NAV1.5 channels are primarily expressed in cardiac muscle where they control the fast upstroke of the cardiac action potential. The normal biophysical properties of fast activation and inactivation results in a transient influx of Na and subsequent membrane depolarization. This depolarization activates cardiac Ca and K channels that govern strength and duration of contraction. In conditions of cardiac ischemia, compensatory mechanisms engage to increase oxygen delivery to tissues. One such mechanism is the modulation of NAV1.5 inactivation through 24-hour messenger systems resulting in a "futura" inward current. This sustained inward current can prolong action potential duration and increase net influx of calcium, producing a more forceful ventricular contraction, which over time can result in deleterious cardiac hypertrophy and electrical remodeling. An inherited long QT syndrome, LQT3 arising from mutation of the NAV1.5 channel produces action potential duration and strength of contraction changes similar to those observed during compensatory responses to cardiac ischemia. We report on the biophysical properties of the NAV1.5 1505-1507 △KPQ LQT3 variant recombinantly expressed in HEK background. Using a cardiac AP voltage waveform, we observed a late sustained inward current with ~3% peak current amplitude that was absent in WT NAV1.5 expressing cells. Application of a NAV1.5 inhibitor that blocks pharmacologically activated (ATX-II) WT NAV1.5 currents similarly blocked unmodified late currents carried by the NAV1.5 1505-1507 △KPQ LQT3 variant. Given that both peak and late NAV1.5 currents contribute differently to cardiac rhythm, the NAV1.5 1505-1507 △KPQ LQT3 channel could be a useful reagent for cardiac safety screening and mechanism of action studies. As such the evaluation of NAV1.5 late currents is an important component of the seven key ion channel currents listed in the evolving CPA initiative.

MATERIALS AND METHODS

cDNA constructs and Cell line generation. The SCNSA 1505-1507 △KPQ LQT3 variant was generated by site-directed mutagenesis of the WT SCNSA construct. The cDNA was cloned into pLNCX2 mammalian expression vector using standard molecular biology methods, the cDNA was sequence verified and a HER231 cell line expressing (1±2) subunits was retrovirally infected. The resulting stable pool was diluted and the prefered clone selected on Patchpress automated patch clamp platform (Molecular Devices) based on NAV1.5 late current amplitude, reference agent pharmacology and overall assay performance.

Manual Patch Clamp Electrophysiology

Coverslips with human embryonic kidney 293 cells expressing human NAV1.5 1505-1507 △KPQ LQT3 were placed in a recording chamber on the stage of an inverted microscope and perfused with an extracellular solution containing (in mM): 132 NaCl, 1.8 CaCl2, 5.4 KCl, 0.8 MgCl2, 5 glucose, and 10 HEPES, pH 7.4, with Na2CO3. Tetrodotoxin (100 nM) was included to prevent contamination from endogenous TTX-sensitive Na currents. Records from patch pipettes were filled with an intracellular solution containing [in mM]: 110 CsF, 35 CsCl, 5 NaCl, 10 TEA, 10 Hepes, 7.3 with CsOH, and had a resistance of 3 to 5 MΩ. All reagents used for buffers were purchased from Sigma-Aldrich. All recordings were made at room temperature (22±2°C) using Axopatch200B amplifiers, digitized using a Digitizer 1440A and acquired on computer with PC-CLAMP software (Molecular Devices). Sodium currents were measured using the whole-cell configuration of the patch-clamp technique. All test compounds were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions, which were then diluted into extracellular solution to attain the final concentrations desired. The final concentration of DMSO was found to have no significant effect on peak or late NAV1.5 currents. Assessment of biophysics and pharmacology in response to various voltage protocols are described in the appropriate figure legends.

SyncroPatch 384 Automated Electrophysiology

WT NAV1.5 and the 1505-1507 △KPQ LQT3 variant currents were recorded on the SyncroPatch 384 automated patch clamp platform (Narren). For WT NAV1.5 channels, ATX-II (Alomone labs) or FF-6536290 (Sigma) was used as a chemical modifier to introduce the late current. Test compounds were evaluated in the continued presence of these chemical modifiers. NAV1.5 1505-1507 △KPQ LQT3 currents were not modified as this variant has an enhanced late current that is ~3% peak current amplitude. Extracellular solutions contained [in mM]: 105 NaCl, 40 NaNO3, 13 KCl, 2 MgCl2, 1 CaCl2, 10 HEPES, 5 glucose, pH 7.4 with NaOH. Tetrodotoxin 200 nM was included to prevent contamination from endogenous TTX-sensitive Na currents. The intracellular solution contained [in mM]: 155 CsF, 10 CsCl, 5 NaCl, 5 EGTA, 10 HEPES, pH 7.2 with KOH.

Data Analysis

For manual patch experiments, current amplitudes were analyzed using Clampfit 10.3 (Molecular Devices). SyncroPatch recordings were collected using PatchControl software (Narren) and processed and analyzed using DataControl Software (Narren). All graphs and curve fits were generated using GraphPad Prism 7.0 (GraphPad Software).

CONCLUSION

- NAV1.5 late currents can be induced by chemical modification of WT channels by or using a disease variant such as NAV1.5 1505-1507 △KPQ LQT3
- NAV1.5 peak and late currents show different inactivation profiles with the late current being more sensitive to frequency and being slower to recover. Thus, for compounds that interact with the inactivated state, selectivity over NAV1.5 peak current does not guarantee selectivity over NAV1.5 late currents.
- icagen offers a WT NAV1.5 late current (ATX-II or FF-6536290) screening assay on Patchpress automated patch clamp platform.
- icagen can measure chemically modified WT NAV1.5 late currents and those produced by NAV1.5 1505-1507 △KPQ LQT3 channels on SyncroPatch 384 automated patch clamp platform. icagen is working to fully develop these assays.
- icagen can provide the NAV1.5 1505-1507 △KPQ LQT3 channel to others looking to include this current in their safety panel.

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Advancing Early Drug Discovery

Matthew Fuller, Sonia Santos, Mark Chapman, Aaron Gerlach
icagen, 4222 Emperor Boulevard, Suite 350, Durham, NC 27703 USA

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