Simultaneous Measurement of Cardiac hERG and Nav1.5 Currents Using an Automated Qube® Patch Clamp Platform

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ABSTRACT

With the ever increasing emphasis on ion channel profiling in safety screening there has been a drive towards increased throughput with respect to patch clamp profiling. The advent of automated 384 well plate based planar patch clamp instruments such as the Qube® have significantly aided in this effort, allowing for a significant increase in the number of compounds that can be profiled in a time and cost effective manner.

To further explore the possibility of increasing efficiencies in these systems we examined whether it would be possible to measure multiple currents from separate cell lines in a single well of a 384 well plate using the Qube® instrument. Theoretically the instrument lends itself to this possibility due to the virtue of being able to select plates that contain multiple holes per well. Hence by adding two independent cell lines, each independently stably expressing a recombinant ion channel, it should be possible to patch two or more cell lines per well.

We have compared a variety of conditions in the study in order to generate a robust assay that allows for the simultaneous profiling of both the Nav1.5 peak and hERG currents. Nav1.5 and hERG currents were separated temporally by virtue of the design of the voltage protocol. Our results show that the success rates of simultaneously recording both Nav1.5 and hERG currents in each well were 34.4%, 87.5%, 93.8%, 93.8% and 93.8% in 2, 6, 10, 16 and 36 hole/well QChips, respectively. The potencies of 28 CiPA compounds on Nav1.5 peak and hERG currents were determined individually in a single cell line format or in a mixed cell format using a 10 hole/well QChip. The correlation of the IC_{50} for the compounds was excellent for both hERG (R^2=0.96) and Nav1.5 (R^2=0.86) when comparing the single cell line format with the mixed cell format.

Our data demonstrate that is possible to generate a robust and reproducible assay using a single voltage protocol and buffer combination that allows for the measurement of the effects of compounds on both hERG and Nav1.5 currents simultaneously. Such a format can both significantly increase assay throughput and reduce assay costs while maintaining data quality.

METHODS

Automated Electrophysiology

Nav1.5 and hERG currents were recorded on the Qube ® 384 well automated patch clamp platform (Sophion) using QChip D (variable hole) and QChip X (10 hole/well chip).

Recombinant Cell Lines

Nav1.5 (Charles Rivers, Cleveland, OH) and hERG (Millipore, Burlington, MA) channels were expressed in CHO cells. Nav1.5 and hERG cells were harvested separately and mixed before the experiment at a ratio of 1:1 (2 million total cells/mL final).

Solutions

Extracellular (in mM): 145 NaCl, 2 $CaCl_2$, 1 $MgCl_2$, 4 KCl, 10 HEPES, 10 Glucose, pH=7.4 (NaOH), ~305 mOsm.

Intracellular (in mM): 120 KF, 20 KCl, 10 HEPES, 10 EGTA, pH=7.2 (KOH), ~300 mOsm.

Compounds

28 CiPA compounds were purchased from Sigma-Aldrich (St Louis, MO) and applied in a cumulative format to individual wells, with six concentrations from 0.001 to 100 µM in ten-fold increments.

Data analysis

Success criteria for a positive recording were defined as a current >100pA during the recording of a baseline response, as well as cell patch that was maintained throughout the time course of compound addition. Concentration-dependent drug effects (Hill fit and IC_{50}) analyses were performed using Analyzer Software 6.2 (Sophion, Denmark) and GraphPad Prism 7.0.4 (GraphPad Software, La Jolla, CA).

RESULTS

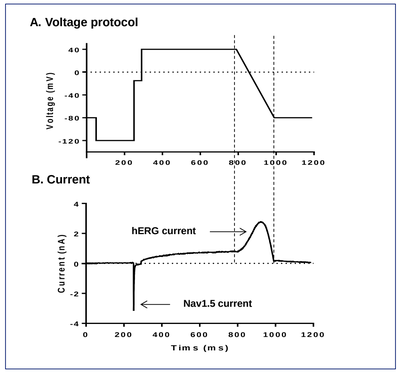

Figure 1. Temporal current separation based on discrete recording time windows with mixed cell lines using 10 hole QChip. **(A)** The voltage protocol for recording Nav1.5 and hERG currents. The step pulse from -120 mV to -15 mV was used to elicit the Nav1.5 current. The ramp from 40 mV to -80 mV was used to induce the hERG current. **(B)** An example of current trace with both Nav1.5 and hERG currents.

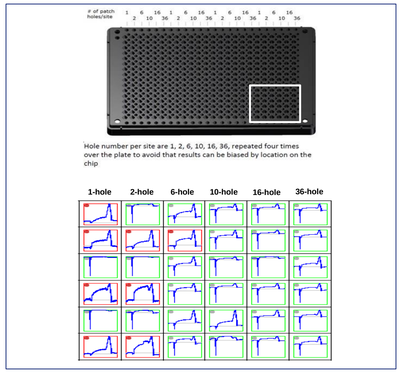

Figure 2. Nav1.5 and hERG currents recording with mixed cell lines using a QChip384 D with a variation of hole per well. The upper panel shows the assay development QChip384 D with hole numbers covering 1 to 36 per well. The lower panel shows example current traces in the lower right 36 wells of the QChip. Either hERG or Nav1.5 was seen in 1-hole well. hERG and Nav1.5 currents were seen in multiple hole wells

	Current Measured (% Success Rate)			
	hERG Only	Nav1.5 Only	hERG + Nav1.5	No Current
1-hole	60.9	31.3	-	7.8
2-hole	34.4	26.6	34.4	4.7
6-hole	7.8	3.1	87.5	1.6
10-hole	1.6	0	93.8	4.7
16-hole	0	0	93.8	6.3
36-hole	0	0	93.8	6.3

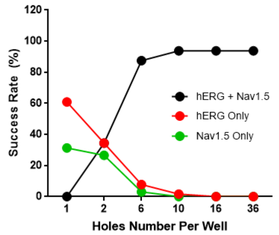

Figure 3. Success rate of recordings for hERG or Nav1.5 only or both (hERG + Nav1.5) currents using QChip 384 D at different hole number per well. Success rate reach a maximum at the 10 holes/well

	Current Measured (% Success Rate)		
	hERG Only	Nav1.5 Only	hERG + Nav1.5
1-hole	92.2	92.2	-
2-hole	96.9	95.3	34.4
6-hole	96.9	96.9	87.5
10-hole	98.4	100	93.8
16-hole	100	98.4	93.8
36-hole	100	100	93.8

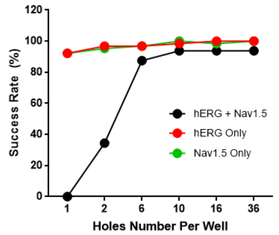

Figure 4. Comparison of success rate for measurement of hERG and Nav1.5 currents in either a single cell format or combined assay format

	hERG IC_{50} (µM)						Nav1.5 IC_{50} (µM)					
	hERG Cell Only			Mixed Cells			Nav1.5 Cell Only			Mixed Cells		
Astemizole	0.18	±	0.01	0.58	±	0.03	3.2	±	0.3	3.2	±	0.1
Azimilide	0.22	±	0.03	0.41	±	0.01	29.6	±	3.7	37.8	±	3.5
Bepridil	0.64	±	0.02	2.25	±	0.18	11.9	±	1.1	12.3	±	0.9
Chlorpromazine	1.33	±	0.11	3.93	±	0.22	9.2	±	0.8	14.4	±	1.3
Cisapride	0.14	±	0.01	0.25	±	0.02	10.9	±	0.9	10.8	±	1.3
Clarithromycin	53.33	±	8.89	98.4	±	10.4	540.7	±	136.7	299.2	±	41
Clozapine	0.79	±	0.06	2.01	±	0.21	15.8	±	2.4	21.7	±	1.5
Diltiazem	5.90	±	0.40	9.71	±	0.43	24.1	±	3.3	20.0	±	0.9
Disopyramide	6.24	±	0.89	8.16	±	0.51	176.3	±	32.2	120.8	±	13.0
Dofetilide	0.28	±	0.01	0.32	±	0.03	213.4	±	27.4	382	±	84
Domperidone	0.12	±	0.01	0.22	±	0.01	13.1	±	0.7	12.7	±	0.8
Droperidol	0.13	±	0.02	0.36	±	0.03	9.7	±	1.4	11.4	±	0.7
Ibutilide	0.07	±	0.01	0.12	±	0.01	29.5	±	3.8	37.3	±	5.0
Loratadine	6.53	±	0.39	3.98	±	0.37	31.2	±	2.9	16.7	±	1.3
Metoprolol	18.88	±	1.10	42.46	±	4.44	240.5	±	32.3	227.4	±	33
Mexiletine	20.13	±	1.06	37.70	±	2.54	40.4	±	6.8	29.6	±	3.2
Nifedipine	41.20	±	2.12	37.23	±	1.62	54.0	±	4.1	69.5	±	8.3
Nitrendipine	16.75	±	1.95	36.88	±	2.54	11.4	±	1.4	14.4	±	0.8
Ondansetron	0.68	±	0.07	1.05	±	0.08	28.7	±	5.0	25.4	±	2.2
Pimozide	0.48	±	0.03	1.36	±	0.22	2.7	±	0.4	5.0	±	0.5
Quinidine	0.32	±	0.03	0.50	±	0.04	13.0	±	1.2	10.9	±	0.9
Ranolazine	4.28	±	0.51	8.17	±	0.47	51.5	±	4.7	60.0	±	6.6
Risperidone	0.26	±	0.03	0.47	±	0.05	77.3	±	9.2	62.2	±	9.8
Sotalol	111.9	±	15.75	321.9	±	34.0	330.8	±	70.7	529.0	±	103
Tamoxifen	6.95	±	0.55	46.09	±	3.93	192.1	±	17.1	259.0	±	26.5
Terfenadine	1.01	±	0.17	2.26	±	0.26	2.7	±	0.2	6.4	±	0.6
Vandetanib	0.49	±	0.05	1.09	±	0.11	20.1	±	1.7	15.6	±	1.1
Verapamil	0.50	±	0.07	1.12	±	0.04	15.0	±	1.2	17.5	±	1.2

Table 1. Summary of IC_{50} values for 28 CiPA compounds on hERG and Nav1.5 currents under single cell line and mixed cell line conditions. Mean ± SEM (N=6-10)

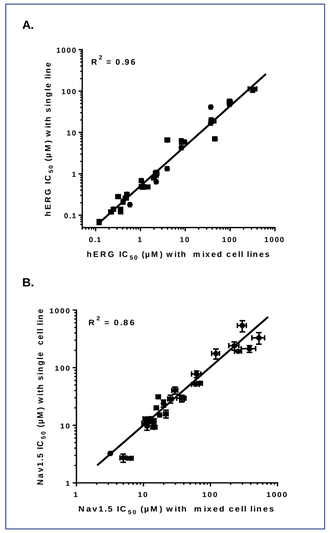

Figure 5. Correlations of potency data of 28 CiPA compounds for **(A)** hERG and **(B)** Nav1.5 under single cell line and mixed cell line conditions. Data represent mean ± SEM.

CONCLUSIONS

- The Qube® 384 technology not only enables high throughput ion channel profiling of compounds, but as demonstrated in this current study it also offers the opportunity to profile multiple cell lines expressing different ion channels within a single well by utilizing the multi-hole chip design
- Nav1.5 and hERG currents can be separated temporally by the fact that Nav1.5 is an inward fast voltage-gated ion channel (full activation within 0.5 msec and inactivation within 20 msec) and hERG is a outward slowly activating voltage-gated ion channel (full activation in the several 100 ms)
- Analysis comparing conventional single cell type recording versus the multi-cell recordings show a high correlation when comparing a series of compound IC_{50} values
- Our data demonstrate that is possible to generate a robust and reproducible assay using a single voltage protocol and buffer combination that allows for the measurement of the effects of compounds on both hERG and Nav1.5 currents simultaneously using a Qchip 384 X (10 hole/well)

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