BIOPHYSICAL AND PHARMACOLOGICAL PROFILING OF MULTIPLE NA_V SUBTYPES ON QPATCH HT

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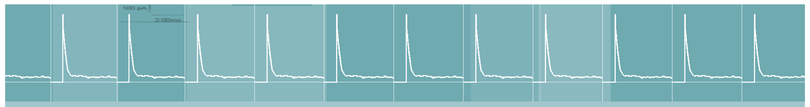


NEW CELL CLONE SCREENING FEATURE ON QPATCH

The new clone screening feature developed for QPatch HT and QPatch 16 allows running up to eight different cell lines (clones or subtypes) at the same time, thus ensuring that the exact same conditions - such as temperature, Ringer's, pH etc. - are applied for each of the cell lines tested. On QPatch HT each of the eight cell lines are applied to six separate sites on the measurement plate, QPlate 48. After the experiment is finished the data for each cell type can be tracked and compared easily using the advanced analysis functionalities in the QPatch Assay Software. In our study, seven subtypes of the voltage gated sodium channel, $Na_v1.1$ $\alpha\beta_1$, 1.2 α, 1.3 $\alpha\beta_1$, 1.4 $\alpha\beta_1$, 1.6 $\alpha\beta_1$, 1.7 $\alpha\beta_1$ and $Na_v1.8$ α, were tested in parallel on QPatch HT, using the cell cone screening feature. Experiments were designed to explore 1) TTX sensitivity, 2) IV-relationship for activation and inactivation, for the entire panel of Na_v channel subtypes in a single experiment. Thus several different voltage protocols were used in the same experiment.

MATERIALS AND METHODS

Cells: All Na_v subtypes tested are stably expressed in HEK293 cells. $Na_v1.1$, 1.3, 1.4, 1.6 and 1.7 consists of their respective α subunits co-expressed with $\beta1$, and were obtained from Scottish Biomedical (Glasgow, UK). $Na_v1.8$ is the α subunit alone, and also obtained from Scottish Biomedical (Glasgow, UK). $Na_v1.2$ is the α subunit alone, and property of Neurosearch (Ballerup, DK).

Ringer's solutions: Intracellular Ringer (in mM): 135 CsF, 1/5 EGTA/ CsOH, 10 HEPES, 10 NaCl. Osmolarity adjusted to approximately 320 mOsm with sucrose, pH 7.4. Extracellular Ringer (in mM): 2 CaCl2, 1 MgCl2, 10 HEPES, 4 KCl, 145 NaCl, 10 TEA-Cl, 10 Glucose. Osmolarity adjusted to approximately, 320 mOsm with sucrose, pH 7.4

Compounds: Tetrodotoxin (Alomone Labs, Jerusalem, Israel).

Voltage protocols: For all concentration-response experiments, a depolarization to +10 mV for 30 msec from a holding potential of -100 mV was used (Figure 1). For IV relationships, a protocol with incremental steps of +10 mV from -120 to +70 mV of 1000 ms duration was used (Figure 2). Steady-state inactivation was tested at 10 mV after the 1000 ms prepulse at +10 mV for 100 msec. Each incremental sweep took place with 5 second intervals.

Figure 1. Voltage protocol for concentration-response experiments.

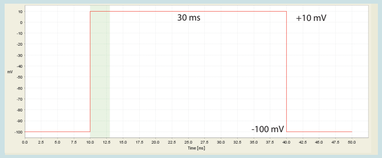
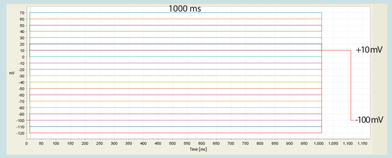


Figure 2. Voltage protocol for determining IV-relationship and steady-state inactivation.



Data analysis:
Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). IV-relationships for activation and inactivation and concentration-dependent drug effects (Hill fit and IC_{50}) were analyzed using QPatch Assay Software.

Analysis features developed side-by-side with the cell clone screening feature enables quick and easy grouping of data from each Na_v channel subtype.

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Figure 3

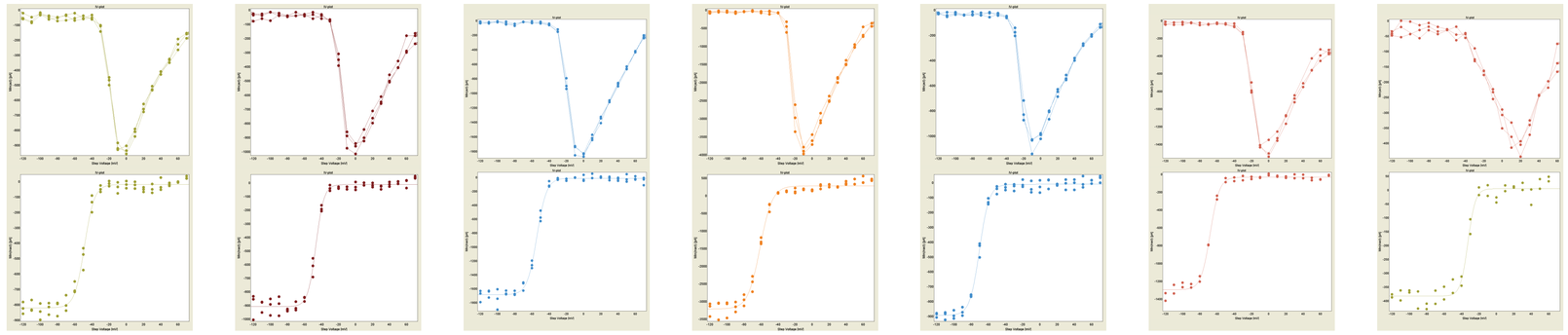

Table 1

	n	Voltage of half-maximal inactivation, $V_{½}$ (mV)		Voltage of half-maximal activation, V_{mid} (mV)	
		QPatch	Literature	QPatch	Literature
$Na_v1.1$	6	-48.1±2.2	-72[1]	-19.8±1.2	-33[1]
$Na_v1.2$	4	-48.7±2.1	-53[2]	-17.5±3.0	-24[2]
$Na_v1.3$	5	-56.9±5.9	-69[3]	-20.0±2.2	-23[3]
$Na_v1.4$	4	-60.0±0.8	-56[2]	-25.6±1.2	-26[2]
$Na_v1.6$	13	-64.4±3.2	-72[4]	-17.3±2.8	-29[1,4]
$Na_v1.7$	5	-69.8±5.4	-74[5]	-21.5±0.9	-24[5]
$Na_v1.8$	2	-32.8	-30[2]	-13.7	-16[2]

Table 1
Voltage of half-maximal inactivation ($V_{½}$) and activation (V_{mid}) ±SD obtained from the Boltzmann fit and extrapolated from the IV-plot, respectively. Data was not adjusted for liquid junction potential. Literature values for data obtained under comparable experimental conditions are also shown.

Table 2
IC_{50} values for TTX obtained from the grouped Hill fits shown in Figure 4. For comparison, average values of individual Hill fits ±SD and literature values for data obtained under comparable experimental conditions are also shown.

Figure 3
IV characteristics of sodium channel subtypes. Representative examples of data for each type of voltage gated sodium channel tested on QPatch HT in the cell clone screening mode. Top: IV plots of the sodium channel current elicited at different voltages. Bottom: Boltzmann fits for steady-state inactivation.

Figure 4
Concentration-response characteristics of sodium channel subtypes. Top: IT plots for each type of voltage gated sodium channel tested on QPatch HT in the cell clone screening mode. Bottom: Corresponding Hill fits based on the current at steady-state compound response for several cells ('group Hill fit').

Figure 4

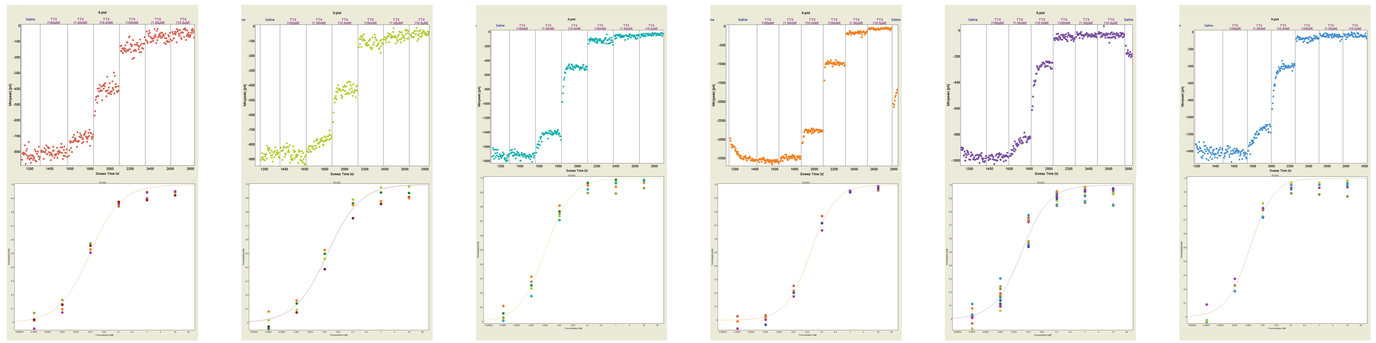

Table 2

	n	IC_{50} (nM)		
		grouped Hill fit	Hill fit	Literature
$Na_v1.1$	5	9.4	9.5±1.2	6[1]
$Na_v1.2$	4	12.5	13.6±4.5	12[2]
$Na_v1.3$	5	3.1	3.2±0.8	4[3,6]
$Na_v1.4$	4	39.2	39.8±8.2	25[7]
$Na_v1.6$	13	5.7	6.1±2.4	2.5[1,4]
$Na_v1.7$	5	3.8	4.6±2.3	26[8]
$Na_v1.8$	-	-	-	TTX insensitive

SUMMARY

The QPatch cell clone screening feature and its direct integration with the QPatch Assay Software data analysis is a strong experimental tool. It allows simultaneous testing of cell clones expressing the same gene(s) of interest, or simultaneous testing of a panel of ion channel subtypes or a group of ion channels (e.g. heart channels). Importantly, the QPatch cell clone screening features ensures identical experimental conditions thereby allowing a direct high quality comparison between the obtained results - be it pharmacology or success rates. The experiments presented here allowed us to obtain pharmacological and biophysical data on a panel of voltage-gated sodium channel subtypes expressed in cell lines previously untested on QPatch. For each subtype, the experiments clearly identified the expected pharmacology (TTX IC_{50} values), and biophysical properties (IV-relationships for activation and inactivation). Based on the pharmacological and biophysical data obtained under identical experimental conditions, each subtype was successfully distinguished from the others. In this way, the QPatch cell clone screening feature enabled a quick and efficient path for validating seven different sodium channel subtypes.