Application Note

Channel: $hNa_V1.7$
Cells: CHO
Tools: Port-a-Patch®

Patch clamp recordings of $hNa_V1.7$ on Nanion's Port-a-Patch®

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Cells kindly provided by Anaxon.

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Summary

The $Na_V1.7$ gene (SCN9A) encodes a voltage-gated sodium (Na_V) channel, primarily expressed in the peripheral nervous system and has been isolated from rat dorsal root ganglion (DRG) neurons[1], human medullary thyroid cancer cells (hNE-Na)[2] and PC12 cells[3,4].

Different Na_V channels play a key role in modulation of action potentials in the central and peripheral nervous systems. In particular, the fast upstroke of the action potential is mediated by Na_V channels. Na_V channels are in part characterized by their TTX-sensitivity (TTX-resistant [TTXr], TTX-sensitive [TTXs]). $Na_V1.7$ is a TTXs channel and is sensitive to TTX in the nanomolar range[1,2]. The role of $hNa_V1.7$ has yet to be fully elucidated but is proposed to play an important role in nociception and pain sensing. $Na_V1.7$ has been implicated to play a role in disease pain states, in particular inflammatory pain[5] and hypersensitivity to heat following burn injury[6]. Common to many of the voltage-gated ion channels, a number of compounds display a higher affinity for the inactivated state of the channel. For this reason, it is important to be able to reliably record both activation and inactivation kinetics of the channel.

In this Application Note we present data using the Port-a-Patch® characterizing CHO cells stably expressing $hNa_V1.7$. The $hNa_V1.7$ activation and inactivation properties and TTX sensitivity are consistent with those reported in the literature[1,2,7,8].

Results

Figure 1 shows the raw current responses to a voltage step protocol from an exemplar CHO cell expressing $hNa_V1.7$ recorded on the Port-a-Patch®. The average activation curve for an average of 16 cells is also shown. $Na_V1.7$ currents started to activate at about -40 mV, peak response was elicited at around 0 mV and Vhalf of activation was -11 mV (n = 16).

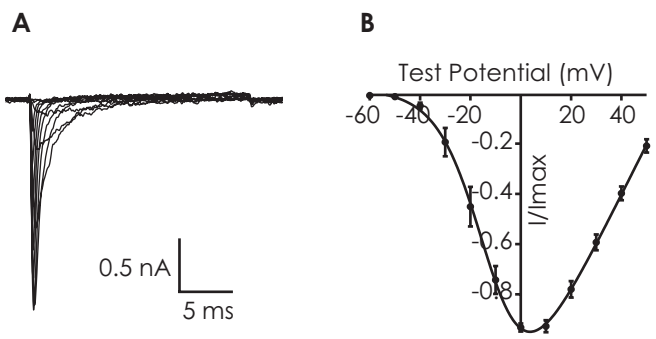

Figure 1:
A Raw current traces of an exemplar CHO cell expressing $hNa_V1.7$ recorded on the Port-a-Patch®. Currents were elicited using a voltage step protocol from a holding potential of -120 mV to -60 mV for 20 ms increasing in 10 mV steps up to 50 mV. **B** Corresponding IV activation plot for an average of 16 cells.

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Figure 2 shows current responses of an example cell to an inactivation voltage protocol. A 5 s pre-pulse to different test potentials was followed by a step to 0 mV for 10 ms. Shown are current responses at 0 mV. The inactivation plot for an average of 5 cells is also shown. The Vhalf of inactivation was -73 mV (n = 5), in good agreement with the literature[1,2,7,8].

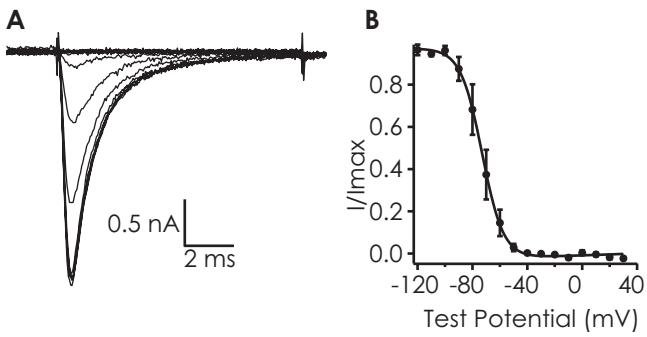

Figure 2:
A Raw current traces of an exemplar CHO cell expressing $hNa_V1.7$ to an inactivation protocol. Currents were elicited using a voltage step protocol to -120 mV for 5 s increasing in 10 mV steps, followed by a step to 0 mV for 10 ms. Currents at 0 mV are shown. **B** Relative peak amplitude at 0 mV was plotted against the test potential. Shown is an inactivation plot for an average of 5 cells. Points were fitted with a Boltzmann equation. Vhalf of inactivation was -73 mV.

$Na_V1.7$ is a TTX-sensitive channel and TTX was applied using the external perfusion system for the Port-a-Patch®.

Current responses in the absence and presence of TTX are shown in Figure 3. A concentration response curve was constructed revealing an IC_{50} = 43 ± 7 nM (n = 5), in good agreement with the literature[2].

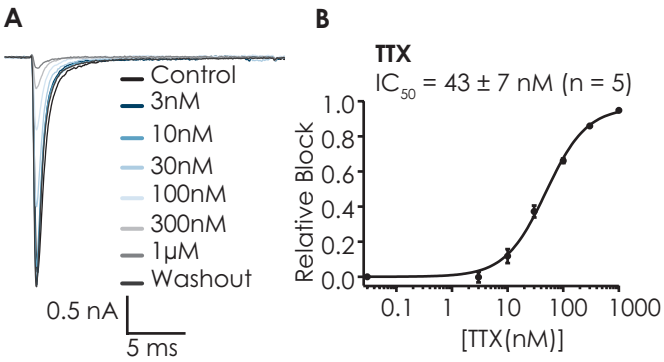

Figure 3:
A Raw current traces of $hNa_V1.7$ and subsequent block by TTX recorded on the Port-a-Patch. Shown are raw current traces from an exemplar CHO cell expressing $hNa_V1.7$, elicited using a voltage step protocol from a holding potential of -120 mV to 0 mV for 20 ms, and increasing block by increasing concentrations of TTX. **B** Concentration response curve for block of $hNa_V1.7$ by TTX for an average of 5 cells. IC_{50} = 43 ± 7 nM (n = 5).

In conclusion, $hNa_V1.7$ expressed in CHO cells provided by Anaxon can be reliably recorded on the Port-a-Patch® with activation, inactivation properties and TTX sensitivity as expected[1,2,7,8].

References

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Methods

Cells

CHO cells stably expressing $hNa_V1.7$ were supplied by Anaxon.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch®. Current-voltage recordings were made using voltage steps from -60 mV to 50 mV for 20 ms increasing in 10 mV steps, from a holding potential of -120 mV. Inactivation protocol used a 5 s pre-pulse to the voltage indicated (-120 mV to 30 mV in 10 mV increments) followed by a step to 0 mV for 10 ms, 20 s sweep interval. Pharmacology experiments used a voltage step protocol from -120 mV to 0 mV for 20 ms, then back to -120 mV, sweep interval 3 s (P/4 leak subtraction used).

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