Automated High Throughput Na+ Late Current Assay on QPatch HT Platform for CiPA28

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Abstract # 0030

Abstract

In cardiac muscle, sustained inward Na+ currents, also known as late Na+ currents (INa-L), occur under physiological conditions during the cardiac Action Potential AP (AP). In addition to extending the plateau duration before AP recovery, an increased INa-L can lead to the development of various triggers and substrates (early after depolarization, EAD) for arrhythmogenesis. Inhibition of INa-L current can prevent proarrythmia by reducing or reversing EAD even in the presence of a prolonged QTc interval (e.g. Ranolazine). Therefore, INa-L is one of the seven ion channels selected for evaluation in the comprehensive *in vitro* proarrythmia assay (CiPA).

Eurofins is a contributor to the Ion-Channel Work-Group of the FDA/HESI/CIPA initiative, supporting validation of automated high throughput methods on the QPatch HT to support the program consortium. These methods measure 1) enhancement or 2) inhibition of INa-L current, using either step pulse or ramp pulse protocol and reference pharmacology, ATXII. Three key parameters, INa-L current, leak current, time-matched vehicle control were measured. The data generated in this study demonstrate that measuring INa-L current charge measurement is superior to measuring INa-L current amplitude for both test and ramp pulse. We investigated the effects of CiPA-28 compounds on INa-L at the EC_{50} of ATXII (30nM). With the un-blinding of CiPA Phase I (12) compounds, Chlorpromazine, Diltiazem, and Mexiletine were identified as hits. Our results indicate that none of the CiPA-28 compounds potentiated the INa-L current.

Methods

HEK-293 cell lines stably-expressing exogenous human Cav1.2 and KCNQ1/mink, were cultured according to internal protocols. Briefly, Cav1.2-HEK cells were grown in DMEM/F12 + Glutamax media supplemented with non-essential amino acids (NEA) and 10% FBS, while KCNQ1/mink-HEK cells were grown in IMDM (Iscove's Modified Dulbecco's Medium) supplemented with 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 80%.

Automated patch-clamp (APC) electrophysiology assays for both hCav1.2 and hKCNQ1/mink were conducted on the QPatch HT platform (Sophion, Denmark). Standard single hole 48 well plates (~2.5MΩ) were used in all experiments. Voltage protocols are described in the figure legends.

Figure 1

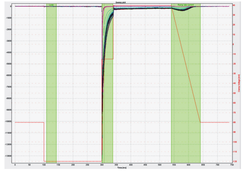

Figure 1. hNav1.5 late current assay on the Qpatch HT with 30nM ATXII
Onset and steady state block of Nav1.5 late current were measured using a pulse pattern, repeated every 5 seconds, consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 40ms duration, followed by step to 40mV for 200ms and finally a 100ms ramp (1.2V/s) to a holding potential of -80mV. Leak current was measured on the step from -80mV to -120mV. Late current was measured during the test pulse (see Figure 3a) as well as during the ramp (see Figure 3b). 30nM ATXII was used to elicit hNav1.5 late current.

Figure 2

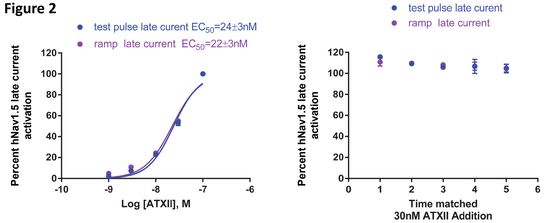

Figure 2: hNav1.5 late current concentration response curve for ATXII and sequential ATXII additions to mimic the test article additions
Normalized hNav1.5 late current activation is plotted against ATXII concentration and fit with an equation to estimate the E_{50} value with ± SEM and time-matched ATXII additions to act as vehicle controls are plotted for four additions at 5 minute intervals.

Figure 3a **Figure 3b** **Figure 3c** **Figure 3d**

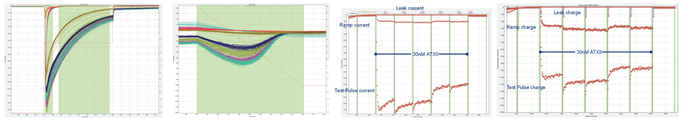

Figure 3a. hNav1.5 late current and charge measurement during test pulse
Peak hNav1.5 late current amplitude and charge were measured 5 seconds after the start of test pulse to measure the time-matched addition of the four (4) vehicle controls.

Figure 3b hNav1.5 late current and charge measurement during ramp pulse
hNav1.5 late current amplitude and the charge flow through the channel during ramp pulse was measured to determine the effect of time-matched addition of the four (4) vehicle controls on the late current.

Figure 3c. hNav1.5 late current amplitude stability for the entire assay duration during test and ramp pulse
IT plot is presented to illustrate the stability of hNav1.5 late current amplitude.

Figure 3d. hNav1.5 late current charge stability for the entire assay duration during test and ramp pulse
IT plot is presented to illustrate the stability of hNav1.5 late current charge.

Table 1 Comparison of test and ramp pulse IC_{50} values for Phase I (manual patch clamp IC_{50} values added due to un-blinding of Phase I compounds) and Phase II compounds

Phase I				Phase II		
Compound ID	Estimated IC_{50} value, µM			Compound ID	Estimated IC_{50} value, µM	
	Test Pulse	Ramp Pulse	MPC		Test Pulse	Ramp Pulse
Ondansetron	>10	>10	19.1	TA1	26	24
Bepridil	>0.3	>0.3	1.8	TA2	11.5	10.3
Verapamil	>1	>1	7	TA3	165	241
Chlorpromazine	2.04	2.46	4.6	TA4	3.4	5.5
Terfenadine	>0.3	>0.3	20	TA5	2.3	3.4
Ranolazine	40.9	38.4	7.9	TA6	33.4	26.4
Sotalol	>300	>300	NA	TA7	9	5.8
Diltiazem	32.3	44	21.9	TA8	>70	>70
Cisapride	>0.3	>0.3	NA	TA9	0.102	0.236
Dofetilide	>0.1	>0.1	753	TA10	12.5	12.2
Quinidine	>10	>10	9.4	TA11	>100	>100
Mexilitine	18.1	20.3	8.9	TA12	>17	>17
				TA13	4.9	4.8
				TA14	>2	>2
				TA15	>18	>18
				TA16	0.448	0.639

Figure 4

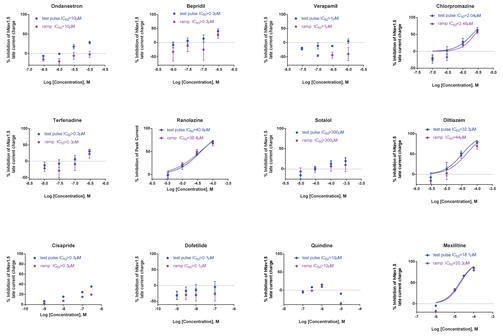

Figure 4. Concentration response curves for CiPA Phase I compounds from three independent assays
30nM ATXII was used to elicit hNav1.5 late current to determine the effect of Phase I compounds. Normalized hNav1.5 late current measured from test pulse and ramp pulse is plotted against compound concentrations and fit with an equation to estimate the IC_{50} value.

Figure 5

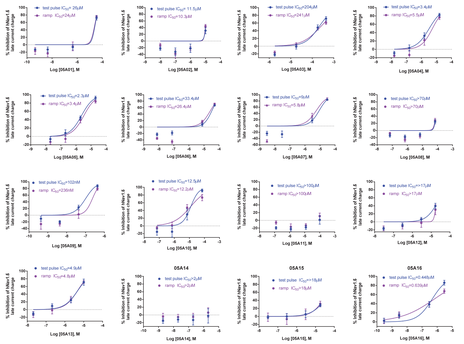

Figure 5. Concentration response curves for CiPA Phase II compounds from three independent assays
30nM ATXII was used to elicit hNav1.5 late current to determine the effect of Phase II compounds. Normalized hNav1.5 late current measured from test pulse and ramp pulse is plotted against compound concentrations and fit with an equation to estimate the IC_{50} value.

Figure 6a **Figure 6b**

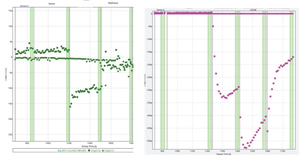

Figure 6a. Example of hNav1.5 late current activation by a test article without ATXII
Addition of test article to a cell expressing hNav1.5 current without 30nM ATXII treatment elicited hNav1.5 late current indicating the test article is hNav1.5 late current activator.

Figure 6b. Example of hNav1.5 late current activation by a test article with ATXII
30nM ATXII was added to the voltage clamped cell to elicit hNav1.5 late current first to act as a vehicle, subsequently, 30µM test article was added together with 30nM ATXII which elicited a further potentiation of hNav1.5 late current indicating the test article is hNav1.5 late current activator.

Conclusions

1. hNav1.5 late current inhibition was measured using step or ramp pulse protocol with 30nM ATXII; potentiation was measured with or without 30nM ATXII.
2. Three key parameters, INa-L current, leak current, with time-matched vehicle control were measured.
3. The IC_{50} values were generated from hNav1.5 late current inhibition by step or ramp pulse for Phase I and Phase II compounds.
4. The IC_{50} values generated for Phase I and Phase II compounds were compared between test and ramp pulses and test pulse IC_{50} values were more potent than the ramp pulses for some compounds, further test pulse IC_{50} values were used as quality control parameter for ramp pulse IC_{50} values.
5. With the un-blinding of CiPA Phase I (12) compounds, Chlorpromazine, Diltiazem, and Mexiletine were identified as hits.

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