Functional Assessment of hNav1.x Ion Channels Using State-Dependent Protocols on the QPatch HT Automated Patch Clamp System

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Abstract

Voltage-gated sodium channels are transmembrane proteins that are responsible for the rapid depolarization that elicits the upstroke of action potentials in neurons and cardiac cells. Molecular cloning techniques have identified nine related voltage-gated sodium channels subtypes with distinct biophysical properties, interacting proteins, and cellular patterns of expression that are involved in electrical signaling. The goal of many research groups is to target specific sodium channels subtypes to develop selective inhibitors that could, in theory, produce safer and more efficacious drugs. To address compound sodium subtype profiling, we used a two-pulse state-dependent protocol to develop a sub-type specific sodium channel (hNav1.1 to hNav1.8) panel on the QPatch HT patch clamp system. Additionally, we tested three stimulus protocols on hNav1.5 with known sodium inhibitors: 1) CiPA protocol for cardiac safety testing (tonic), 2) two-pulse state-dependent protocol (inactivation-dependent), 3) three-pulse protocol (tonic, inactivation-dependent, and $V_{1/2}$ dependent inactivation). The CiPA protocol identified state-independent compounds (e.g. Flecainide) within three-fold of published literature values, but didn't identify state-dependent compounds such as Lidocaine. The two-pulse protocol identified both state-dependent and independent compounds at pulse 2 with IC_{50} values within 4-fold published literature values. Data from the first two pulses of the three-pulse protocol had comparable pharmacology to the two-pulse protocol. The functional activity from $V_{1/2}$ inactivation (a delayed 3rd pulse) was less predictive of state-dependent inhibition than the two-pulse protocol but may reflect the *in vivo* inhibition. The hNav1.x panel using the two pulse protocol can be useful to profile target identification, selectivity and state-dependence of novel compounds. (QPatch Automated Patch Clamp System is manufactured by Sophion Bioscience)

Methods

HEK-293 cell lines stably expressing exogenous hNav1.5, were cultured according to internal protocols. Briefly, hNav1.5-HEK cells were grown in DMEM/F12 + GlutaMAX media supplemented with non-essential amino acids (NEA) and 10% FBS. T-150 flasks were seeded 48-72 hours (maintained at 37C, 5% CO_2) prior to the experiment to achieve a cell confluency of 60-90%.

Automated patch-clamp (APC) electrophysiology assays for hNav1.5 were conducted on the QPatch HT platform. Standard single hole 48-well plates (~2.5MΩ) were used in all experiments.
Voltage protocols are described in the figure legends (Figures 1A, 1C, 1E, Figure 2).

hNav1.5 – Three Unique Protocols

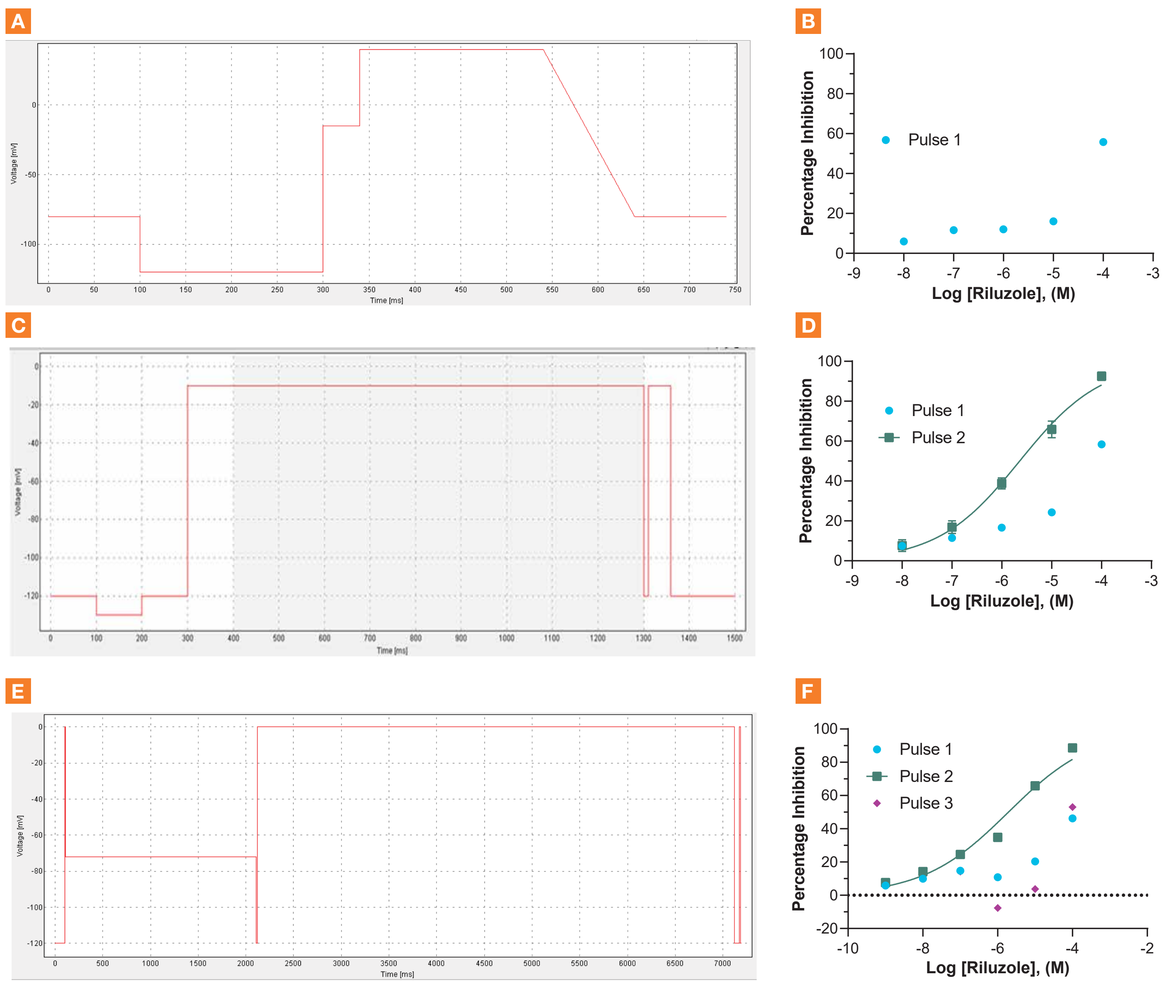

Figure 1. Different stimulus protocols can identify state-dependent compounds. The CiPA pulse protocol (A.) is a single pulse most commonly used to test cardiac safety liability. The voltage protocol is applied every 5s, which will induce a full response in state-independent compounds but a limited response in state-dependent compounds such as Riluzole (n=9) (B.). A Two-Pulse Protocol (C.) was developed to better identify compounds that are inactivation dependent, and is applied every 10s. Results from the inactivation pulse (Pulse 1) were similar to results from the CiPA pulse protocol, while results from the recovery pulse (Pulse 2) showed a more potent full dose response in Riluzole (n=7) (D.). The Three-Pulse Protocol (E.) is applied every 12s and identifies compounds potent after fast and slow channel inactivation. Riluzole (F.) lost potency following the $V_{1/2}$-dependent inactivating pulse (Pulse 3) and the data (n=5) reflected the initial tonic pulse.

hNav1.5 – Reference Panel Comparison

A

Compound Name	CiPA Protocol			Inactivation Dependent Protocol			$V_{1/2}$ Dependent Inactivation Protocol		
	IC_{50} (Pulse 1, μM)			IC_{50} (Pulse 1, μM)	IC_{50} (Pulse 2, μM)		IC_{50} (Pulse 1, μM)	IC_{50} (Pulse 2, μM)	IC_{50} (Pulse 3, μM)
Carbamazepine	-			-	23.4		-	41.9	-
Flecainide	8.8			31.8	12.4		24.7	14.9	10.3
Lamotrigine	-			-	28.6		-	27.1	223.8
Lidocaine	-			-	8.4		-	10.4	108.2
Phenytoin	-			-	4.9		-	26.7	-
Riluzole	84.1			67.2	2.3		-	1.9	92.1
Tetracaine	0.82			17.8	0.25		57.8	0.31	0.47

A panel of well characterized hNav1.5 antagonists was run on three different pulse protocols – CiPA (A.) Two-Pulse (Inactivation-dependent) (B.), and a Three-Pulse ($V_{1/2}$ inactivation) protocol (C.). Compounds that are state-independent (ex: Flecainide) or have limited state-dependence (Tetracaine, Riluzole) produced IC_{50} values on the CiPA pulse protocol. Compounds with state-dependence (ex: Lamotrigine) showed a similar response to the CiPA Protocol results on Pulse 1 and a full dose-dependent response on Pulse 2 with calculable IC_{50} values. Results from Pulses 1 and 2 of the $V_{1/2}$ Inactivation protocol were comparable to compound results from the Two-Pulse Protocol, while results from the delayed 3rd pulse were less reflective of the inactivation of the channel as state-dependent compounds produced a less potent or no IC_{50} value.

hNav1.5 on QPatch HT – Activation and Inactivation

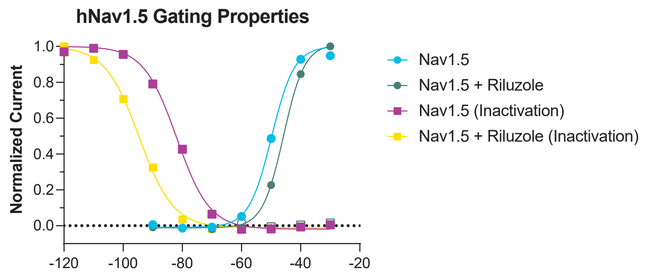

Figure 2. Channel sensitivity to voltage on the QPatch HT when treated with Riluzole. hNav1.5 cells were treated with vehicle buffer (n=11) as a control or 2μM Riluzole (n=11). The activation curve was induced via 50ms depolarizing steps of 10mV starting at -90mV. Steady-state inactivation was induced via a 500ms pre-pulse ranging from -120mV to -30mV and measured during a subsequent 4ms test pulse to -30mV. Application of Riluzole had no significant effect on the activation curve, but did cause a -12.7mV shift in the inactivation curve.

Summary

A panel of sodium channel inhibitors profiled on hNav1.5 on the QPatch HT demonstrated its effectiveness in investigating compounds with potential state-dependence.

The activation and inactivation curves for hNav1.5 were characterized on the QPatch HT. Riluzole was evaluated to confirm that a state-dependent blocker could identify an inactivation voltage shift on the QPatch platform.

A sodium subtype panel (hNav1.1-hNav1.8) was developed for investigating state-dependence of compounds using Tetracaine and Lidocaine (data not shown).