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. VGSCs are responsible for the initiation and propagation of action potentials in excitable cells. During this process, the VGSC transitions through distinct kinetic states that determine its drug sensitivity. 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 different VGSC subtypes (Naᵥ1.1-8). A first step was to determine the half-inactivation potential V½ (inactivation) for each individual cell. This value was then used during the next steps as preconditioning pulse. Such an adaptive protocol allowed for in vivo-like conditions and the inactivated state and reduce heterogeneity of the cells. Both V½ 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 Naᵥ1.1 to Naᵥ1.8 were cultured according to the SOP for the respective cell line. HEK292S cells heterogeneously expressing Naᵥ1.1, Naᵥ1.2, Naᵥ1.3 and Naᵥ1.4 were obtained from MB Drug Discovery (Shanghai, LR); CHO-Naᵥ1.5 from The Jackson Laboratory (Bar Harbor, ME); CHO-Naᵥ1.6 from Charles River Laboratories (Cleveland, OH) and CHO-Naᵥ1.7 from Amazon AG (Bern, CH).

The cells were harvested using detachin (HEK293 cells) or treponin (CHO cells) and transferred to serum-free medium (EX-CELL 400 medium, Sigma-Aldrich, Brøndby, DK) supplemented with HEPES 25 mM, glucose 10 mM, and resuspended in the extracellular buffer using QPatchII's onboard 40 μg/ml trypsin inhibitor and P/S. The cells were automatically washed and resuspended in QPatchIII's onboard cell preparation unit.

Experimental protocol:
Voltage protocol (VP 1):

The voltage was clamped at the half-inactivation voltage V½. This value was determined for each individual cell using VP2.

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 +45 mV. Next, a family of voltage steps was applied to the cells (VP 3). Before the families of voltage steps, a 3 min and 5 min compound exposure times (12 repetitions of VP1). The experiment was finished with application of 10 µM TTX as positive reference.

Intracellular solution (IC) (in mM): CsF 135, NaCl 10, EGTA 5, HEPES 10, P/S.

Solutions:

Positive reference.

Results

Steady-state slow inactivation

Activation

Table 1: Summary of V½ of activation and inactivation.

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<th>Subtype</th>
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<th>IC₅₀ [µM]</th>
<th>IC₅₀ [µM]</th>
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<td>16</td>
<td>5.10</td>
<td>0.10</td>
</tr>
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References

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