Pharmacology on hNa\textsubscript{v}1.7 performed on Nanion’s Patchliner\textsuperscript{®} at Vhalf

The electrophysiology team at Nanion Technologies GmbH, Munich. 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 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\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 Vhalf of inactivation.

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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\textsubscript{V}1.7 current by increasing concentrations of tetracaine. The timeplot of the experiment is also shown.

![Figure 2: Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of hNa\textsubscript{V}1.7 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.](image)

Figure 2:
A Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of hNa\textsubscript{V}1.7 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\textsubscript{V} channel blockers at different holding potentials are shown in Figure 3. The V\textsubscript{half} was determined and set individually for each cell. The IC\textsubscript{50}s for each compound are shown on each individual graph using two different holding potentials (V\textsubscript{half} or -120 mV). In all cases, holding the cells at V\textsubscript{half} caused a leftward shift of the concentration response curve.

In conclusion, hNa\textsubscript{V}1.7 expressed in CHO cells provided by Anaxon can be reliably recorded on the Patchliner® with activation and inactivation properties as expected\textsuperscript{1,2,7,8}. Compound potencies can be reliably measured at V\textsubscript{half}.

### References

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

### Methods

#### Cells

CHO cells stably expressing hNa\textsubscript{V}1.7 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 V\textsubscript{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\textsubscript{half}, interval 10 s (P/4 leak subtraction used).