Biophysical and pharmacological profiling of multiple voltage-gated sodium channel subtypes on QPatch II

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Introduction

Voltage-gated sodium channels (VGSC) are in the spotlight of drug development as strong evidence is available linking different subtypes to various disease states.

VGSC is responsible for the initiation and propagation of action potentials in excitable cells. During this process, the VGSC transitions from a closed to an open into an inactivated state. Interestingly, inhibitory compounds often exhibit different pharmacological profiles dependent upon the conformational state of the ion channel.

In the present work, the second generation QPatch (QPatch II; Sophion Bioscience) was used in combination with adaptive voltage protocols to investigate state-dependent inhibition of tetrodotoxin (TTX), amitriptyline and tetracaine on 8 different VGSC subtypes ($Na_V1.1$-8). A first step was to determine the half-inactivation potential $V_{½}$(inactivation) for each individual cell. This value was then used during the next steps as preconditioning pulse. Such an adaptive protocol allowed to determine IC_{50} values for both the closed and the inactivated state and reduce heterogeneity of the cells. Both IC_{50} values and biophysical parameters of the different subtypes align well with literature values.

Materials and methods

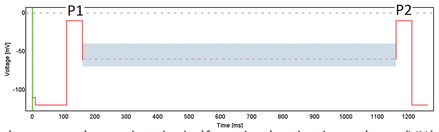
Cell lines and cell culture:

Cells expressing sodium channel isoforms $Na_V1.1$ to $Na_V1.8$ were cultured according to the Sophion SOP for the respective cell line. HEK293 cells heterogeneously expressing $Na_V1.1$, $Na_V1.2$, $Na_V1.3$ and $Na_V1.4$ were obtained from SB Drug Discovery (Glasgow, UK), CHO-$Na_V1.5$ from B'SYS GmbH (Witterswilw, CH), HEK293-$Na_V1.6$ and CHO-$Na_V1.8$ from Charles River Laboratories (Cleveland, OH) and CHO-$Na_V1.7$ from Anaxon AG (Berne, CH).

The cells were harvested using detachin (HEK293 cells) or trypsin (CHO cells) and transferred to serum-free medium (EX-CELL® ACF CHO Medium, Sigma-Aldrich, Brøndby, DK) supplemented with HEPES 25 mM, 40 µg/ml trypsin inhibitor and P/S. The cells were automatically washed and resuspended in the extracellular buffer using QPatchII's onboard cell preparation unit.

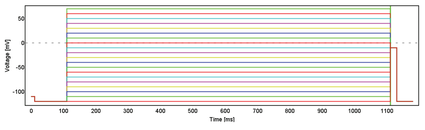
Experimental protocol:

Voltage protocol (VP) 1



The voltage was clamped at the half-maximal activation voltage (V½). This value was determined for each individual cell using VP2.

VP 2



Extracellular saline was applied two times to gain a stable baseline. Each application was followed by 6 repetitions of voltage protocol (VP) 1 with 10 s intersweep interval. During this initial period, the blue shaded voltage segment was clamped to a fixed value of -65 mV. Next, a family of voltage steps was applied to the cells (VP 2). Before the application of test compounds, VP1 was repeated 3 times and the average current obtained in this period was used as baseline value. 6 concentrations of each compound were applied to the cells in an increasing manner with each 3 min compound exposure times (12 repetitions of VP1). The experiment was finished with application of 10 µM TTX as positive reference.

Solutions:

Intracellular solution (IC) (in mM): CsF 135, NaCl 10, EGTA 5, HEPES 10, adjusted to pH 7.2 (CsOH).
Extracellular solution (EC) (in mM): NaCl 145, KCl 4, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10, glucose 10, adjusted to pH 7.4 (NaOH).

Compounds:

Tetrodotoxin was purchased from Alomone Labs (Jerusalem, Israel), all other compounds were obtained from Sigma-Aldrich (Darmstadt, Germany). The analysis was carried out using the Sophion Analyzer software and Qrigin7.5 (OriginLab, Northampton, MA, USA).

Results

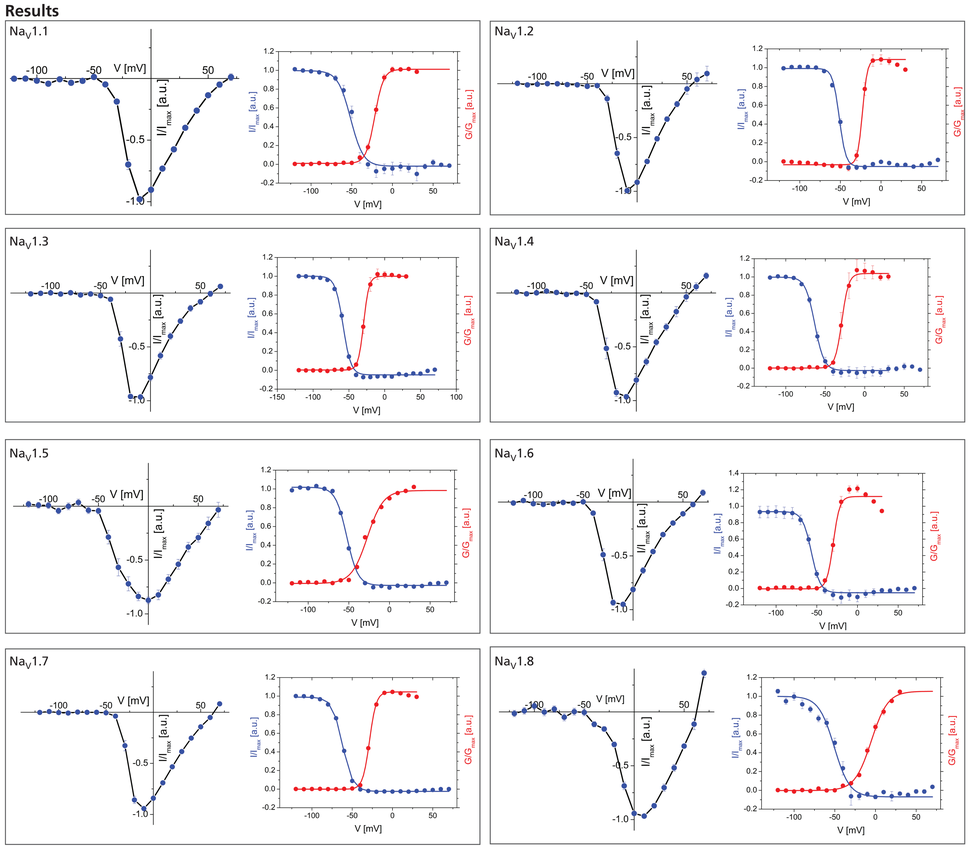


Fig. 1: Biophysical characteristics of 8 different sodium channel subtypes. 1st and 3rd column: Normalized average IV plots of $Na_V1.1$-1.8. Currents were elicited using VP2. 2nd and 4th column: Activation and inactivation kinetics. Slow, steady-state Inactivation curves are shown in blue and activation curves in red. A Boltzmann function was fitted to the data to estimate $V_{½}$ values. Data are presented as mean ± s.e.m.

Table. 1: Summary of $V_{½}$ of activation and inactivation.

Subtype	Steady-state slow inactivation			Activation		
	n	$V_{½}$ [mV]	Literature [mV]	n	$V_{½}$ [mV]	Literature [mV]
$Na_V1.1$	21	-51	-72[1]	21	-22	-33[1]
$Na_V1.2$	15	-51	-53[2]	13	-23	-24[2]
$Na_V1.3$	12	-58	-69[3]	12	-29	-23[3]
$Na_V1.4$	6	-63	-56[2]	7	-29	-26[2]
$Na_V1.5$	25	-53	-81[9]	24	-27	-34[9]
$Na_V1.6$	15	-57	-72[4]	13	-40	-29[1.4]
$Na_V1.7$	26	-72	-74[5]	26	-29	-24[5]
$Na_V1.8$	16	-51	-30[2]	17	-5	-16[2]

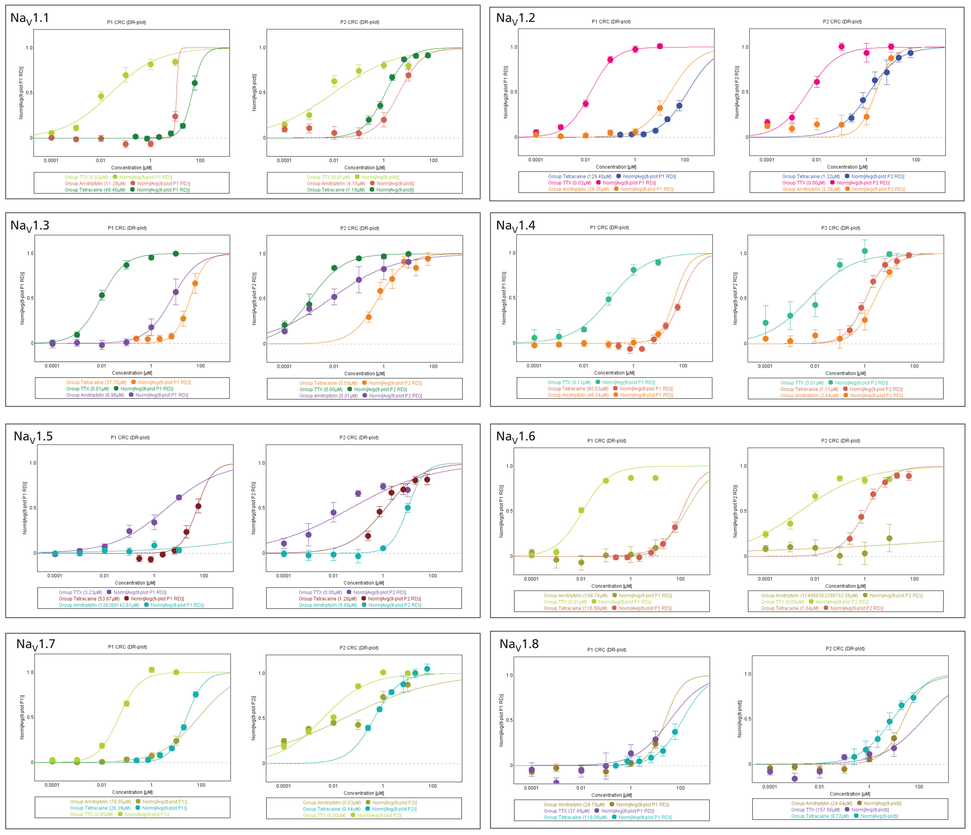


Fig. 2: Pharmacological characterization of different sodium channel subtypes. Different concentrations of amitriptyline, tetrodotoxin (TTX) and tetracaine were applied to the cells in a cumulative fashion (see experimental set up). Current inhibition was monitored using the voltage protocol 1 shown under materials and methods. The 1st and 3rd column show data recorded during the first stimulation (P1) to -10 mV and the 2nd and 4th column show data from the second stimulation (P2). The voltage was clamped at $V_{½}$ of inactivation between P1 and P2. $V_{½}$ was estimated for each individual cell at the beginning of the experiment.

Table. 2: Summary of all IC_{50} values.

Subtype	Amitriptyline			TTX			Tetracaine		
	n	IC_{50} (P1) [µM]	IC_{50} (P2) [µM]	n	IC_{50} (P1) [µM]	IC_{50} (P2) [µM]	n	IC_{50} (P1) [µM]	IC_{50} (P2) [µM]
$Na_V1.1$	12	>10	4.15	11	0.03	0.01	14	48.46	1.18
$Na_V1.2$	14	>10	2.38	13	0.02	0.008	12	26.26	1.32
$Na_V1.3$	13	>10	0.01	12	0.01	0.001	12	6.96	0.59
$Na_V1.4$	7	>10	2.64	5	0.12	0.01	5	46.04	1.11
$Na_V1.5$	18	>10	9.88	15	3.23	0.05	14	53.87	1.28
$Na_V1.6$	14	>10	>10	14	0.01	0.003	13	>60	1.04
$Na_V1.7$	32	>10	0.03	32	0.05	0.004	30	26.39	0.44
$Na_V1.8$	15	>10	>10	16	>10	>10	14	>60	8.72

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