

Characterization of rNa_v1.8 (ND7-23) on Nanion's Patchliner®

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by Millipore.

Summary

The Na_v1.8 gene (originally named PN3 or SNS; gene symbol SCN10A) encodes a voltage-gated sodium (Na_v) channel, selectively expressed in dorsal root ganglion (DRG) neurons. DRGs transmit peripheral stimuli to the central nervous system and are involved in nociception.

Different Na_v channels play a key role in modulation of DRG action potentials. 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_v1.8 is a TTXr channel. Compared with other Na_v channels, Na_v1.8 has slow activation and inactivation kinetics and is opened at relatively high voltages¹. It is an interesting drug target for inflammatory and neuropathic pain, because modulation of Na_v1.8 by inflammatory mediators seems to be a key mechanism of DRG nociceptor sensitization and activation². Interestingly, Na_v1.8 has been reported to play an important role in the perception of cold pain³.

In this Application Note we present data recorded on the Patchliner® characterizing ND7-23 cells (a rat DRG/mouse neuroblastoma hybrid) stably transfected with rat Na_v1.8. All experiments were performed in the presence of 100 nM TTX to block the endogenous TTXs Na⁺ current present in these cells. The Na_v1.8 activation and inactivation properties and tetracaine sensitivity recorded on the Patchliner® were consistent with those reported in the literature¹⁻⁷.

Results

Figure 1 shows current responses to increasing voltage steps for an exemplar ND7-23 cell expressing Na_v1.8 and the corresponding activation and inactivation curves for an average of 19 cells. Na_v1.8 currents started to activate at about -40 mV, peak response was elicited at around 20 mV and V_{half} of activation was -9 mV (n = 19). The V_{half} of inactivation was -24 mV (n = 4), in good agreement with the literature^{5,6}.

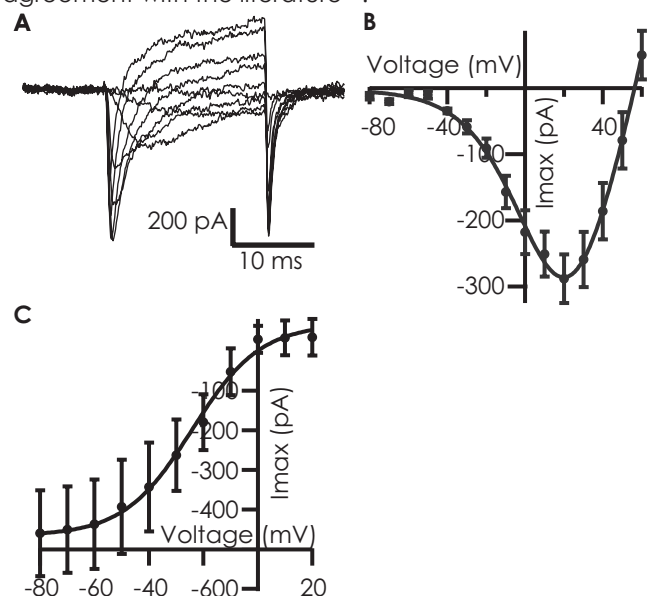


Figure 1:

A Raw traces from an exemplar cell recorded on the Patchliner®. Shown are current responses to increasing voltage steps from -80 to +60 mV. **B** Average current-voltage plot, V_{half} of activation was -9 mV (n = 19). **C** Average inactivation plot, V_{half} of inactivation was -24 mV (n = 4).

Application Note

Figure 2 shows current responses to a single step protocol to 20 mV and inhibition of the $\text{Na}_v1.8$ current by increasing concentrations of tetracaine. The timeplot of the experiment is also shown.

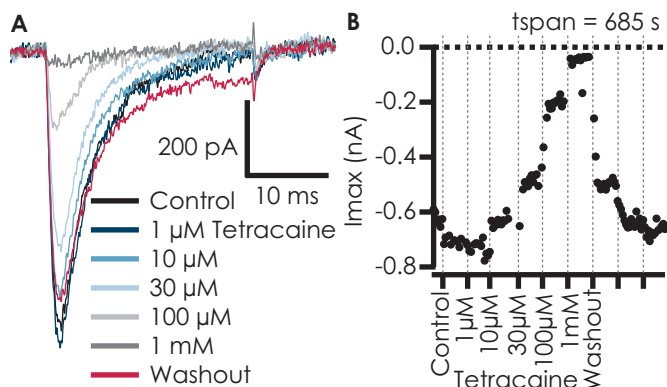


Figure 2: **A** Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of current by increasing concentrations of tetracaine. Shown are current responses to a single step protocol to 20 mV for 25 ms from a holding potential of -90 mV. Current amplitude was completely recovered upon washout of tetracaine (red trace). **B** Timeplot of the experiment.

The corresponding concentration response curve is shown in Figure 3 revealing an IC_{50} for tetracaine of $35 \pm 8 \mu\text{M}$ ($n = 3$) with a holding potential of -90 mV. This is in good agreement with the literature⁷.

References

1. Sangameswaran *et al.*, 1996. JBC. 271(11): 5953-5956
2. Porreca *et al.*, 1999. PNAS. 96: 7640-7644
3. Zimmermann *et al.*, 2007. Nature. 447: 856-859
4. Goldin AL., 2001. Annu. Rev. Physiol. 63: 871-894
5. Catterall *et al.*, 2005. Pharmacol. Rev. 57:397-409
6. Leffler *et al.*, 2007. JPET. 320:354-364
7. John *et al.*, 2004. Neuropharmacology. 46(3):425-38

Methods

Cells

ND7-23 cells stably expressing rat $\text{Na}_v1.8$ were supplied by Millipore.

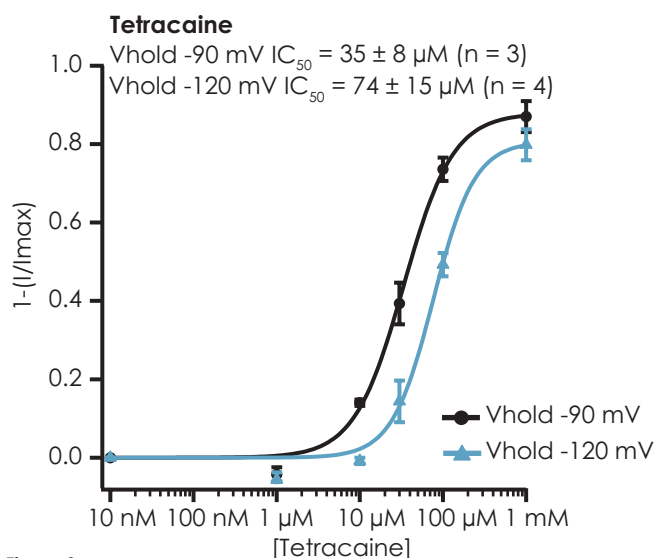


Figure 3: Average concentration response curve for tetracaine, $\text{IC}_{50} = 35 \pm 8 \mu\text{M}$ ($n = 3$) for $V_{\text{hold}} = -90 \text{ mV}$ and $74 \pm 15 \mu\text{M}$ ($n = 4$) for $V_{\text{hold}} = -120 \text{ mV}$.

The potency of tetracaine was affected by holding potential, becoming less potent with a more negative holding potential, $\text{IC}_{50} = 74 \pm 15 \mu\text{M}$ ($n = 4$) with a holding potential of -120 mV.

In conclusion, $\text{Na}_v1.8$ expressed in ND7-23 cells can be reliably recorded on the Patchliner® with activation and inactivation properties, and tetracaine sensitivity, in good agreement to those reported in the literature^{5,6,7}.

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®. All recordings were made in the presence of 100 nM TTX to block endogenous TTXs Na_v currents. Current-voltage recordings were made using voltage steps from -80 mV to 60 mV for 20 ms increasing in 10 mV steps, from a holding potential of -120 mV. Inactivation protocol used a 100 ms pre-pulse to the voltage indicated followed by a step to 20 mV for 25 ms. Pharmacology experiments used a single voltage step protocol to 20 mV for 25 ms from a holding potential of -90 mV or -120 mV (as indicated), repeated every 5 s.