Application Note

Channel: $hNa_V1.7$
Cells: CHO
Tools: Patchliner

Pharmacology on $hNa_V1.7$ performed on Nanion's Patchliner at Vhalf

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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 measure the effects of compounds at Vhalf of inactivation, the voltage at which 50% of the channels are inactivated.

In this Application Note we present data using an 8-channel Patchliner® characterizing CHO cells stably expressing $hNa_V1.7$. The $hNa_V1.7$ activation and inactivation properties are consistent with those reported in the literature[1,2,7,8]. The potency of sodium channel blockers mexiletine, tetracaine, amitriptyline and lidocaine were compared using a holding potential of -120 mV vs the Vhalf or inactivation.

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Results

Figure 1 shows the activation and inactivation curves for an average of 8 CHO cells expressing $hNa_V1.7$. $Na_V1.7$ currents started to activate at about -40 mV, peak response was elicited at around -10 mV and Vhalf of activation was -19 mV (n = 8). The Vhalf of inactivation was -74 mV (n = 8), in good agreement with the literature[1,2,7,8]. TTX was applied revealing an IC_{50} = 20 ± 9 nM (n = 6), in good agreement with the literature[2].

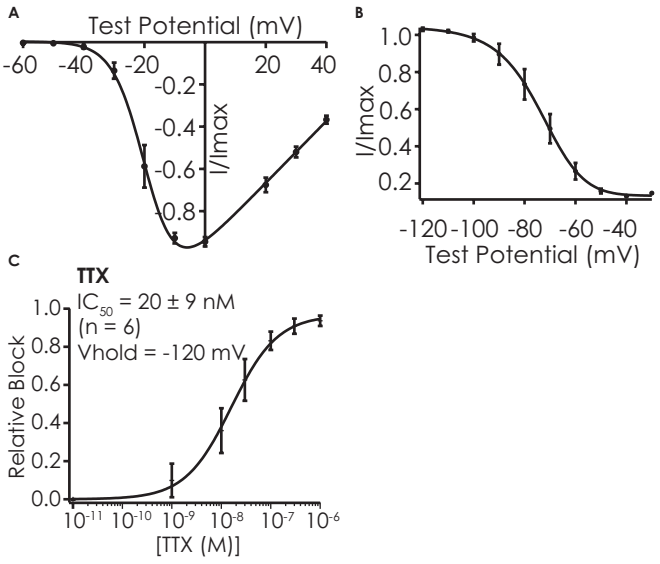

Figure 1:
A Average current-voltage plot of activation, Vhalf of activation was -19 mV (n = 8). **B** Average inactivation plot, Vhalf of inactivation was -74 mV (n = 8). **C** Concentration response curve for TTX (Vhold = -120 mV) reveals an IC_{50} = 20 ± 9 nM (n = 6) in good agreement with the literature[2].

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Figure 2 shows current responses to a voltage step protocol from -120 mV to 0 mV and inhibition of the $hNa_V1.7$ current by increasing concentrations of tetracaine. The timeplot of the experiment is also shown.

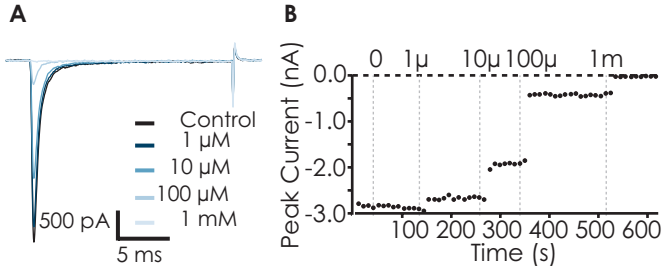

Figure 2:
A Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of $hNa_V1.7$ current by increasing concentrations of Tetracaine. Shown are current responses to a voltage step protocol from -120 mV to 0 mV for 20 ms. **B** Timeplot of the experiment.

The concentration response curves for 4 known Na_V channel blockers at different holding potentials are shown in Figure 3. The Vhalf was determined and set individually for each cell. The IC_{50}s for each compound are shown on each individual graph using two different holding potentials (Vhalf or -120 mV). In all cases, holding the cells at Vhalf caused a leftward shift of the concentration response curve.

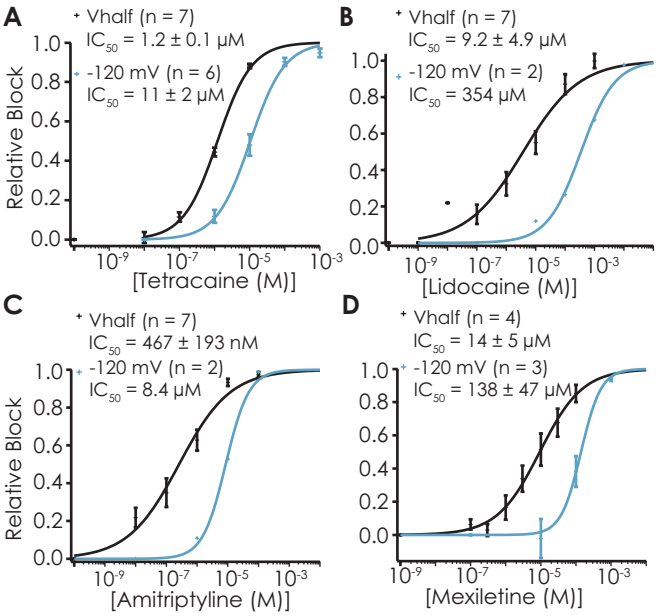

Figure 3:
Average concentration response curves for **A** Tetracaine, **B** Lidocaine **C** Amitriptyline, and **D** Mexiletine at Vhold = Vhalf (black) and Vhold = -120 mV (blue), IC_{50} values are shown on individual plots.

In conclusion, $hNa_V1.7$ expressed in CHO cells provided by Anaxon can be reliably recorded on the Patchliner® with activation and inactivation properties as expected[1,2,7,8] and compound potencies can be reliably measured at Vhalf.

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 Patchliner®. 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 or Vhalf (set individually for each cell) to 0 mV for 20 ms, then to -120 mV for 2 s then back to -120 mV or Vhalf, interval 10 s (P/4 leak subtraction used).

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