Contrasting Nav1.8 Activity in Scn10a−/− Ventricular Myocytes and the Intact Heart

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Background—Genome-wide association studies have implicated variants in SCN10A, which encodes Nav1.8, as modulators of cardiac conduction. Follow-up work has indicated that the SCN10A sequence includes an intronic enhancer for SCN5A. Yet the role of the Nav1.8 protein in the myocardium itself is still unclear. To investigate this, we use homozygous knockout mice (Scn10a−/−) generated by disruption of exons 4 and 5, leaving the Scn5a enhancer intact.

Methods and Results—We previously reported that pharmacologic blockade of Nav1.8 in wild-type animals blunts action potential prolongation by ATX-II at slow drive rates (≤1 Hz). Here we present evidence of the same blunting in Scn10a−/− compared to wild-type ventricular myocytes, supporting the conclusion that Nav1.8 contributes to late sodium current at slow rates. In contrast to earlier studies, we found no differences in electrocardiographic parameters between genotypes. Low-dose ATX-II exposure in lightly anesthetized animals and Langendorff-perfused hearts prolonged QTc and generated arrhythmias to the same extent in wild-type and Scn10a−/−. RNA sequencing failed to identify full-length Scn10a transcripts in wild-type or knockout isolated ventricular myocytes. However, loss of late current in Scn10a−/− myocytes was replicated independently in a blinded set of experiments.

Conclusions—While Scn10a transcripts are not detectible in ventricular cardiomyocytes, gene deletion results in reproducible loss of late sodium current under extreme experimental conditions. However, there are no identifiable consequences of this Scn10a deletion in the intact mouse heart at usual rates. These findings argue that common variants in SCN10A that affect ventricular conduction do so by modulating SCN5A. (J Am Heart Assoc. 2016;5:e002946 doi: 10.1161/JAHA.115.002946)

Key Words: sodium channels • transgenic mice • ventricular arrhythmia

Multiple genome-wide association studies have identified common variants in SCN10A as modulators of PR and QRS durations in humans,1,2 but the underlying mechanisms have remained uncertain.3,4 An initial study reported little immunostaining for Nav1.8, the sodium channel encoded by SCN10A, in ventricular myocytes but noted expression in the cardiac ganglia and atrial myocytes.5,6 However, the antibodies used were not validated by Western blots or in tissue from Scn10a null animals. Other groups used reverse transcription polymerase chain reaction (RT-PCR) to identify expression of the gene in ventricular myocytes and more extensively in the conduction system.1,2,7

Initial reports using telemetry in mice reported PR, QRS, and HV interval prolongation after treatment with the specific Nav1.8 blocker A-803467.2 By contrast, initial analysis of the Scn10a−/− mouse model used in the present studies described a shorter PR interval in the knockouts relative to the wild type.1 Our in vitro studies in mouse and rabbit ventricular myocytes showed that A-803467 shortened action potential duration (APD) at slow drive rates; furthermore, A-803467 suppressed arrhythmogenic afterdepolarizations elicited at slow rates by the sea anemone toxin ATX-II, an enhancer of late sodium current generated by multiple voltage-gated sodium channels including SCN5A and SCN10A.7

SCN10A transcripts are much less abundant in heart than those for SCN5A, encoding the canonical cardiac sodium channel Nav1.5 thought to mediate cardiac conduction velocity.7 Thus, alternate hypotheses suggesting an indirect
effect of SCN10A on conduction have been advanced. One possibility is that Nav1.8, which is known to be highly expressed in dorsal root ganglia, modulates conduction through a neurally mediated effect. Other studies have identified intronic variants in SCN10A that regulate SCN5A expression.

We report here the in vivo and in vitro electrophysiologic consequences of deleting Scn10a; in the mouse we studied, the sequence regulating Scn5a expression has been left intact. While we extend and reproduce our previous in vitro findings, we find no electrophysiologic effect of deleting Scn10a in the intact ventricle. Thus, we conclude that SCN10A variants regulate conduction through indirect mechanisms.

Methods

Animal Model

The mouse model used in the present report was originally generated by, and rederived by, MRC Harwell. In this animal, the majority of exons 4 and 5 have been replaced with a neomycin-resistance cassette. Previous studies indicate transcription of the null allele results in a low-level expression of truncated Scn10a in mouse dorsal root ganglia.

The animals are genotyped using 2 forward primers, 1 in the 5’ (intact) region of exon 4 and the other in the neomycin cassette, and a common reverse primer in intron 5. In homozygous wild-type (WT) animals these primers generate an 1100-bp band, and in homozygous knockouts (Scn10a<sup>−/−</sup>) a 900-bp band. The SCN10A region implicated by van den Boogaard et al as an SCN5A enhancer is located further 3’ and is intact in these animals. The use of animals was approved by the Institutional Review Board and Animal Care at Vanderbilt Medical Center and studies were performed according to National Institutes of Health guidelines. Approximately 220 animals were used in this study.

Quantitative RT-PCR

Total mRNA from adult mice atria and ventricles was isolated using the TRIzol method (Invitrogen). Gene-specific cDNA was amplified using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Thermo Fisher Scientific) and gene-specific primers; SNS, X92184, 5’-CGAGATCGAAGGAGAGTCGAG-3’, 5’-GCGTGCCCTACTGAGTGCCATC-3’ by following the kit protocol. PCR products were imaged using a 2% ethidium bromide agarose gel.

Isolation of Mouse Ventricular Cardiomyocytes

Given the recognition of a sex effect on repolarization in mice by our work and others, experiments were conducted in isolated ventricular myocytes from adult female C57 mice. Myocytes were isolated by a modified collagenase/protease method, as previously reported. All procedures were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Peak and Late Sodium Current Recordings

Whole-cell voltage clamp experiments for sodium current recordings were conducted at 18°C. To better voltage control sodium current recordings in mouse myocytes, the external K<sup>+</sup>- and Ca<sup>2+</sup>-free bath solution contained (in mmol/L) NaCl 20, CsCl 5, TEA-Cl 1.0, glucose 10, and HEPES 10; the pH was 7.4, adjusted with CsOH. The pipette (intracellular) solution contained (in mmol/L) NaF 5, CsF 110, CsCl 20, EGTA 10, and HEPES 10; the pH was 7.4, adjusted with CsOH. To eliminate the possible L- or T-type inward calcium currents and transient outward potassium current (I<sub>TO</sub>) in mouse myocytes, nisoldipine (1 μmol/L), NiCl<sub>2</sub> (200 μmol/L), and 4-aminopyridine (4-AP, 500 μmol/L) were added into the bath solution, respectively.

Glass microelectrodes were heat polished to tip resistances of 0.5 to 1 MΩ. Data acquisition was carried out using an Axopatch 200A patch-clamp amplifier and pCLAMP version 9.2 software (MDS Inc, Mississauga, Ontario, Canada). Currents were filtered at 100 kHz (~3 dB, 4-pole Bessel filter) and digitized using an analog-to-digital interface (DigiData 1322A, MDS Inc). To minimize capacitive transients, capacitance and series resistance were corrected (~80%). Cell size (in μm<sup>2</sup>) was calculated by Membrane Test (OUT 0) in pClamp 9.2. Clamp protocols used are shown in figure 3.

Since late sodium current (I<sub>Na-L</sub>) in cardiac cells is very small (~0.5% of peak sodium current, even under a physiologic concentration of extracellular sodium), it is technically challenging to establish a typical current–voltage (I–V) relationship for I<sub>Na-L</sub>. In order to establish the I–V relations for I<sub>Na-L</sub> in WT and Scn10a<sup>−/−</sup> cardiomyocytes, therefore, we pretreated myocytes with the INa-L enhancer ATX-II (3 nmol/L) for 15 minutes before the electrophysiologic studies. A 200-ms voltage clamp protocol to +60 mV in 10-mV increments
from the holding potential of −120 mV was used to record sodium current. \(I_{Na-L}\) was measured in a 3-ms time window (195–198 ms after the pulse) before the capacity transient at the end of the 200-ms pulse and expressed as the percentage of peak sodium current or the current density (in pA/pF).

### Action Potential (AP) Recordings

In current-clamp mode, APs from isolated mouse ventricular myocytes were elicited by injection of a brief stimulus current (1–2 nA, 2–6 ms, variable stimulation frequencies). For AP experiments, the bath (extracellular) solution contained (in mmol/L): NaCl 135, KCl 4.0, CaCl\(_2\) 1.8, and MgCl\(_2\) 1.0, HEPES 5.0, and glucose 10, with a pH of 7.4 (adjusted by NaOH). The pipette-filling (intracellular) solution contained (in mmol/L): KCl 110, ATP-K\(_2\) 5.0, MgCl\(_2\) 1.0, BAPTA-K\(_2\) 5.0, and HEPES 10, with a pH of 7.2 (adjusted by KOH). Microelectrodes with tip resistances of 3 to 5 MΩ were used. Ten successive traces were averaged for analysis of APDs at 50% and 90% repolarization (APD\(_{50}\) and APD\(_{90}\)). For experiments in which enhancement of late current, the action potential prolongation, and early afterdepolarizations were examined, variable concentrations of ATX-II (1 and 3 nmol/L) were added into the external solution. Electrophysiological data were analyzed using pCLAMP version 9.2 software and the figures were prepared by using Origin 8.5.1 software (OriginLab Corp, Northampton, MA).

### Surface ECG Collection and Analysis

ECGs were recorded during inhaled administration of isoflurane vapor titrated to maintain light anesthesia. Mice were anesthetized initially under a concentration of 2% isoflurane and then held under a constant flow of roughly 1%, ventilated with \(O_2\). The heart rate was continuously monitored and isoflurane levels were adjusted to keep the heart rate between 350 and 450 bpm. Baseline ECG (leads I and II) was recorded for 5 minutes following which the animals were injected intraperitoneally with ATX-II (0.03 mg/kg) dissolved in water and measurements were acquired for 20 to 25 minutes post–drug administration. This dose was identified in preliminary experiments defining no effect (0.01 mg/kg) and acutely toxic (0.06 mg/kg) doses.

ECG intervals were determined after signal averaging 30 s of data in each lead using a custom-built LabVIEW program (National Instruments, Austin, TX) and analyzed using an electronic caliper by an investigator blind to the genotype. Heart rate was determined as the average during 30 s. QRS duration was measured from the first deflection of the Q-wave to the end of the S-wave, defined as the point of minimum voltage in the terminal phase of the QRS complex. QT interval was measured from the beginning of the QRS complex to the end of the T-wave, defined as the point where the T-wave merges with the isoelectric line. Heart rate–corrected QT interval (QTc) was calculated using a formula developed for mice: QTc–QT/(RR/100). For each interval, the larger value from each lead is reported.

For arrhythmia analysis, the ECG was examined for arrhythmia activity starting 10 minutes after ATX injection. The amount of time spent not in sinus rhythm was totaled for each mouse.

### Langendorff Preparation

Mice aged 10 to 12 weeks received an intraperitoneal injection of heparin (0.5 U/g) followed by vaporized isoflurane (5%) in oxygen titrated to a deep level of anesthesia. Cervical dislocation was performed, followed by thoracotomy and excision of the heart and aorta cannulation. Hearts were perfused retrograde with modified Tyrode's (130 mmol/L NaCl, 4 mmol/L KCl, 23 mmol/L NaHCO\(_3\), 1.5 mmol/L NaH\(_2\)PO\(_4\), 1 mmol/L MgCl\(_2\), 2 mmol/L CaCl\(_2\), and 10 mmol/L glucose) bubbled with 95% \(O_2/5%\) \(CO_2\) in the Langendorff mode at a constant perfusion pressure of 70 mm Hg at 37°C or at room temperature.

### Langendorff Data Analysis

The ECGs were viewed using LabChart 7 Reader (ADInstruments v 7.3.4) and the heart rate was determined for 1 minute prior to ATX injection. The number of separate arrhythmia events and total time not in sinus rhythm were analyzed for 10 minutes immediately following ATX injection.

### RNA-Seq

RNA was extracted from 2 freshly harvested mouse myocyte samples, using Qiagen’s Rneasy Mini Kit. The kit protocol was followed with the additional step of on-column DNase digestion.

The short-read sequence files generated by Illumina’s CASAVA pipeline were first processed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Mean base-call quality scores were above pred-like values of 32 in all positions of all samples, and no hard trimming of the reads was necessary. The sequence files were then aligned to mouse assembly build mm10 using the TopHat2 splice junction mapper (http://ccb.jhu.edu/software/tophat/index.shtml). Transcripts were assembled by and differential expression analysis was performed with Cufflinks (http://cole-trapnell-lab.github.io/cufflinks/) using the bias-correction (\(-u\)) and stranded-library (fr-firststrand) options. RefSeq gene annotation was incorporated during the “cuffmerge” step to allow for quantification of known as well...
as novel transcript isoforms. In the final “cuffdiff” step, individual samples were merged into WT and Scn10a−/− replicate groups.

## Chemicals

Nisoldipine, NiCl₂, 4-aminopyridine (4-AP), and ATX-II were purchased from Sigma-Aldrich Co (St. Louis, MO). Stock solutions for the drugs were prepared according to the vendor’s instructions and then diluted for studies as needed.

## Statistical Analysis

Results are presented as mean±SEM, and statistical comparisons were made using the unpaired and paired Student t test. A value of P<0.05 was considered statistically significant. Statistical significance of ECG parameters and arrhythmia was calculated using the Wilcoxon rank-sum test and median and interquartile range are reported.

## Results

### Loss of Nav1.8 Function Does Not Affect Expression of Scn5a

The exon 4 to 5 deletion leaves the SCN10A sequences implicated as enhancing SCN5A expression intact in these animals. Figure 1A shows that there was no significant difference in the level of Scn5a expression between WT and Scn10a−/− animals. To confirm the loss of Scn10a in our mouse line, we performed RT-PCR using dorsal root ganglion tissue across exons 3 to 8 wherein cassette insertion removes a portion of the exons 4 and 5 resulting in a smaller cDNA fragment in Scn10a−/− mice (Figure 1B).

### Expression of Scn10a in Isolated Myocytes

To assess expression of Nav1.8 in isolated cardiomyocytes, we sequenced both directly from cDNA isolated from cardiomyocytes and using RNASeq. Under both experimental conditions, no Scn10a transcripts from exons 1 to 8 and exons 13 and 14 were detected in either WT or Scn10a−/− myocytes.

### In Vitro Arrhythmogenic Effects of ATX-II

There was no significant difference in baseline APDs between WT and Scn10a−/− (Figure 2A): 24.1±0.4 ms versus 23.5±0.4 ms (P>0.05, n=10 each; 1 Hz). Interestingly, and despite the RNA data, we did identify differences in electrophysiologic behavior in WT and Scn10a−/− myocytes under more extreme experimental conditions. Following 1 nmol/L ATX-II exposure, early afterdepolarizations (occurred as a positive upstroke arising from early phase 3 of the action potential) were present in 8 out of 12 WT cells but only 1 out of 12 Scn10a−/− cells (P<0.01, Figure 2B through 2D; 1 Hz).

### Late Sodium Current in WT and Scn10a−/− Isolated Cardiomyocytes

Our previous work using heterologous expression and A-803467 in WT cells defined differences in current–voltage (IV)
relationships for Nav1.5- and Nav1.8-mediated currents. Figure 3A and 3B highlight differences in late currents between WT and Scn10a−/− ventricular myocytes. Summary and digital subtraction data (Figure 3C) show the signature of Nav1.8-mediated late current is very similar to that we previously described using heterologous expression. In contrast to the changes in late current, peak sodium current sizes in WT and Scn10a−/− ventricular myocytes remained unchanged (Figure 3D). To gain further confidence in these results, they were repeated and replicated (Figure 4) in a separate laboratory in experiments in which the electrophysiologist was blinded to genotype. The data reproduce the obvious Nav1.8-like difference current between WT and Scn10a−/− cardiomyocytes.

These differences between current–voltage relations for Nav1.5 and Nav1.8 translate into changes in APD. Figure 5 shows that after ATX-II treatment, action potentials were markedly prolonged at 0 mV and at −30 mV in WT cells. However, in Scn10a−/− cells, APD at 0 mV was unchanged by ATX-II, while APD at −30 mV was prolonged as seen in WT cells. When APD at 20%, 50%, and 90% repolarization was assessed, there was no change in APD20 after ATX-II in Scn10a−/− cells but both APD50 and APD90 were prolonged. These findings further support a role for Nav1.8 in mediating repolarization especially at more positive potentials, and demonstrate that the prolongation at more negative potentials, where Nav1.5 makes a major contribution, was intact.

No Changes in ECG Parameters Between WT, Scn10a+/−, and Scn10a−/− Mice

Unlike previous reports,1,2 and despite the APD changes we describe above, we found no significant differences in PR interval, P-wave duration, QRS interval, or QTc among WT, heterozygotes, and Scn10a−/− anesthetized animals (Table 1).

ATX-II exposure in vivo resulted in PR, QRS, and QTc prolongation, changes in T-wave morphology, and polymorphic ventricular arrhythmias, but again there was no difference between WT and Scn10a−/− animals (Table 2, Table S1). Interestingly, and in keeping with recent reports of an association between atrial fibrillation and SCN10A...
variants in humans, there was an increase in the number of premature atrial contractions in the Scn10a/C0/C0 (19.2 ± 5.6, n=11) animals compared to WT (8.4 ± 2.3, n=11).

To exclude the possibility of incomplete action of ATX-II as a result of intraperitoneal injection, we isolated hearts and directly exposed them to 3 nmol/L ATX-II using Langendorff perfusion. Again, there was no difference between WT and Scn10a/C0/C0 hearts in ECG intervals or arrhythmia episodes or total duration. Since the in vitro studies were performed at room temperature, we performed another set of Langendorff experiments at 37°C, and again observed no differences.

Discussion

The contribution of the SCN10A encoded sodium channel Nav1.8 to cardiac conduction has been a topic of significant interest since association of common SCN10A variants with modulation of ECG parameters. Expression of the channel has previously been reported in cardiomyocytes using RT-PCR but at a very low level. Studies suggest it is enriched in the Purkinje fibers, while immunostaining indicates expression in the cardiac ganglia. Our investigations have focused on the possible cell-autonomous role for Nav1.8 in ventricular myocytes. We have reported that, in vitro, pharmacological blockade or genetic loss of function of Nav1.8 reduces late sodium current, thus preventing ATX-II-induced early afterdepolarizations. However, there is no effect on peak sodium current (Figure 3D), a major determinant of cardiac conduction velocity.

While others have reported that A803467 or Scn10a knockout produces ECG changes in intact mice, we found no such differences. Possible explanations include our use of light anesthesia as opposed to conscious mice. We also performed more experiments than in previous studies in WT mice (n=14 versus n=4) and in a smaller age range, 8 to 12 weeks, as opposed to 8 to 34 weeks. Under physiological conditions in the mouse, we detect no difference in repolarization or response to ATX-II between genotypes. Given the in vitro data, we infer that the most likely explanation is that the channel is expressed at a level

**Figure 3.** Voltage-dependence of late sodium currents in wild-type (WT) and Scn10a<sup>−/−</sup> myocytes. A, In a WT mouse myocyte, ATX-II (3 nmol/L) augmented late INa at broad membrane potentials, shown as an inset. B, In a Scn10a<sup>−/−</sup> mouse myocyte, late INa magnitude increased by ATX-II (3 nmol/L) became dramatically smaller at positive membrane potentials, also shown as an inset. C, Summary of the current–voltage relations from WT, Scn10a<sup>−/−</sup> and difference current (as the SCN10A-encoded Nav1.8 component) are presented. D, Comparison of peak sodium current (INa) in WT and Scn10a<sup>−/−</sup> mouse myocytes (n=4 each).
below the detection of our RNA-Seq analysis. Therefore, we conclude that for ventricular myocytes, Nav1.8 exerts its modulatory effect primarily through alternate means, likely as acting as an enhancer for SCN5A expression.8 However, this awaits further studies testing the role of specific SCN10A SNPs on SCN5A expression for confirmation that it acts as the primary mechanism by which SCN10A impacts conduction.

Figure 4. A, Voltage ramp protocol was applied to elicit ramp current. B, Ramp currents were recorded from wild-type (WT) and Scn10a−/− cardiomyocytes. There is an inward late Na current present in WT cell, not in Scn10a−/− cell. Current traces were normalized to the peak current. C, The difference current between WT and Scn10a−/− cells based on (B).

Figure 5. Summary of the effects of ATX-II (1 nmol/L) on various action potential durations (APDs at 1 Hz) in WT and Scn10a−/− mouse myocytes. A, The left panel shows APD change at 0 mV (≈AP plateau) in 2 groups of myocytes before and after ATX-II; the right panel shows APD change at −30 mV (≈AP phase 3) in 2 groups of myocytes before and after ATX-II. B, The effects of ATX-II on APD20, 50, and 90 in 2 groups of myocytes are shown. Note that (1) all basal APDs in WT and Scn10a−/− myocytes are nonsignificant statistically; (2) in Scn10a−/− myocytes, ATX-II (1 nmol/L) had no significant effects on the APD measured at 0 mV and APD20. Each group had 12 myocytes.
The enhancer theory also does not preclude an action for SCN10A or Nav1.8 in the cardiac ganglion. Neuronal expression could contribute to the increased atrial fibrillation inducibility seen in vitro with both gain and loss of function SCN10A variants. Indeed, we noted that the Scn10a−/− mice demonstrate an increase in premature atrial contractions but have not pursued this further, as this study was focused on Nav1.8 in the ventricle. However, the notion is supported by

Table 1. Surface ECGs of Wild-Type, Scn10a+/−, Scn10a−/− Mice at 8 and 12 Weeks of Age

<table>
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<tr>
<th></th>
<th>WT</th>
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<th>Scn10a−/−</th>
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<td>8 weeks</td>
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</tr>
<tr>
<td>N</td>
<td>14</td>
<td>33</td>
<td>14</td>
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<td>58.2 (55.3–64.3)</td>
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bpm indicates beats per minute; HR, heart rate; IQR, interquartile range; WT, wild type.

The enhancer theory also does not preclude an action for SCN10A or Nav1.8 in the cardiac ganglion. Neuronal expression could contribute to the increased atrial fibrillation inducibility seen in vitro with both gain and loss of function SCN10A variants. Indeed, we noted that the Scn10a−/− mice demonstrate an increase in premature atrial contractions but have not pursued this further, as this study was focused on Nav1.8 in the ventricle. However, the notion is supported by

Table 2. Treatment of Scn10a−/− In Vivo and Ex Vivo With ATX-II Does Not Confer Arrhythmia Protection

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IP Injection at RT</th>
<th>Langendorff at 37°C</th>
<th>Langendorff at RT</th>
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<tr>
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<td>Scn10a−/−</td>
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<td>11</td>
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<td>Time in arrhythmia</td>
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<td>164</td>
<td>213</td>
</tr>
<tr>
<td>Median (IQR)</td>
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<td>152 (56.6–230)</td>
<td>249 (0.0–294)</td>
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</table>

bpm indicates beats per minute; HR, heart rate; IP, intraperitoneal; IQR, interquartile range; RT, room temperature; WT, wild type.

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recent work connecting the ablation of the ganglionic plexuses with decreased inducibility of supraventricular arrhythmia in patients with atrial fibrillation.\textsuperscript{16–18}

Taken together, the expression data, the in vitro differences, and the lack of in vivo effects suggest that the channel is expressed, but at levels below the limits of our detection, in myocytes. This results in detectible, reproducible electrophysiologic changes at extreme experimental conditions, but not translated to any change under in vivo conditions.

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Disclosures

None.

References


Table S1. ATX-II effects PR and QRS as anticipated in WT and Scn10a⁻/⁻ mice.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th></th>
<th>Scn10a⁻/⁻</th>
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<tr>
<td></td>
<td>preATX-II</td>
<td>postATX-11</td>
<td>preATX-II</td>
<td>postATX-II</td>
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<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>453</td>
<td>502</td>
<td>485</td>
<td>477</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>476 (410-484)</td>
<td>526 (473-546)</td>
<td>489 (446-538)</td>
<td>488 (396-571)</td>
</tr>
<tr>
<td>P wave duration, ms</td>
<td>12.9</td>
<td>12.3</td>
<td>11.9</td>
<td>11.1</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>12.9 (12.5-13.4)</td>
<td>12.1 (11.2-12.9)</td>
<td>11.1 (11.0-13.4)</td>
<td>11.1 (10.4-11.7)</td>
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<tr>
<td>PR, ms</td>
<td>39.5</td>
<td>43.9*</td>
<td>40.7</td>
<td>43.7*</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>38.5 (35.6-42.5)</td>
<td>42.4 (41-46.8)</td>
<td>36.5 (35.3-37.8)</td>
<td>38.8 (38.0-44.6)</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>12.5</td>
<td>14.4*</td>
<td>11.8</td>
<td>12.6</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>12.6 (12.1-12.9)</td>
<td>14.0 (13.9-14.5)</td>
<td>11.4 (11.0-12.1)</td>
<td>12.8 (12.4-13.5)</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>49.4</td>
<td>64.9*</td>
<td>50.0</td>
<td>67.0*</td>
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<tr>
<td>Median (IQR)</td>
<td>49.9 (46.5-52.6)</td>
<td>72.8 (63.0-76.3)</td>
<td>50.4 (46.0-54.2)</td>
<td>68.7 (65.7-74.5)</td>
</tr>
</tbody>
</table>

bpm (beats per minute), milliseconds (ms), Interquartile Range (IQR)
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Dina Myers Stroud, Tao Yang, Kevin Bersell, Dymtro O. Kryshtal, Satomi Nagao, Christian Shaffer, Laura Short, Lynn Hall, Thomas C. Atack, Wei Zhang, Bjorn C. Knollmann, Franz Baudenbacher and Dan M. Roden

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