Application Note

Channel: $hNa_V1.5$
Cells: HEK293
Tools: Patchliner®

Pharmacology of $hNa_V1.5$ recorded on Nanion´s Patchliner®

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Cells were kindly provided by EMD Millipore, USA.

Summary

Voltage gated sodium channels (Na_V) are important elements of action potential initiation and propagation in excitable cells. The channels are activated upon a depolarization of the membrane. Their activation leads to further depolarization of the membrane which constitutes the upstroke of the action potential.

Na_V currents generally activate very fast (within 1-2 ms) upon depolarization of the membrane. Hence, a good and stable access resistance is critical for high quality pharmacological patch clamp recordings. Also, for automated patch clamp devices, it is not a given that applied compound concentrations are accurately delivered to the cell. This is a pre-requisite for accurately reproducible dose-response curves.

Here we present data collected on the 8-channel Patchliner®. Tetrodotoxin and lidocaine dose-response curves on $hNa_V1.5$ expressed in HEK293 cells are shown. Lidocaine has been shown to block $hNa_V1.5$ in its inactivated state (Bean *et al.* 1983) which means that the IC_{50} of lidocaine becomes dependent on the holding potential. This dependence was investigated.

We also demonstrate the stability and reproducibility of the data collected with the Patchliner®. Using two sequential dose responses of $hNa_V1.5$ to TTX we demonstrate that the compound concentrations are accurately delivered to the cells and that recordings are stable with robust access resistance.

Results

Current responses of an individual cell to 20 ms voltage pulses (-40 mV) in the presence of TTX concentrations as indicated are shown in Figure 1. A single application of external solution (Figure 1, washout) led to full recovery of the peak control current.

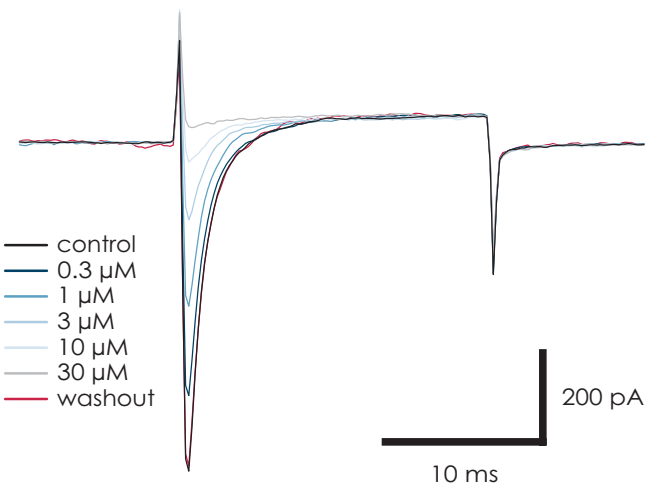

Figure 1:
TTX dose-response curve on an individual cell.

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Figure 2 shows the time-course of the peak Na^+ current during a single experiment. Two full TTX dose responses, including washout, were performed on a single cell. The reduction in peak currents at the different TTX concentrations are highly reproducible indicating accurate compound application. Washes were performed twice after application of the highest TTX concentration. Full recovery of the peak current after the first wash is also obvious from the trace. Washing a second time did not lead to a significant change in peak current. This observation is also an indication that solution changes are close to complete.

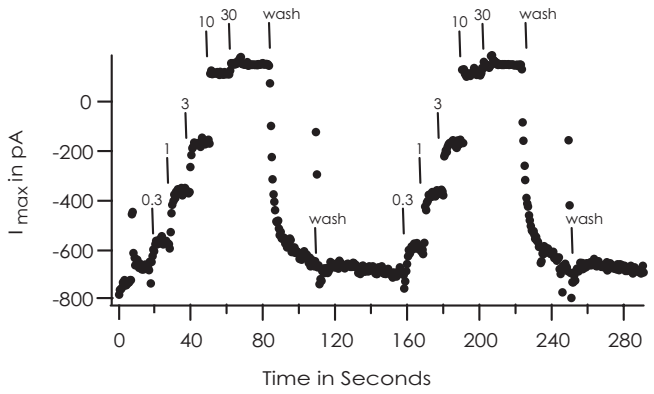

Figure 2:
Time-course of the peak Na current in a single experiment. TTX was applied and washed out as indicated by the arrows. Concentrations shown are given in µM.

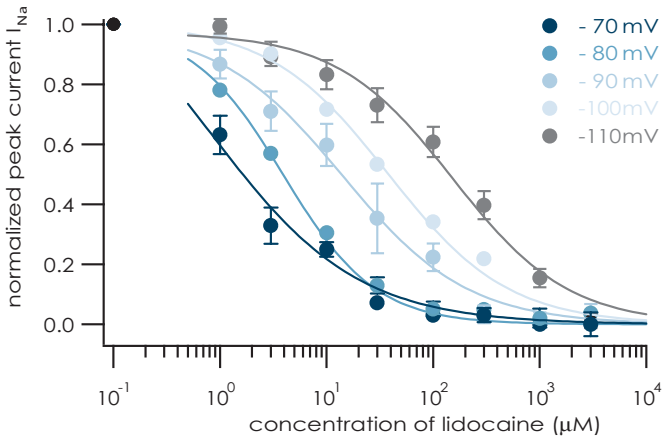

Figure 3:
The IC_{50} of lidocaine depends on the holding potential. Average IC_{50}s of 1.7 µM (-70 mV), 4.0 µM (-80 mV), 21.7 µM (-90 mV), 37.8 µM (-100 mV), and 194.6 µM (-110 mV) were obtained.

Full lidocaine dose-response curves (in µM: 1, 3, 10, 30, 100, 300, 1000, 3000) at all holding potentials (in mV: -70, -80, -90, -100, -110) were obtained on all cells. The IC_{50} of 195 µM at the holding potential of -110 mV is in good agreement with 353 µM at -120 mV determined by Bean *et al.* (1983).

In summary, whole cell recordings on the Patchliner® are stable so that long lasting, high quality recordings can be obtained. This, in combination with precise compound application, ensures reliable, reproducible dose-response curves.

References

1. Bean, B.P., Cohen, C.S., and Tsien, R.W. 1983. Lidocaine Block of Cardiac Sodium Channels. J.Gen. Physiol. 81: 613 - 642.

Methods

Cells

PrecisION hNav1.5-HEK recombinant cell line (CYL3004) were kindly supplied by EMD Millipore, USA.

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®. In the tetrodotoxin (TTX) experiments, currents were elicited every 1 s by 20 ms voltage steps to -40 mV from the holding potential of -90 mV. In the lidocaine experiments, cells were held at the potentials as indicated. Currents were elicited by 10 ms steps to 0 mV every 2 s.

Both TTX and lidocaine were diluted in external solution at the indicated concentrations.

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