Blocking Scn10a Channels in Heart Reduces Late Sodium Current and Is Antiarrhythmic

Tao Yang, Thomas C. Atack, Dina Myers Stroud, Wei Zhang, Lynn Hall, Dan M. Roden

Rationale: Although the sodium channel locus SCN10A has been implicated by genome-wide association studies as a modulator of cardiac electrophysiology, the role of its gene product Nav1.8 as a modulator of cardiac ion currents is unknown.

Objective: We determined the electrophysiological and pharmacological properties of Nav1.8 in heterologous cell systems and assessed the antiarrhythmic effect of Nav1.8 block on isolated mouse and rabbit ventricular cardiomyocytes.

Methods and Results: We first demonstrated that Scn10a transcripts are identified in mouse heart and that the blocker A-803467 is highly specific for Nav1.8 current over that of Nav1.5, the canonical cardiac sodium channel encoded by SCN5A. We then showed that low concentrations of A-803467 selectively block “late” sodium current and shorten action potentials in mouse and rabbit cardiomyocytes. Exaggerated late sodium current is known to mediate arrhythmogenic early afterdepolarizations in heart, and these were similarly suppressed by low concentrations of A-803467.

Conclusions: Scn10a expression contributes to late sodium current in heart and represents a new target for antiarrhythmic intervention. (Circ Res. 2012;111:322-332.)

Key Words: SCN10A • sodium channels • heart • afterdepolarizations • arrhythmia • cardiac electrophysiology • genome-wide association study • heart • SCN5A

The opening of cardiac sodium channels initiates action potentials in most cardiac cells. The canonical cardiac sodium channel, Nav1.5, is encoded by SCN5A, and Nav1.5 blockers reduce sodium current (I_{Na}) in individual cells and conduction velocity in the heart.1 The latter action can be antiarrhythmic but can also provoke arrhythmias, probably by engaging reentrant circuits. Mutations in SCN5A cause a range of human disease including type 3 long-QT syndrome characterized by defective fast inactivation, enhanced “late” sodium current (I_{Na-L}) during the plateau of the action potential, prolonged action potential duration (APD), and arrhythmogenic early afterdepolarizations (EADs).2

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The idea that sodium channels may not inactivate completely after opening was recognized in the node of Ranvier in the mid-1970s,3 and a late plateau current in heart sensitive to tetrodotoxin (TTX) was identified shortly thereafter.4–6 This late sodium current represents either channels that do not inactivate completely after opening or a “window” current flowing in the restricted voltage range where channels are activated and also not completely inactivated. The plateau current is sensitive not only to TTX but also to clinically used antiarrhythmics and other agents,7–9 including the antianginal ranolazine10; this effect of ranolazine is not specific for SCN5A channels, as the drug is known to block multiple other voltage-gated sodium channel isoforms.11–13

The genome-wide association study (GWAS) paradigm has been used to examine variability in PR and QRS durations, electrocardiographic indices of atrioventricular and intraventricular conduction velocity, respectively.14–18 In both cases, the top associations have been with common single-nucleotide polymorphisms (SNPs) not in SCN5A, but in a directly adjacent gene, SCN10A, which encodes the Nav1.8 channel known to be expressed in dorsal root ganglia (DRG)19,20 and in retina,21 but not previously implicated in cardiovascular function.

Preliminary studies14 included in one of the PR GWAS reports detected expression of the gene by PCR in mouse and...
human heart and indicated shorter PR interval but no effect on QRS duration in Scn10a−/− mice. By contrast, the QRS meta-analysis reported that the Nav1.8 blocker A-8046722 prolonged both PR and QRS in wild-type mice and that the gene was expressed in heart and highly enriched in cells isolated from the conduction system. Notably, cells in the conduction system have the longest action potentials in the heart and a physiological INa-L may contribute.23,24 Our findings demonstrate that Nav1.8 channels contribute to INa-L in heart and that Nav1.8 block suppresses arrhythmogenic afterdepolarizations. These results not only reveal a direct role for Scn10a expression in cardiac electrophysiology but also identify its gene product as a potential target for antiarrhythmic intervention.

Methods

FuGENE6-Mediated Channel Expression and Cell Transfection
ND7/23 cells (Sigma-Aldrich, St Louis, MO), a cell line formed by fusion between mouse neuroblastoma and rat DRG cells,25 were cultured in Dulbecco modified Eagle’s medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen), 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C under 95% air and 5% CO2. After growth to ∼70% confluence on 35-mm polystyrene culture dishes in culture, cells were transiently transfected as follows: 2 to 3 μg of the cDNA encoding human Scn10a or Scn5a (plus 0.5 μg of a plasmid for green fluorescent protein, GFP) were mixed with 10 μL FuGENE6 (Roche) in 0.5 mL serum-free DMEM medium and incubated for 30 minutes, after which the standard medium was replaced for a 48-hour incubation. GFP was used as a marker to identify successfully transfected cells for the electrophysiology studies. The cells were harvested by brief wash-off without trypsinization and stored in standard medium for use within 12 hours.

Isolation of Mouse and Rabbit Ventricular Cardiomyocytes
Adult mouse and rabbit cardiac ventricular myocytes were isolated by a modified collagenase/protease method (Online Data Supplement Methods);26 in these studies, we used mice in which the murine gene has been ablated and the human cardiac sodium (H) channel gene has been placed in the murine Scn5a locus.27 We have previously shown that these H/H mice display sodium current and electrocardiograms identical to those from wild-type mice.27 All procedures were approved by the Institutional Animal Care and Use Committee. For electrophysiology experiments, mice (12 wild-type and 3 Scn10a−/−) and rabbits (n=5) were used.

Ion Current Recordings
The biophysical properties of sodium currents were determined by voltage-clamp protocols shown in the Figures. Voltage dependence of inactivation was studied using 500-ms prepulses from −20 mV (for the Nav1.5 channel) or −20 mV (for the Nav1.8 channel) or −20 mV (for the Nav1.5 channel). Two types of voltage-clamp experiments were conducted. First, we applied regular 200-ms square-wave pulses to record peak and late INa in mouse or rabbit ventricular myocytes before and during drug application. The second protocol was a slow ramp to specifically examine late current without the need for a previously depolarizing pulse; this approach allows the experiment to be conducted in physiological extracellular sodium concentration. In both instances, the perfusate contained 1 mmol/L nisoldipine and 200 μmol/L NiCl2 to block other inward current through L- and T-type calcium channels.

Current-voltage relations for steady-state activation and inactivation were determined by fitting a Boltzmann function (I/Imax=1/[1+exp((V−V1/2)/k)]−1), yielding the membrane potential of the half-maximal activation (V1/2−activation) and inactivation (V1/2−inactivation) and slope factor (k). The time course of inactivation of macroscopic current was fit with a monoeponential Chebyshev equation: I=A1*exp[−(t−k)*τ1]+C.

Action Potential Recordings
To record action potentials from isolated ventricular myocytes, cells were studied in current-clamp mode (Axopatch 200B amplifier, Molecular Diagnostics, Sunnyvale, CA) by injecting stimulus current (1–2 nA, 2–4 ms) at different frequencies (5, 2, 1, and 0.1 Hz). Junction potentials between pipette and bath solutions were compensated electronically.

Data acquisition was carried out using an Axopatch 200B patch-clamp amplifier and software pCLAMP 9.2. Currents were filtered at 5 kHz (~3 dB, 4-pole Bessel filter) and digitized using an analog-to-digital interface (Digidata 1322A, Molecular Diagnostics). To minimize capacitive transients, capacitance was compensated ~80%. Series resistance was 1 to 4 mol/LΩ. In all voltage-clamp experiments, the protocols are presented schematically in the Figures. Linear leakage currents were digitally subtracted online by the P/N protocol. All ion currents and action potential (AP) parameters were analyzed using pCLAMP9.2 software.

RNA Isolation and Real-Time PCR
An expanded Methods section is available in the Online Data Supplement.

Solutions and Drugs
To record Nav1.8 and Nav1.5 currents transiently expressed in ND7/23 cells, we used an external K+-free solution that contained (in mmol/L): 135 NaCl, 1.8 CaCl2, MgCl2 1.0, TEA-Cl, 10 HEPES, and 10 glucose, with a pH of 7.4 adjusted with NaOH. Endogenous TTX-sensitive INa and L-/T-type ICa were eliminated with tetrodotoxin 200 mol/L, nisoldipine 0.5 μmol/L, and NiCl2 200 μmol/L, respectively. Glass electrodes were filled with the internal solution that contained (in mmol/L): 120 CsF, 10 NaCl, 1 CaCl2, 2 MgCl2, 10 EGTA, 10 TEA-Cl and 10 HEPES, with a pH of 7.3 adjusted with CsOH. To better control INa recording in mouse and rabbit ventricular myocytes, the internal and external Na+ concentrations were lowered to 5 and 20 mol/L, respectively. The experiments for INa recordings in myocytes were conducted at 18°C. Other experiments were done at 22–23°C.

To record the action potentials in mouse and rabbit ventricular myocytes, external Tyrode solution contained (in mmol/L): 135 NaCl, 4 KCl, 1.8 CaCl2, and 1 MgCl2, 5 HEPES, and glucose 10, with a pH of 7.4 (adjusted by NaOH). The pipette (intracellular) solution had (in mmol/L): 120 Aspirate-K, 25 KCl, 4 ATP-Na2, 1

Non-standard Abbreviations and Acronyms

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<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>APD</td>
<td>action potential duration</td>
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<tr>
<td>DRG</td>
<td>dorsal root ganglia</td>
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<td>EAD</td>
<td>early afterdepolarization</td>
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<td>GWAS</td>
<td>genome-wide association study</td>
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<td>H/H</td>
<td>humanized sodium channel</td>
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<td>INa-L</td>
<td>“late” sodium current</td>
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<td>ND7/23</td>
<td>dorsal root ganglion-derived cell line</td>
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<td>SNPs</td>
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MgCl₂, 2 Phosphocreatine-Na₂, 2 GTP-Na, 1 CaCl₂, 10 EGTA, and 5 HEPES, with a pH of 7.3 (adjusted by KOH). A-803467, a specific Nav 1.8 channel blocker, was purchased from Sigma-Aldrich Corp (St Louis, MO) and dissolved in 5% dimethyl sulfoxide (DMSO) and 95% polyethylene glycol (PEG 400) to make a stock solution of 1 mmol/L. During the experiments, the drug solution was further diluted at the desired test concentrations. The final concentrations of solvents in the cell chamber were 0.01% or less, with no effects on Nav1.8 and action potentials in separate experiments. For experiments in which the antiarrhythmic effect of Nav1.8 block was examined, the external Tyrode solution with lower external potassium (2 mmol/L), and a low concentration of ATX-II (3–5 nmol/L) was used to induce EADs.

Experiments evaluating the effects of drug were conducted by first establishing a predrug stable baseline and then adding drug to the extracellular solution until a new steady state was evident during repetitive pulsing.

**Generation of Scn10a−/− Mice**

Founder mice were regenerated by MRC Harwell, using the same construct as previously reported. Mice were genotyped using PCR amplification of fragment that includes the targeted region: a 1100-bp band was seen in wild-type mice and a 900-bp band in the Scn10a−/− mice. Ventricular myocytes were isolated and studied using the same procedures as those outlined above.

**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 for quantitative real-time PCR was determined by the Wilcoxon rank-sum test.

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**Results**

**Contrasting Electrophysiological and Pharmacological Properties of Nav1.8 and Nav1.5 in a Heterologous Expression System**

As previously reported, expression of SCN10A in CHO or HEK cells, heterologous systems conventionally used to study SCN5A, yielded very small and inconsistent currents, even with coexpression of any of the 4 sodium channel β-subunits or incubation at low temperatures. However, in ND7/23 cells (a cell line formed by fusion between mouse neuroblastoma and rat DRG cells), robust currents were readily detected. We confirmed expression of the construct and the absence of any endogenous SCN10A by RT-PCR (Online Figure I). These cells do express a TTX-sensitive channel so all the experiments reported here were conducted in the presence of a low concentration (200 nmol/L) of TTX. Both Nav1.5 and Nav1.8 are designated TTX-resistant because a high concentration (30 μmol/L) is required to suppress Nav1.5 current and does not affect Nav1.8 currents. In addition, the cells express both β1 and β3 sodium channel subunits.

Figure 1 shows currents recorded with transfection of SCN10A or SCN5A in ND7/23 cells. The SCN5A-mediated current displays fast activation and inactivation typical for Nav1.5 current (and shown in Figure 1A) recorded in other cells transfected with SCN5A plus SCN1B, encoding the β1 subunit. By contrast, SCN10A-mediated current activates and
inactivates much more slowly. Online Table I contrasts the Nav1.5 and Nav1.8 currents in ND7/23 cells. Not only is the time to peak Nav1.8 current strikingly delayed compared with that for Nav1.5, but the postpeak (late) current also represents a much larger fraction of peak current than with Nav1.5. In addition, Nav1.8 channels activate at much more positive potentials than do Nav1.5 channels; the peak current amplitudes were at $+20 \text{ mV}$ compared with $-30 \text{ mV}$ for Nav1.5. Both channels displayed an overlap of activation and inactivation voltage relationships that would enable a “window” current, but in different voltage ranges (Figure 1E).

Figure 2A and 2B show the selectivity of the blocker A-803467 for Nav1.8 over Nav1.5 currents in this system. Single 50-ms repetitive pulses from $-120 \text{ mV}$ to $+20 \text{ mV}$ (the potential at which peak Nav1.8 current is observed) were used to elicit Nav1.8 current, and block was near-complete with 30 nmol/L A-803467 (Figure 2A). However, this concentration of drug produced no effect on Nav1.5 current studied with single pulses to $+30 \text{ mV}$ (the potential at which peak Nav1.5 current is recorded). Indeed, even at 1000 nmol/L, the blocker had minimal effect on Nav1.5 current (Figure 2B). No effect of A-803467 on peak sodium current was also seen in mouse cardiomyocytes (Online Figure IIA).

As described below, experiments using A-803467 in cardiomyocytes implicated Scn10a as a mediator of late $I_{\text{Na}}$. However, late $I_{\text{Na}}$ is commonly more sensitive to drug block than is peak $I_{\text{Na}}$. Accordingly, we conducted further experiments to examine the effects of A-803467 on physiological and pathologically increased Nav1.5-mediated late current.

Figure 2C shows that in ND7/23 cells, application of the sodium channel opener ATX-II augments Nav1.5-mediated sodium current but that this augmented current is not highly sensitive to A-803467: ATX-augmented SCN5A-mediated late current was no more sensitive to A-803467 than was peak current (50% block at $5000 \text{ nmol/L}$). Another setting in which SCN5A-mediated late current is pathologically enhanced is in type 3 long-QT syndrome. Figure 2D shows that 1000 nmol/L A-803467 had no effect on late currents recorded with heterologous expression of LQT3 mutant SCN5A channel F1486L; the same lack of effect on LQT3 late current was observed with the mutants R1544H and T1304L.

Figure 3. Quantitative PCR showing relative expression of Scn10a to SCN5A. Data for both genes were normalized to those for the housekeeping gene Rpl19. Scn10a expression was significantly lower than in the atria and was markedly lower in left versus right ventricle; $n=4$ hearts. LA indicates left atria; RA, right atria; LV, left ventricle; RV, right ventricle.
T1304. This blocker at high concentrations (1 and 10 \( \mu \)mol/L) also had minor effect on L-type calcium current in mouse myocytes (Online Figure IIB).

We also examined the relative abundance of \textit{Scn10a} transcripts in mouse heart compared with those for human cardiac sodium channel allele by quantitative RT-PCR (Figure 3). \textit{Scn10a} transcripts are readily detected in both atria and right ventricle but were markedly reduced in left ventricle. The ND7/23 cell data and the expression data establish low-dose A-803467 as a tool to dissect the contribution of \textit{Scn10a} to ionic currents recorded in cardiomyocytes.

**A Component of Sodium Current in Cardiomyocytes Is Sensitive to Low Concentrations of A-803467**

Figure 4A shows the effects of A-803467 (30 nmol/L) on peak and late sodium current in mouse ventricular myocytes. The right-hand panels contrast, at expanded scales, the prominent suppression of late current with minimal effects on peak current; late currents such as these were observed in approximately half of the cells studied. A 2-second voltage command ramp from \(-120\) mV to \(+40\) mV (Figure 4B) elicited an inward current with 2 minima, one at \(-70\) mV and the second at \(-10\) mV. A-803467 reduced the component at the more positive potential, and the A-803467 sensitive-current obtained by digital subtraction is shown as difference current. Data summarizing the effect of A-803467 on these 2 minima in the ramp current are presented in Figure 4C and 4D; the drug reduced current at \(-70\) mV by 7.3 \( \pm \) 1.3% and at 0 mV by 39.2 \( \pm \) 6.2% (n=8, \( P<0.01 \)). These data further confirm that Nav1.8 current contributes to late sodium current (\( I_{Na,L} \)) in heart.

**A-803467 Shortens Ventricular APD and Suppresses EADs**

Figure 5 shows the effects of 30 nmol/L A-803467 on action potentials recorded in mouse ventricular myocytes. The drug produced little effect at fast rates (5 and 1 Hz), but at slower...
ones, action potential shortening was evident (right, Figure 5A). The effects on APD at 50% and 90% repolarization (APD$_{50}$ and APD$_{90}$) in individual cells (n=20) are shown in Figure 5B through 5E: these differences are statistically significant at slower rate, although no effect by A-803467 was observed in 9 of 20 cells. This is consistent with the notion that INa-L is a more prominent contributor to action potential duration at slow rather than at fast rates and that not all ventricular myocytes express Scn10a.

Figure 6 shows the results of experiments conducted in rabbit myocytes. As in mouse cells, 30 nmol/L A-803467 preferably inhibited late current during 200-ms pulsing and had only a minor effect on peak current in rabbit cardiomyocytes. The drug-sensitive component (as difference current) was separated from the mixed sodium currents by digital subtraction (Figure 6A and insets). The difference current displays very slow activation and inactivation with prominent late current, features similar to those seen with heterologous expression of SCN10A in ND7/23 cells.

Figure 6B shows action potential traces in a rabbit ventricular myocyte recorded at 2 Hz before and during exposure to 30 nmol/L A-803467, which shortened APD. The effect on APD$_{50}$ and APD$_{90}$ in 7 rabbit myocytes are summarized in Figure 6C.

The action potential prolongation generated by enhanced INa-L can generate EADs that have been implicated in arrhythmias in both congenital long-QT syndromes and in acquired settings. We exposed myocytes to a low concentration of ATX-II and then demonstrated that 30 nmol/L A-803467 suppressed EADs elicited under these conditions in mouse and rabbit myocytes. Figure 7A shows action potentials in a mouse myocyte recorded under EAD-promoting conditions: low extracellular potassium (2 mmol/L) and exposure to a low concentration (3 nmol/L) of the sodium channel opener ATX-II. Using 1-Hz stimulation rate under these conditions elicited EADs and triggered activity (Figure 7B through 7D), and these abnormal action potentials were promptly suppressed by 30 nmol/L A-803467. This effect was observed in 7 of 8 mouse preparations.

Very similar effects were observed in rabbit myocytes (Figure 7G through 7I). Exposure to 5 nmol/L ATX-II for 15 minutes induced EADs that lasted longer than the drive cycle.
When the cell was pretreated with A-803467 for 15 minutes, addition of 5 nmol/L ATX-II did not cause long-lasting EADs, although the recorded action potentials still were much longer than that in control (indicated by an arrow in Figure 7I).

Effects of A-803467 in Scn10a<sup>−/−</sup> Mouse Cardiomyocytes

Figure 8 shows that in ventricular myocytes isolated from Scn10a<sup>−/−</sup> mice, no late sodium current was recorded and there was no effect of 30 nmol/L A-803467 on current recorded at the peak or at 200 ms after the onset of the depolarizing pulse. Action potentials were shorter than those in wild-type animals, and there was similarly no effect of A-803467 on APD.

Discussion

One of the key scientific advances enabled by the GWAS paradigm is the identification of genes and pathways contributing to important human phenotypes and not previously implicated in human physiology. GWAS provides a very robust and reproducible signal that variation at the SCN10A locus, not previously implicated in cardiac physiology, modulates conduction in the heart. However, a function of the SCN10A gene product Nav1.8 in cardiac electrophysiology had not, to date, been defined. We used the Nav1.8-specific blocker A-803467 to not only define a role of Nav1.8 channels in control of the cardiac action potential but also to demonstrate that block of Nav1.8-mediated current can exert antiarrhythmic actions.

One hurdle that we had to overcome in these experiments was to identify and validate a system to compare Nav1.5 and Nav1.8 pharmacology.
robust Nav1.5 currents with transfection did not generate similar Nav1.8 currents. The likely explanation for this finding is that ND7/23 cells express some key but as yet unidentified cofactor necessary for Nav1.8 expression and absent from HEK or CHO cells. The major findings in the ND7/23 experiments we present are that A-803467 is specific for Nav1.8 over Nav1.5 and that the channels display striking differences in gating and late current.

Although our findings demonstrate a role for Nav1.8 in the control of cardiac repolarization as assessed by action potential duration, the GWAS results implicate variants in SCN10A as modulators of cardiac conduction. The functional characteristics of Nav1.8 current in the heterologous expression experiments indicate that the current activates much more slowly than does Nav1.5 current, and this difference may in turn contribute to variable cell-to-cell charge transfer that underlies fast conduction in heart. Available data raise the possibility that the SCN10A signal may be mediated by a nonsynonymous coding region SNP, resulting in V1073A. Preliminary reports indicate V1073A does demonstrate gating characteristics that differ from the wild-type channel, but these experiments have only been conducted in heterologous expression systems and so the way in which this variant modulates Nav1.8 function awaits further studies in myocytes.

The quantitative RT-PCR data unexpectedly show that Scn10a expression is much higher in right compared with left ventricle. It is conceivable that this signal reflects enrichment in specialized cell types (e.g., conduction tissue). Indeed, the QRS GWAS report included data that expression was enriched in mouse cardiac Purkinje cells compared with ventricle; Purkinje cells have longer action potentials than those in ventricle, so it is conceivable that Nav1.8 contributes to this difference. We only observed a late current sensitive to 30 nmol/L A-803467 in about half of the myocytes we studied, consistent with the idea that many myocytes will lack Nav1.8 expression. In the electrophysiological experiments, we did not distinguish myocytes of left versus right ventricular origin.

Interestingly, whereas Nav1.5 is the canonical cardiac sodium channel, other channels are known to be expressed at lower levels and in specific regions in heart. One report indicates that Nav1.8 is detectible in heart and pericardial ganglia by immunostaining, but, using Western analyses, we have been unable to establish the specificity of any commercially available antibody. Thus, a key unanswered question is the cellular and subcellular localization of the Nav1.8 channel in heart and other tissues. The possibility remains, for example, that the GWAS

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Figure 7. Antiarrhythmic effects of A-803467 in mouse and rabbit ventricular myocytes. A through F, Changes in action potentials recorded at 1 Hz in the absence and presence of ATX-II alone and then ATX-II + A-803467 in a mouse myocyte. G and H, ATX-II induced long-lasting early afterdepolarizations (EADs) at 0.5 Hz in a rabbit myocyte. I, Pretreatment of a rabbit myocyte with A-803467 eliminated ATX-II induced EADs. Two action potential traces indicated by arrows are for comparison.
conduction velocity signal reflects neural modulation of cardiac conduction by Nav1.8 channels expressed in cardiac ganglia. Further, our data (Figure 5B through 5E) indicate that the extent to which A-803467 shortens action potential duration varies from cell to cell, suggesting that the current plays a variable role, perhaps across cell subtypes, in cardiac repolarization. The qRT-PCR data demonstrate that there is striking variability in the detection of Scn10a transcripts across chambers but do not address cell-type specific expression of the gene.

A low concentration of A-803467 suppressed EADs and associated triggered activity. ATX-II interferes with inactivation of multiple sodium channel isoforms, including Scn5a. However, the antiarrhythmic effect of A-803467 cannot be attributed to block of Nav1.5 channels because we have shown that the concentration used is far below that required to even minimally block physiological or pathological late Nav1.5 current. Thus, we infer that the effect of A-803467 in this setting is to eliminate a component of arrhythmogenic inward current—presumably mediated by Nav1.8—to a sufficient extent to suppress EADs and triggering. The failure of A-803467 to alter APD in multiple ventricular myocytes from Scn10a−/− mice lends further support to this hypothesis.

The demonstration that Nav1.8 channels play a role in cardiac repolarization and that Nav1.8 block can suppress arrhythmogenic EADs represents a key proof-of-principle finding for further development of this novel antiarrhythmic strategy. Many currently available antiarrhythmics block both peak and late current mediated by Nav1.5,7,8 but these can provoke arrhythmias.39 Ranolazine appears to be more selective for late current,10 and, to date, less proarrhythmic.40,41 Indeed, ranolazine exerts antiarrhythmic effects in some settings,42–44 including suppressing EADs. However, the drug is not selective for Nav1.5 currents. The interesting difference between Nav1.5 and Nav1.8 activation raises the possibility that selective block of late Nav1.8-mediated current could be antiarrhythmic without proarrhythmic liability.

**Acknowledgments**

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Disclosures

None.

References

Novelty and Significance

What Is Known?

- Genome-wide association studies have implicated variation in the sodium channel gene SCN10A as modulators of cardiac conduction (PR and QRS durations).
- SCN10A was originally cloned from dorsal root ganglion, and the encoded channel (termed Nav1.8) is thought to play a role in pain perception.

What New Information Does This Article Contribute?

- A specific Nav1.8 blocker eliminates the persistent sodium current recorded after long depolarizing pulses in mouse and rabbit cardiomyocytes and shortens the action potential duration.
- These effects are absent in myocytes from Scn10a knockout mice.
- The Nav1.8 blocker reverses arrhythmogenic early afterdepolarizations elicited by experimental conditions that increase persistent sodium current.

Genome-wide association studies of cardiac conduction suggest a role for variation in SCN10A, not previously implicated in heart function. We show that Scn10a transcripts are present in the mouse heart. Also, we confirmed the selectivity of the Nav1.8 blocker A-803467 over Nav1.5 encoded by the canonical cardiac sodium channel gene SCN5A. In mouse and rabbit ventricular myocytes, a low concentration of A-803467 had little effect on peak sodium current but eliminated “late” current recorded after a long depolarizing pulse and shortened action potential duration. These effects were absent in cells from Scn10a knockout mice. The blocker also eliminated arrhythmogenic early afterdepolarizations elicited when the late current was increased. These results identify a role for the SCN10A gene product in mediating late current in the heart and suggest that inhibition of this current may be antiarrhythmic under some conditions.
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Blocking SCN10A channels in heart reduces late sodium current and is antiarrhythmic

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Supplemental Methods

Isolation of mouse and rabbit ventricular cardiomyocytes

After intraperitoneal injection of 500 IU of heparin, adult mice and rabbits were anesthetized using inhaled isoflurane/oxygen mixture, hearts excised, and their aortae rapidly cannulated and perfused with modified Tyrode’s solution (MTS) for 3 min followed by MTS containing collagenase (Liberase Blendzyme-4, Roche, 0.04 mg/ml) for 5~7 min at a constant pressure of 80 mmHg and temperature of 34ºC. The MTS contained (in mmol/L) NaCl 130, HEPES 10, glucose 10, KCl 5.4, MgCl2 1.2, NaH2PO4 2, 3-butanedione monoxime 10, pH of 7.2. The digested ventricles were minced in MTS containing 1 mg/ml bovine serum albumin and 0.2mmol/L CaCl2 and triturated by gently pipetting. The resulting solution was strained and the myocytes allowed to sediment in MTS of increasingly higher Ca2+ concentrations (0.2, 0.5, and 1 mmol/L). A modified procedure was used to isolate rabbit ventricular myocytes.

RNA isolation and Real-time Polymerase Chain Reaction (PCR)

Total RNA was isolated from both SCN10A-transfected as well as untransfected ND7/23 cells using TRIzol (Invitrogen) following the manufacturer’s directions. cDNA was amplified from total RNA with the Transcriptor First Strand cDNA Synthesis Kit (Roche) using 2.5 µg total RNA following the instructions provided. Real Time quantitative PCR (qPCR) was performed using the TaqMan method to measure quantities of Scn5a (Hs00165693_m1) and Scn10a (Mm01342496_m1) with Rpl19 (Mm02601633_g1) as an internal reference. Samples were run on an Applied Biosystems 7900HT and analyzed with SDS 2.3 software.

For standard RT-PCR, SCN10A was amplified from cDNA with the Expand Hi Fidelity PCR Kit (Roche) using the forward primer (5’-3’) TGTGTCATGAAGATGTTCGCTTTG and the reverse primer GCGTTGGGGAGAAGTAACTTTGAA. The reaction began with an initial denature step of 94º C for 2 minutes followed by 40 cycles (tissue) or 32 cycles (cells) of 30º seconds at 94º C, 30 seconds at 58º C, and 45 seconds at 72º C with a final extension time of 7 minutes at 72º C. Reactions were loaded onto a 2% agarose-1xTBE gel and imaged. Specificity of products was confirmed by sequencing.
**Supplemental Table I: Comparisons of the electrophysiological properties of cardiac Nav1.5 and Nav1.8 channels expressed in ND7/23 cells.** All differences between Nav1.8 (at +20 mV) and Nav1.5 (at -30 mV) are statistically significant (n=7-9 each, p<0.01).

<table>
<thead>
<tr>
<th></th>
<th>Nav1.5</th>
<th>Nav1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak $I_{Na}$ (pA/pF)</td>
<td>-48.7±3.2</td>
<td>-27.6±1.4</td>
</tr>
<tr>
<td>Late $I_{Na}$ (% of Peak $I_{Na}$)</td>
<td>0.74±0.09</td>
<td>9.43±1.0</td>
</tr>
<tr>
<td>$V_{1/2}$-activation (mV)</td>
<td>-49.1±0.8</td>
<td>-6.1±1.1</td>
</tr>
<tr>
<td>$V_{1/2}$-inactivation (mV)</td>
<td>-92.6±1.2</td>
<td>-54.9±2.4</td>
</tr>
<tr>
<td>Time-to-peak $I_{Na}$ (ms)</td>
<td>1.36±0.04</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>Inactivation rate (ms)</td>
<td>$\tau_{fast}$: 1.3±0.1</td>
<td>$\tau_{fast}$: 3.2±0.2</td>
</tr>
<tr>
<td></td>
<td>$\tau_{slow}$: 2.9±0.2</td>
<td>$\tau_{slow}$: 18.2±0.9</td>
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</tbody>
</table>

Supplemental Figure I. RT-PCR showing Scn10a expression transfected ND7/23 cells. Transcripts are absent from untransfected ND7/23 cells (ND) and in or negative (water) controls (Ctrl).
Supplemental Figure II: Minor effects of A-803467 at high concentrations on peak sodium and calcium currents in WT mouse ventricular myocytes. A, Sodium current traces recorded from a holding potential of -120 mV to 0 mV for 200 ms pre- and post-drug in same myocyte. A-803467 at 10 µmol/L mainly inhibited late current and had minor effect on peak current (boxed). B, Calcium current traces recorded from a holding potential of -40 mV to 0 mV for 200 ms in same myocyte in the absence and presence of A-803467 first and then nisoldipine, an L-type calcium channel blocker.