Simultaneous Measurement of Cardiac Nav1.5 Peak and Late Currents in an Automated QPatch Platform

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ABSTRACT

High throughput in vitro profile of the cardiac Nav1.5 peak sodium current (I_{Na}) is widely used in cardiac safety screening. However, there is no standardized high throughput method to measure late I_{Na}. A variety of differing protocols used across industry and academia may contribute to variation in the data.

The objectives of this study were to (1) assess pharmacology and biophysical properties of veratridine- and ATX-II-induced late INa together with that induced by a mutation in the channel (ΔKPQ-Nav1.5), (2) develop a protocol to allow simultaneous measurement of both peak and late INa under a single protocol using automated QPtach system.

The planar patch clamp technique (QPatch) was applied to record the peak and late I_{Na} from the Nav1.5 channel (human SCN5A gene) or ΔKPQ-Nav1.5 mutant channel expressed in mammalian cells.

When measured at the maximal response during the ramp of the voltage protocol, the ΔKPQ-Nav1.5 mutant produced a small late I_{Na} (41.9 ± 5.4 pA). Veratridine and ATX-II induced concentration-dependent increases in the late I_{Na}. The amplitude of late I_{Na} were 1162.2 ± 258.5 pA and 392.4 ± 71.3 pA in the presences of 100 μM veratridine and 100 nM ATX-II, respectively. Veratridine inhibited the peak I_{Na} (IC_{50} = 84.1 ± 10.5 μM) and altered the biophysical properties of the I_{Na}. ATX-II showed minimal effects on the peak I_{Na} and preserved the biophysical properties of the I_{Na}. In the presence of 100 nM ATX-II, potencies of 25 clinical I_{Na} blockers on peak and late I_{Na} were characterized. In addition, the IC_{50} values of these clinical I_{Na} blockers on peak I_{Na} correlated well with and without ATX-II. The results also demonstrated that the potency of a compound blocking late I_{Na} could be either overestimated or underestimated if the late I_{Na} was measured at the end of the depolarizing pulse versus during the ramp.

In conclusion, in the presence of ATX-II, both peak and late I_{Na} could be assessed simultaneously under a single protocol. Our results suggest that late I_{Na} may be best assessed using the maximum response obtained during the ramp after 200 ms depolarizing pulse at 40 mV.

METHODS

Automated Electrophysiology

WT Nav1.5 and ΔKPQ-Nav1.5 mutant currents were recorded on the QPatch HTX automated patch clamp platform (Sophion). For WT Nav1.5 channels, ATX-II (Alomone labs) or veratridine (Sigma) was used as a chemical modifier to induce the late I_{Na}. Test compounds were evaluated in the continued presence of these chemical modifiers. Test compounds were purchased from Sigma-Aldrich (St Louis, MO).

Recombinant Cell Lines

Wild type Nav1.5 channels were expressed in CHO cells (Charles Rivers, Cleveland, OH) and ΔKPQ-Nav1.5 mutant channels were expressed in HEK 293 cells (Icagen, Durham, NC).

Solutions

Extracellular solution (in mM):137 NaCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 4 KCl, 10 HEPES, 10 Glucose, pH=7.4 (NaOH), ~295 mOsm.
Intracellular solution (in mM): 92 CsF, 55 CsCl, 10 HEPES, 10 EGTA, 2 $MgCl_2$, 2 MgATP pH=7.2 (KOH), ~300 mOsm.

Data analysis

Analyses of current (I) / Voltage (V)-relationships and concentration-dependent drug effects (Hill fit and IC_{50}) were performed using QPatch Assay Software (Sophion, Denmark) and Graphpad Prism 7.0.4 (GraphPad Software, La Jolla, CA).

RESULTS

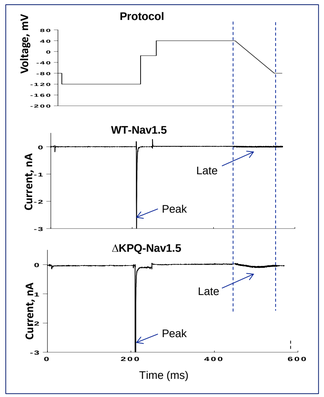


Figure 1. Late I_{Na} in WT and ΔKPQ Nav1.5 channels

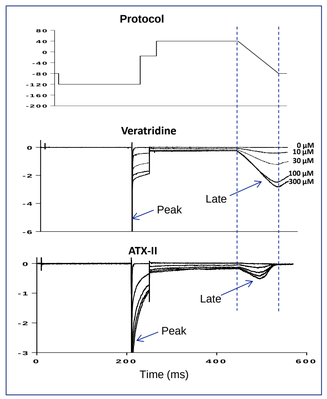


Figure 2. Veratridine and ATX-II-induced late I_{Na} in WT-Nav1.5 channel

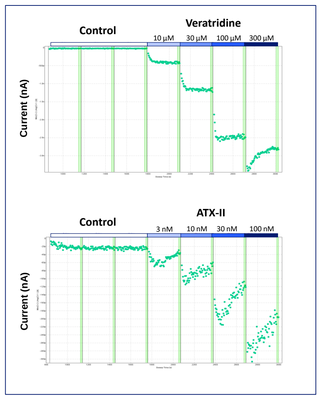


Figure 3. Current-time (I-T) plots of Veratridine and ATX-II-induced late I_{Na}

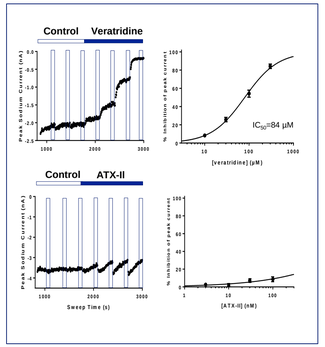


Figure 4. Effects of Veratridine and ATX-II on peak I_{Na}. Mean ± SEM (N=6-8)

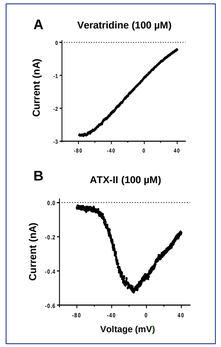
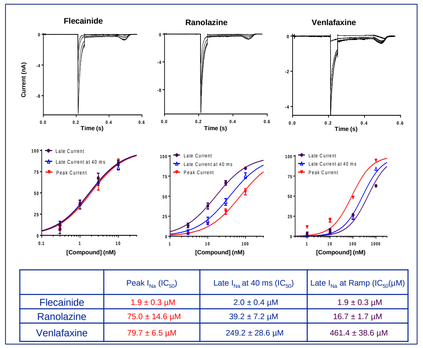


Figure 5. Veratridine and ATX-II induced late I_{Na} current-voltage relationship



	Peak I_{Na} (IC_{50})	Late I_{Na} at 40 ms (IC_{50})	Late I_{Na} at Ramp (IC_{50}/μM)
Flecainide	1.9 ± 0.3 μM	2.0 ± 0.4 μM	1.9 ± 0.3 μM
Ranolazine	75.0 ± 14.6 μM	39.2 ± 7.2 μM	16.7 ± 1.7 μM
Venlafaxine	79.7 ± 6.5 μM	249.2 ± 28.6 μM	461.4 ± 38.6 μM

Figure 6. Compounds-mediated inhibition of peak/late I_{Na} in the presence of 100 nM ATX-II. Mean ± SEM (N=6-10)

	IC_{50} (μM)		
	Late I_{Na} with ATX-II	Peak I_{Na} with ATX-II	Peak I_{Na} without ATX-II
5-OH Propafenone	1.3 ± 0.1 (5)	0.8 ± 0.1 (9)	0.4 ± 0.0 (9)
Amitriptyline	2.3 ± 0.2 (11)	2.7 ± 0.4 (12)	1.1 ± 0.2 (7)
Bupivacaine	2.1 ± 0.3 (8)	3.8 ± 0.8 (9)	4.5 ± 0.5 (9)
Carbamazepine	25.4 ± 2.1 (9)	173.6 ± 34.8 (7)	76.3 ± 10.0 (7)
Clomipramine	4.1 ± 0.6 (11)	6.7 ± 0.8 (12)	7.2 ± 0.7 (9)
Desipramine	2.5 ± 0.3 (9)	2.8 ± 0.5 (9)	2.8 ± 0.4 (12)
Disopyramide	89.1 ± 5.0 (8)	84.3 ± 10.3 (9)	102.6 ± 25.5 (5)
Flecainide	1.9 ± 0.3 (6)	1.9 ± 0.3 (5)	1.8 ± 0.1 (8)
Imipramine	3.6 ± 0.5 (9)	3.8 ± 0.6 (12)	1.2 ± 0.1 (8)
Lamotrigine	34.5 ± 3.2 (8)	36.3 ± 6.8 (9)	31.3 ± 3.6 (7)
Maprotiline	1.8 ± 0.2 (8)	1.9 ± 0.3 (7)	1.6 ± 0.2 (9)
Mesoridazine	3.6 ± 0.3 (6)	2.8 ± 0.5 (6)	3.1 ± 0.3 (9)
Mexiletine	12.2 ± 1.1 (12)	23.9 ± 3.3 (10)	21.9 ± 2.6 (10)
Nortriptyline	3.3 ± 0.6 (7)	2.9 ± 0.5 (10)	1.7 ± 0.1 (11)
Procainamide	554.4 ± 85.2 (9)	515.9 ± 103.8 (5)	487.5 ± 54.2 (12)
Procaine	82.5 ± 7.1 (6)	81.7 ± 6.8 (5)	91.7 ± 7.3 (12)
Propafenone	1.9 ± 0.2 (9)	2.4 ± 0.2 (12)	1.4 ± 0.1 (9)
Quinidine	16.8 ± 1.4 (8)	18.4 ± 0.7 (6)	14.9 ± 2.4 (9)
Quinine	11.4 ± 1.5 (8)	19.5 ± 2.5 (9)	19.0 ± 3.1 (12)
Ranolazine	16.7 ± 1.7 (5)	75.0 ± 14.6 (5)	79.5 ± 5.5 (12)
Risperidone	26.2 ± 5.9 (10)	31.8 ± 6.4 (10)	14.8 ± 3.0 (9)
Ropivacaine	5.6 ± 0.6 (5)	10.2 ± 1.5 (5)	11.5 ± 1.4 (11)
Tetracaine	1.5 ± 0.4 (6)	1.1 ± 0.2 (6)	1.1 ± 0.1 (11)
Thioridazine	2.8 ± 0.2 (10)	4.5 ± 0.5 (9)	6.9 ± 1.0 (12)
Venlafaxine	461.4 ± 38.6 (6)	79.7 ± 6.5 (6)	36.5 ± 3.2 (8)

Table 1. IC_{50} values for sodium channel blockers on peak and late I_{Na}. Data represent mean ± SEM. Values in parenthesis represent the number of individual experiments performed

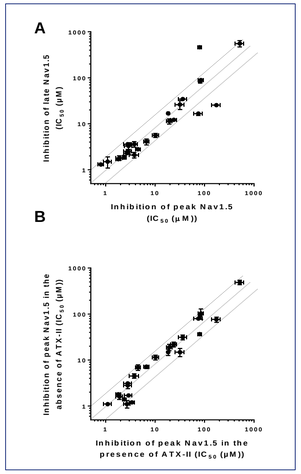


Figure 7. Correlations of compound IC_{50} values for **(A)** peak and late I_{Na} in the presence of ATX-II, and on **(B)** peak I_{Na} in the absence or presence of ATX-II

CONCLUSIONS

- Under control conditions no significant late I_{Na} was detected in cells expressing the WT Nav1.5 channel when measured during the ramp. A small late I_{Na} (41.9 ± 5.4 pA) was observed in cells expressing the ΔKPQ-Nav1.5 mutant channel that was insufficient for high throughput profiling of compounds
- Veratridine significantly enhanced the late I_{Na} (EC_{50} = 27 μM). However, it also inhibited the peak I_{Na} (IC_{50} = 84 μM) and altered the voltage-dependency of the I_{Na}, demonstrating that it is not an ideal enhancer for assessing either the peak or late I_{Na}
- ATX-II produced a concentration-dependent increase in the late I_{Na} (392.4 ± 71.3 pA (ATX-II 100 nM)), but had minimal effect on the peak I_{Na} and preserved the biophysical properties of I_{Na}. ATX-II therefore had a profile that was suitable as an enhancer for measuring both peak and late I_{Na} simultaneously
- The presence of ATX-II did not significantly affect the pharmacology of the peak I_{Na} for a series of sodium channel inhibitors when compared with results obtained in the absence of ATX-II
- We suggest that late I_{Na} should be measured at the maximum response generated during the ramp after a 200 ms depolarizing pulse at +40 mV (which allows for the completely inactivation of peak I_{Na}) in the presence of ATX-II
- Our data demonstrate that in the presence of ATX-II (100 nM), peak and late I_{Na} can be measured simultaneously, allowing for a rapid and cost effective assessment of both currents

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