Online supplemental data

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 34°C. The MTS contained (in mmol/L) NaCl 130, HEPES 10, glucose 10, KCl 5.4, $MgCl_2$ 1.2, NaH_2PO_4, 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 $CaCl_2$ and triturated by gently pipetting. The resulting solution was strained and the myocytes allowed to sediment in MTS of increasingly higher Ca^{2+} concentrations (0.2, 0.5, and 1 mmol/L). A modified procedure was used to isolate rabbit ventricular myocytes.

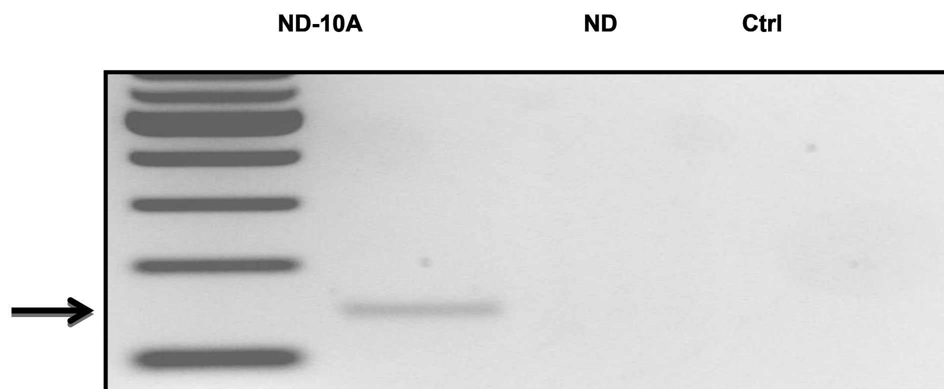
RNA isolation and Real-time Polymerase Chain Reaction (PCR)

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.

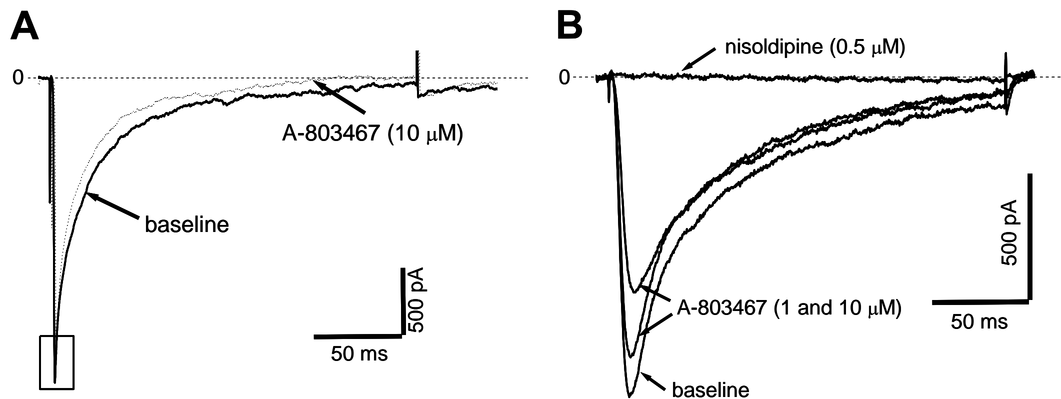
For standard RT-PCR, *SCN10A* was amplified from cDNA with the Expand Hi Fidelity PCR Kit (Roche) using the forward primer (5'-3') TGTGTCATGAAGATGTTCGCTTTG and the reverse primer GCGTTGGGGAGAAGTAACTTTGAA. The reaction began with an initial denature step of 94° C for 2 minutes followed by 40 cycles (tissue) or 32 cycles (cells) of 30º seconds at 94° C, 30 seconds at 58° C, and 45 seconds at 72° C with a final extension time of 7 minutes at 72° 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).

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



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.