

Pharmacology of hNa_v1.5 recorded on Nanion's Patchliner®

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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 activate generally very fast (within 1-2 ms) upon depolarization of the membrane. Hence is a good and stable access resistance critical for high quality pharmacological patch clamp recordings. Also, for automated patch clamp robots it is not a given that applied compound concentrations are accurately delivered to the cell which 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 IC₅₀ of lidocaine becomes dependent on the holding potential. This dependence was investigated.

Here we demonstrate the stability and reproducibility of the data collected with the Patchliner®. It demonstrates that 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.

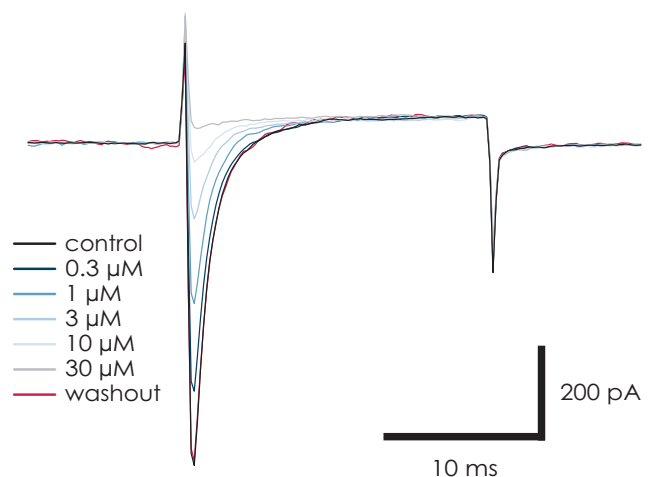


Figure 1:
TTX dose-response curve on an individual cell.

Application Note

Figure 2 shows the time course of the peak Na⁺ current during a single experiment. Two full TTX dose responses including wash out are performed on a single cell. The reduction in peak currents at the different TTX concentrations are highly reproducible indicating accurated compound application. Washes were performed twice after the highest TTX concentration has been applied. 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 .

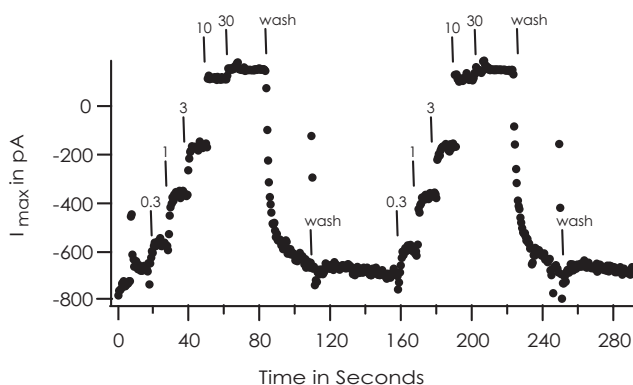


Figure 2: Time course of the peak Na current in a single experiment. TTX was applied and washed out as indicated by the arrows. Concentrations shown are given in mM.

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

HEK293 cells stably expressing Na_v1.5 were used.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocol.

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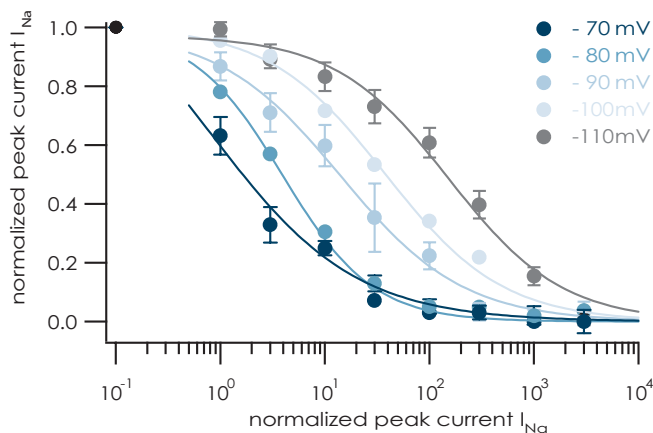


Figure 3: The IC₅₀ of lidocaine depends on the holding potential. Average IC₅₀s of 1.7 mM (-70 mV), 4.0 mM (-80 mV), 21.7 mM (-90 mV), 37.8 mM (-100 mV), and 194.6 mM (-110 mV) were obtained.

Full lidocaine dose-response curves (in mM: 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 IC₅₀ of 195 mM at the holding potential of -110 mV is in good agreement with 353 mM at -120 mV determined by Bean *et al.* (1983).

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

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Patchliner®. Currents were elicited in case of the tetrodotoxin (TTX) experiments every second by 20 ms voltage steps from the holding potential of -90 mV to -40 mV. In case of 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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