Application Note

Channel: $rNa_V1.8$
Cells: ND7-23
Tools: Patchliner®

Characterization of $rNa_V1.8$ (ND7-23) on Nanion´s Patchliner®

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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 voltages[1]. 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 activation[2]. Interestingly, $Na_V1.8$ has been reported to play an important role in the perception of cold pain[3].

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 literature[1-7].

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 Vhalf of activation was -9 mV (n = 19). The Vhalf of inactivation was -24 mV (n = 4), in good agreement with the literature[5,6].

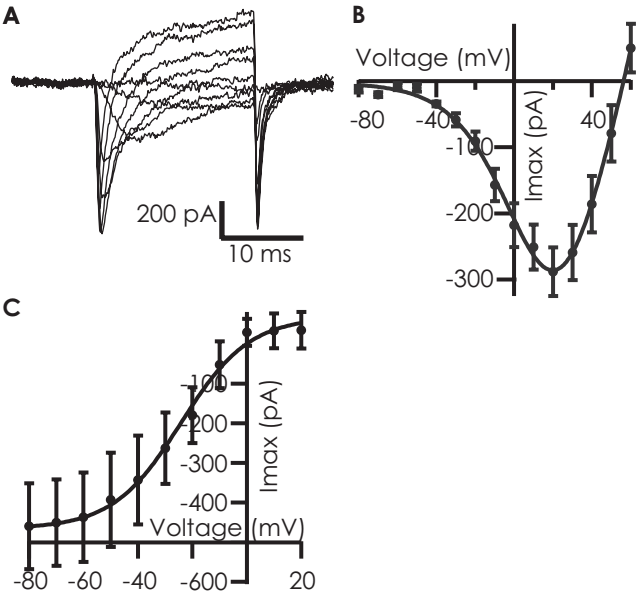

Figure 1:
A Raw traces from an exemplar cell recorded on the Patchliner®. Shown are current responses to increasing voltage steps from -80 to +60 mV. **B** Average current-voltage plot, Vhalf of activation was -9 mV (n = 19). **C** Average inactivation plot, Vhalf of inactivation was -24 mV (n = 4).

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Figure 2 shows current responses to a single step protocol to 20 mV and inhibition of the $Na_V1.8$ current by increasing concentrations of tetracaine. The timeplot of the experiment is also shown.

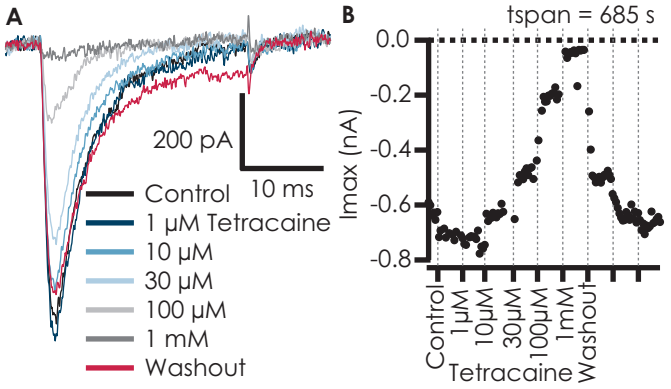

Figure 2:
A Raw traces from an exemplar cell recorded on the Patchliner® showing inhibition of current by increasing concentrations of tetracaine. Shown are current responses to a single step protocol to 20 mV for 25 ms from a holding potential of -90 mV. Current amplitude was completely recovered upon 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 ± 8 µM (n = 3) with a holding potential of -90 mV. This is in good agreement with the literature[7].

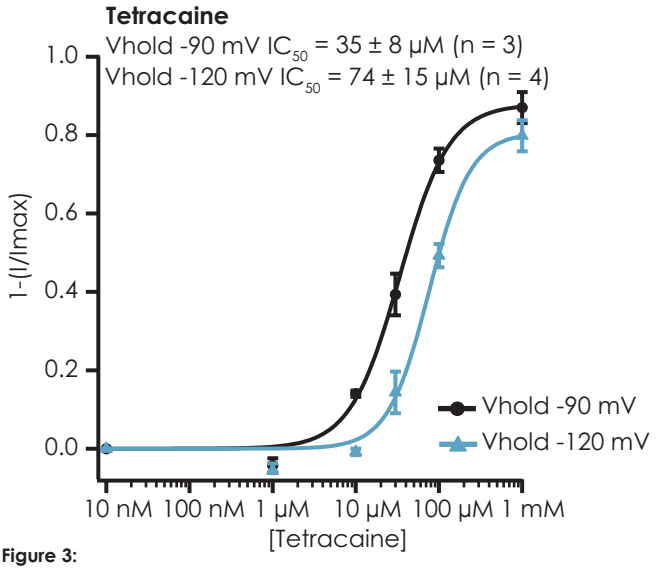

Figure 3:
Average concentration response curve for tetracaine, IC_{50} = 35 ± 8 µM (n = 3) for Vhold - 90 mV and 74 ± 15 µM (n = 4) for Vhold - 120 mV.

The potency of tetracaine was affected by holding potential, becoming less potent with a more negative holding potential, IC_{50} = 74 ± 15 µM (n = 4) with a holding potential of -120 mV.

In conclusion, $Na_V1.8$ expressed in ND7-23 cells can be reliably recorded on the Patchliner® with activation and inactivation properties, and tetracaine senstitivity, in good agreement to those reported in the literature[5,6,7].

References

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Methods

Cells

ND7-23 cells stably expressing rat $Na_V1.8$ were supplied by Millipore.

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.

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