

Pharmacology on hNa_v1.7 performed on Nanion's Patchliner® at Vhalf

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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) neurons¹, 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 pain⁵ and hypersensitivity to heat following burn injury⁶. 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 of 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 peak responses (4.4 ± 0.8 nA @ Vhold = -120 mV, n = 8) were elicited at around -10 mV. 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 IC₅₀ = 20 ± 9 nM (n = 6), in good agreement with the literature².

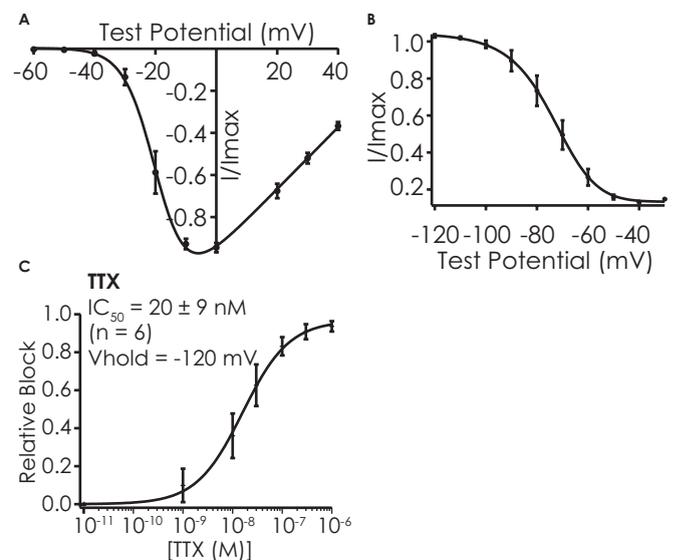


Figure 1:

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 IC₅₀ = 20 ± 9 nM (n = 6) in good agreement with the literature².

Application Note

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.

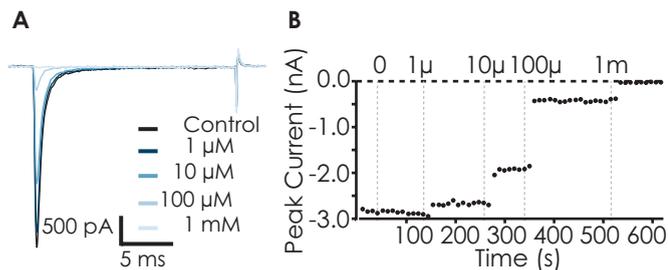


Figure 2: **A** Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of hNa_v1.7 current by increasing concentrations of Tetracaine. Shown are current responses to a voltage step protocol from -120 mV to 0 mV for 20 ms. **B** Timeplot of the experiment.

The concentration response curves for 4 known Na_v channel blockers at different holding potentials are shown in Figure 3. The V_{half} was determined and set individually for each cell. The IC₅₀s for each compound are shown on each individual graph using two different holding potentials (V_{half} or -120 mV). In all cases, holding the cells at V_{half} caused a leftward shift of the concentration response curve.

References

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6. Shields *et al.*, 2012. J. Neurosci. 32 (32): 10819-10832
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8. Cummins *et al.*, 2004. J. Neurosci. 24 (38): 8232-8236

Methods

Cells

CHO cells stably expressing hNa_v1.7 were supplied by Anaxon.

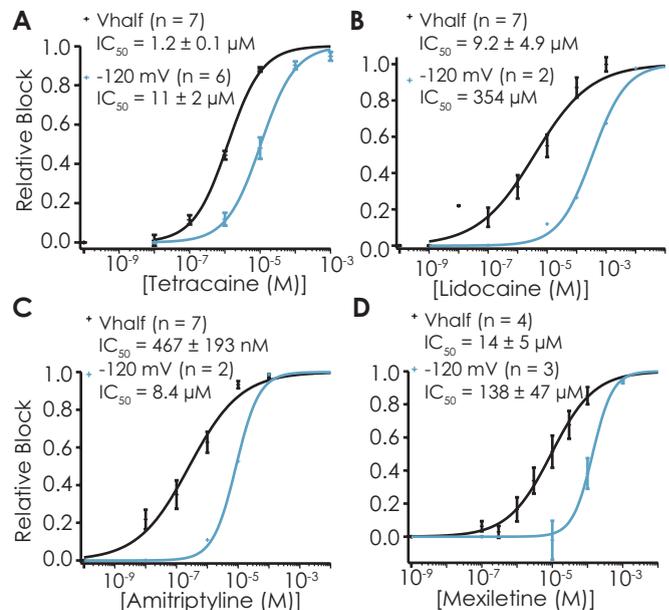


Figure 3: Average concentration response curves for **A** Tetracaine, **B** Lidocaine **C** Amitriptyline, and **D** Mexiletine at V_{hold} = V_{half} (black) and V_{hold} = -120 mV (blue), IC₅₀ values are shown on individual plots.

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}. Compound potencies can be reliably measured at V_{half}.

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