Biophysical and pharmacological characterisation of multiple Na_V subtypes on Qube

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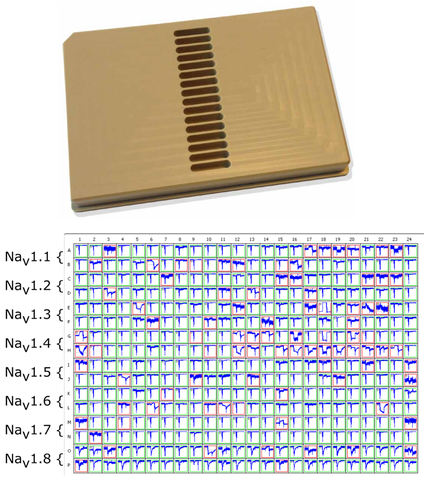
Introduction

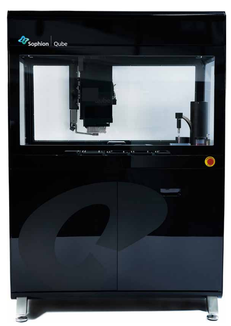
The voltage dependent sodium channel is responsible for the upstroke and directed propagation of action potentials in nerve and muscle cells and is therefore a central ion channel in excitable tissues. The implication of voltage gated sodium channels in pain mediation and diseases such as epilepsy and cardiac arrhythmia has made them very important targets for drug discovery. Here we show eight subtypes of the voltage gated sodium channel tested in parallel on Qube, the Sophion 384-format automated patch clamp system designed for high throughput and high fidelity electrophysiological recordings. On Qube it is possible to run up to 16 different clones or cell lines simultaneously which ensures identical conditions across the experiments.

Three types of experiments were designed to explore 1) TTX sensitivity, 2) IV-relationship for activation and inactivation and 3) pulse train suitable for screening for use dependent sodium channels blockers. For the screening example pharmacology was represented by tetracaine. For every run Na_V channel subtypes $Na_V1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 and 1.8 were tested side by side.



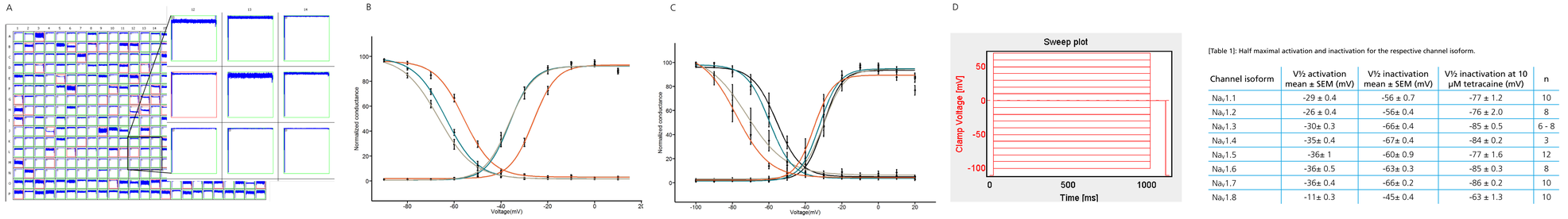
[Fig. 1]: Top, cell clone Cell Transfer Plate (ccCTP) for the Qube instrument. Cells expressing the different sodium channel isoforms were added to the individual wells of the ccCTP and subsequently transferred to the QChip for experiment. Lower panel shows layout and evoked currents from a typical experiment. Currents were elicited by a depolarizing pulse from a holding potential of -110 mV to 0 mV.

Sophion Qube



References

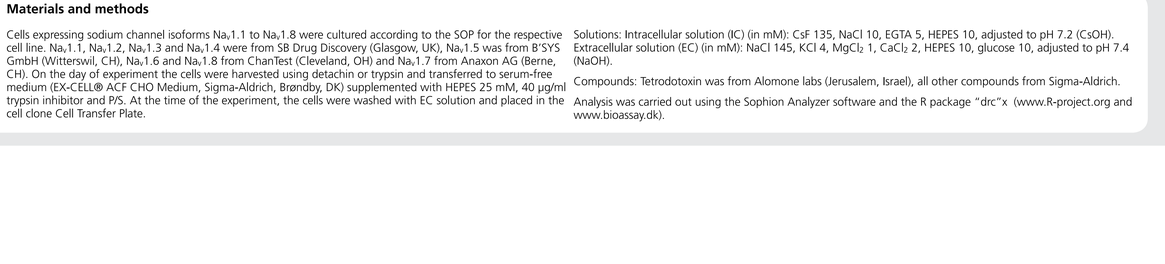
Catteral W.A. et al. Pharmacol Rev. 57:397-409, 2005 and references therein.



[Fig. 2]: Voltage-dependent activation and steady-state inactivation for $Na_V1.X$ isoforms. Figure 2A, typical currents in response to a depolarizing pulse from -110 to 0 mV and figure 2D, voltage protocol. Figure 2B, conductance-voltage relationship for activation and steady-state inactivation for $Na_V1.2$ (blue), $Na_V1.6$ (orange) and $Na_V1.7$ (grey). Figure 2C, activation and steady-state inactivation for $Na_V1.7$ under control conditions (black) and in the presence of 10 µM of amitriptyline (blue), bupivacaine (orange) and tetracaine (grey) respectively.

[Table 1]: Half maximal activation and inactivation for the respective channel isoform.

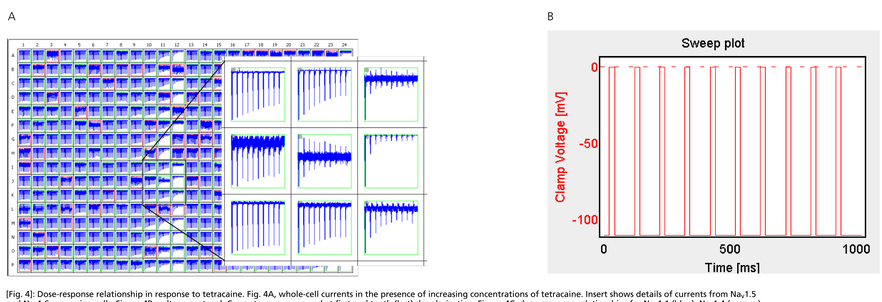
Channel isoform	V½ activation mean ± SEM (mV)	V½ inactivation mean ± SEM (mV)	V½ inactivation at 10 µM tetracaine (mV)	n
$Na_V1.1$	-29 ± 0.4	-56 ± 0.7	-77 ± 1.2	10
$Na_V1.2$	-26 ± 0.4	-56± 0.4	-76 ± 2.0	8
$Na_V1.3$	-30± 0.3	-66± 0.4	-85 ± 0.5	6 - 8
$Na_V1.4$	-35± 0.4	-67± 0.4	-84 ± 0.2	3
$Na_V1.5$	-36± 1	-60± 0.9	-77 ± 1.6	12
$Na_V1.6$	-36± 0.5	-63± 0.3	-85 ± 0.3	8
$Na_V1.7$	-36± 0.4	-66± 0.2	-86 ± 0.2	10
$Na_V1.8$	-11± 0.3	-45± 0.4	-63 ± 1.3	10



[Fig. 3]: Dose-response relationship in response to tetrodotoxin (TTX). Figure 4A, representative whole-cell currents under control conditions and in the presence of 123 nM TTX for $Na_V1.1$ and $Na_V1.5$ respectively and figure 4B, voltage protocol. The depolarizations were elicited at 0.1 Hz. Figure 4D, dose-response relationships for $Na_V1.4$ (blue), $Na_V1.5$ (orange) and $Na_V1.6$ (grey) in response to increasing doses of TTX.

[Table 2]: Concentration dependent inhibition of sodium currents by TTX. Cells were stimulated with depolarizations from -110 to 0 mV at 0.1 Hz.

Channel isoform	IC_{50} ± SEM (nM)	n
$Na_V1.1$	22 ± 5	11 - 14
$Na_V1.2$	33 ± 4	11 - 15
$Na_V1.3$	12 ± 3	7 -14
$Na_V1.4$	44 ± 5	7 - 13
$Na_V1.5$	7800 ±	7 - 12
$Na_V1.6$	9 ± 1	11 - 15
$Na_V1.7$	93 ± 9	12 - 15
$Na_V1.8$	>10000	13 - 16



[Fig. 4]: Dose-response relationship in response to tetracaine. Fig. 4A, whole-cell currents in the presence of increasing concentrations of tetracaine. Insert shows details of currents from $Na_V1.5$ and $Na_V1.6$ expressing cells. Figure 4B, voltage protocol. Currents were measured at first and tenth (last) depolarization. Figure 4C, dose-response relationships for $Na_V1.1$ (blue), $Na_V1.4$ (orange) and $Na_V1.8$ (grey) in response to increasing doses of tetracaine for the first pulse (dashed line) and tenth pulse (solid line).

[Table 3]: Concentration-dependent inhibition of sodium currents by tetracaine. Cells were stimulated with 10 depolarizations from -110 mV to 0 mV at 10 Hz.

Channel isoform	First depolarization IC_{50} ± S.E.M (µM)	Tenth depolarization IC_{50} ± S.E.M (µM)*	n
$Na_V1.1$	>30	8.6 ± 2.8	2 - 8
$Na_V1.2$	>30	9.7 ± 2.9	4 - 6
$Na_V1.3$	>30	9.9 ± 0.3	2 - 8
$Na_V1.4$	>30	3.4 ± 0.4	2 - 4
$Na_V1.5$	>30	3.1± 0.2	5 - 9
$Na_V1.6$	>30	11.4 ± 4.9	6 - 8
$Na_V1.7$	>30	3.9 ± 0.6	3 - 7
$Na_V1.8$	>30	4.3 ± 3.4	4 - 7

Materials and methods

Cells expressing sodium channel isoforms $Na_V1.1$ to $Na_V1.8$ were cultured according to the SOP for the respective cell line. $Na_V1.1$, $Na_V1.2$, $Na_V1.3$ and $Na_V1.4$ were from SB Drug Discovery (Glasgow, UK), $Na_V1.5$ was from B'SYS GmbH (Witterswil, CH), $Na_V1.6$ and $Na_V1.8$ from ChanTest (Cleveland, OH) and $Na_V1.7$ from Anaxon AG (Berne, CH). On the day of experiment the cells were harvested using detachin or trypsin 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. At the time of the experiment, the cells were washed with EC solution and placed in the cell clone Cell Transfer Plate.

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 from Alomone labs (Jerusalem, Israel), all other compounds from Sigma-Aldrich.

Analysis was carried out using the Sophion Analyzer software and the R package "drc"x (www.R-project.org and www.bioassay.dk).

Conclusion

This study presented biophysical and pharmacological characterization of eight different Nav cell lines on Qube simultaneously. Multiple compounds and cell lines were used in parallel on Qube and the results for Tetrodotoxin sensitivity, IV-relationship (activation and inactivation), and screening of use dependent sodium channel blockers were of high and consistent quality, which proves excellent voltage control and cell handling. This Qube feature creates endless opportunities for simultaneous testing of multiple cell lines and compounds on Qube.