Mice

We have generated a heterozygous transgenic mouse carrying the Scn5a mutation 1798insD (Scn5a\(^{1798\text{insD}}\)), equivalent to the human SCN5A mutation 1795insD found in a large Dutch
family presenting with a high incidence of nocturnal sudden death and features of sinus node dysfunction, conduction disease, long-QT syndrome, and Brugada syndrome. This mouse model recapitulates a large part of the diverse clinical phenotype observed in this family. Scn5a1798insD/+ mice display bradycardia and ECG parameters consistent with slow conduction and delayed repolarization. Furthermore, they show signs of sinus node dysfunction, both spontaneously as episodes of bradycardia and pauses lasting longer than 1 second, and after flecainide administration as bradycardia and/or sinus arrest.

Because 12-lead ECG morphology in mice is not similar to man, we could not evaluate whether the typical Brugada ECG pattern was present in this mouse model. Epicardial mapping experiments, however, showed that conduction in Scn5a1798insD/+ mice is predominantly affected in the RV, whereas LV conduction is generally unchanged. The predominant involvement of the RV is clinically well documented in Brugada syndrome. Similar to Scn5a1798insD/+ mice, we and others have previously shown the presence of RV conduction slowing in patients with this disorder.17–20 In addition, Van Veen et al11 have also recently shown the presence of conduction slowing and lengthening of the effective refractory period preferentially in the RV of Scn5a heterozygous knock-out mice. Although regional differences in transmural ion channel distribution as well as RV and LV size differences may play a role,22,23 the mechanism for a predominant role for the RV remains unclear. In contrast to the human phenotype, no sudden cardiac death occurred in Scn5a1798insD/+ mice, which may be attributable to the high intrinsic heart rate of mice compared with humans. We did not observe tachyarrhythmias in Scn5a1798insD/+ mice, but it should be noted that inducibility of ventricular arrhythmias was not tested. In patients carrying the 1795insD mutation, however, tachyarrhythmias have not been documented either, and ventricular arrhythmias may therefore not constitute part of the phenotype of this particular mutation.

**Figure 8.** A, Examples of persistent inward current measurements, using a descending voltage ramp protocol consisting of a 200-ms prepulse to +40 mV followed by a 1000-ms descending voltage ramp to −120 mV (inset). B, Persistent inward current traces obtained by subtraction of the current before and after application of 30 μmol/L tetrodotoxin (TTX). C, Mean values for persistent inward current density (P<0.05 versus wild-type).

Because homozygote mutant mice are not viable, it is not possible to directly measure the biophysical properties of pure mutant current in myocytes. Although these considerations may explain some of the differences in electrophysiological characteristics with previous transfection studies, the

**Electrophysiological Characteristics of Scn5a1798insD/+ Cardiomyocytes**

Patch-clamp experiments showed that AP prolongation in Scn5a1798insD/+ cardiomyocytes was most pronounced at relatively slow pacing rates, whereas sodium channel availability as measured by AP upstroke velocity was progressively decreased at higher stimulation frequencies. These observations are in accordance with the proposed hypothesis of a heart rate–dependent coexistence of both LQT3 and Brugada syndrome, with a prolongation of cardiac repolarization predominantly at slow heart rates and decreased sodium channel availability especially at fast heart rates.14,24

In contrast to previous studies of the biophysical properties of the 1795insD mutation utilizing heterologous expression systems,7,14 no significant changes were observed in the voltage dependence of either activation or inactivation, nor in the development of slow inactivation. However, fast inactivation was disrupted, as evidenced by a delayed time course of current decay in Scn5a1798insD/+ myocytes, potentially underling the observed persistent current. Myocytes from Scn5a1798insD/+ mice display a significant reduction in peak sodium current of 39%, caused either by dysfunctional mutant channels located in the cell membrane or by ineffective trafficking of mutant channels to the cell surface. In any case, the sodium current properties represent largely those of the wild-type sodium channels present in these cells. As a result, small changes in mutant current kinetics (as previously measured in transfection systems) may be masked by the prevailing wild-type channels. The significant increase in persistent inward current in Scn5a1798insD/+ myocytes surely represents a contribution of the mutant channels, indicating that at least some of them are functional on the cell surface. Because homozygote mutant mice are not viable, it is not possible to directly measure the biophysical properties of pure mutant current in myocytes. Although these considerations may explain some of the differences in electrophysiological characteristics with previous transfection studies, the
measurements from the present study represent the true situation in these mice and reflect the situation in patients who carry this mutation heterozygously. Hence, these observations underscore the danger of overinterpretation of electrophysiological properties attributed to ion channel mutations obtained from studies in heterologous expression systems instead of native cardiomyocytes. In Scn5a1798insD+/− mice, the observed disrupted fast inactivation and small persistent inward current in combination with the drastic reduction in sodium current density were sufficient to cause a relevant phenotype, as shown by ECG measurements and epicardial mapping experiments. Interestingly, the size of the persistent inward current measured in myocytes from Scn5a1798insD+/− mice was of similar magnitude compared with that observed in the ΔKPQ heterozygous mice, a model of pure LQT3. In the latter mice, substantial AP prolongation was observed. Although the peak sodium current was increased, only a relatively small persistent current was found, which was obviously sufficient to cause a long-QT phenotype.

The bradycardia and sinus node dysfunction (exacerbated by the sodium channel blocker flecainide) observed in Scn5a1798insD+/− mice appears similar to that described in Scn5a heterozygous knock-out mice.26 Experiments on the sinoatrial node of these mice have indicated an important role for the cardiac sodium channel in coupling of sinoatrial and atrial cells, and thus in heart rate regulation. Although Scn5a1798insD+/− mice share a number of similarities in phenotype with the heterozygous Scn5a knock-out mice, an important difference is the absence of QT prolongation in the latter. Therefore, the observed QT prolongation in our mice must be attributed to the presence of the small persistent inward current. Furthermore, the persistent inward current in Scn5a1798insD+/− mice may also account for the observed bradycardia and sinus pauses resulting from sinoatrial node cells failing to repolarize under conditions of extra net inward current.28

Conclusions

Scn5a1798insD+/− mice recapitulate a large part of the diverse clinical phenotype of patients carrying the human equivalent mutation SCN5A-1795insD+/−, including bradycardia, conduction slowing, and QT prolongation. The Scn5a1798insD+/− mouse thus represents the first transgenic model of a sodium channelopathy evoking multiple cardiac rhythm disturbances. Extensive analysis of Scn5a1798insD+/− cardiomyocytes has provided mechanistic insight into the electrophysiological abnormalities associated with this mutation in the native cardiomyocyte environment. The mutation leads to a drastic reduction in peak sodium current density, a delayed time course of fast inactivation, and a small persistent sodium current, explaining the observed multiple phenotypes. These results demonstrate that the presence of a single SCN5A mutation is indeed sufficient to cause an overlap syndrome of cardiac sodium channel disease. The mouse model in the present study constitutes a useful tool for future studies addressing as yet unanswered questions, such as the role of genetic and environmental modifiers on cardiac conduction and repolarization.

Acknowledgments

We are indebted to Leander Beekman and Tamara T. Koopmann for excellent technical assistance. We thank Wiet Schepers (Neurogenetics Laboratory, Academic Medical Center, Amsterdam, The Netherlands), André T.J. Klein and Phil Barnett (Experimental and Molecular Cardiology Group, Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands) for their expert help on the Western blotting protocol.

Funding Sources

This study was supported by the Netherlands Heart Foundation (Grant 2003/B195). C.R.B. is an Established Investigator of the Netherlands Heart Foundation (Grant 2005/T024).

Disclosures

None.

References

Mutations in the gene encoding the cardiac sodium channel (SCN5A) have been implicated in multiple arrhythmia syndromes, each characterized by distinct electrocardiographic and clinical features, including long-QT syndrome type 3, Brugada syndrome, conduction disease, and sinus node dysfunction. These disorders are now considered to display more similarities than previously appreciated, however, introducing what have become known as “overlap syndromes of cardiac sodium channel disease. This mouse model constitutes a useful tool for future studies addressing as yet unanswered questions such as the role of genetic and environmental modifiers on cardiac conduction and repolarization.
Overlap Syndrome of Cardiac Sodium Channel Disease in Mice Carrying the Equivalent Mutation of Human SCN5A-1795insD


_Circulation._ 2006;114:2584-2594; originally published online December 4, 2006; doi: 10.1161/CIRCULATIONAHA.106.653949

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/114/24/2584

Data Supplement (unedited) at:
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