Pharmacology on hNa\textsubscript{v} 1.7 performed on Nanion’s Patchliner at V\textsubscript{half}

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Cells kindly provided by Anaxon.

Summary

The Na\textsubscript{v}1.7 gene (SCN9A) encodes a voltage-gated sodium (Na\textsubscript{v}) channel, primarily expressed in the peripheral nervous system and has been isolated from rat dorsal root ganglion (DRG) neurons\textsuperscript{1}, human medullary thyroid cancer cells (hNE-Na)\textsuperscript{2} and PC12 cells\textsuperscript{3,4}.

Different Na\textsubscript{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\textsubscript{v} channels. Na\textsubscript{v} channels are in part characterized by their TTX-sensitivity ([TTX-resistant [TTXr], TTX-sensitive [TTXs]]). Na\textsubscript{v}1.7 is a TTXs channel and is sensitive to TTX in the nanomolar range\textsuperscript{1,2}. The role of hNa\textsubscript{v}1.7 has yet to be fully elucidated but is proposed to play an important role in nociception and pain sensing. Na\textsubscript{v}1.7 has been implicated to play a role in disease pain states, in particular inflammatory pain\textsuperscript{5} and hypersensitivity to heat following burn injury\textsuperscript{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 V\textsubscript{half} of inactivation, the voltage at which 50% of the channels are inactivated.

In this Application Note we present data using an 8-channel Patchliner\textsuperscript{®} characterizing CHO cells stably expressing hNa\textsubscript{v}1.7. The hNa\textsubscript{v}1.7 activation and inactivation properties are consistent with those reported in the literature\textsuperscript{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 V\textsubscript{half} of inactivation.

Results

Figure 1 shows the activation and inactivation curves for an average of 8 CHO cells expressing hNa\textsubscript{v}1.7. Na\textsubscript{v}1.7 currents started to activate at about -40 mV, peak response was elicited at around -10 mV and V\textsubscript{half} of activation was -19 mV (n = 8). The V\textsubscript{half} of inactivation was -74 mV (n = 8), in good agreement with the literature\textsuperscript{1,2,7,8}. TTX was applied revealing an IC\textsubscript{50} = 20 ± 9 nM (n = 6), in good agreement with the literature\textsuperscript{2}.

Figure 1:

A Average current-voltage plot of activation, V\textsubscript{half} of activation was -19 mV (n = 8).
B Average inactivation plot, V\textsubscript{half} of inactivation was -74 mV (n = 8).
C Concentration response curve for TTX (V\textsubscript{hold} = -120 mV) reveals an IC\textsubscript{50} = 20 ± 9 nM (n = 6) in good agreement with the literature\textsuperscript{2}.
Figure 2 shows current responses to a voltage step protocol from -120 mV to 0 mV and inhibition of the hNaV1.7 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 hNaV1.7 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 NaV channel blockers at different holding potentials are shown in Figure 3. The Vhalf was determined and set individually for each cell. The IC50s 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.

In conclusion, hNaV1.7 expressed in CHO cells provided by Anaxon can be reliably recorded on the Patchliner® with activation and inactivation properties as expected1,2,7,8 and compound potencies can be reliably measured at Vhalf.

References
5. Nassar et al., 2004. PNAS. 101 (34): 12706-12711

Methods
Cells
CHO cells stably expressing hNaV1.7 were supplied by Anaxon.