Investigating pain pathways by inhibition of voltage-gated sodium channels

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Abstract

Voltage-gated sodium channels (hNa) are attractive targets for investigation of chronic and neuropathic pain, due to their physiological role in action potential generation and propagation and, thus, neuronal excitability. The hNa1.7 is found primarily in the peripheral sensory system and is thought to play a role in nociception and pain sensing. The TX-resistant hNa1.8 is selectively expressed in dorsal root ganglion (DRG) neurons. We present recordings of hNa1.7 and hNa1.8 channels on a high throughput screening patch clamp platform.

hNa1.7 was expressed in CHO cells and the current-voltage relationship recorded, both consistent with hNa1.7 data obtained using other methods. hNa1.7 activation was 20 mV (Vr=275). Using a double-step voltage protocol we were able to investigate whether compounds, such as tetrodotoxin, exerted state dependence. We show that tetrodotoxin exhibited lower IC50 on the second pulse, i.e., the inactivated state of the receptor, compared with the resting state. hNa1.8 expressed in CHO cells started to activate at approximately -40 mV, peaking at between 10 mV and 20 mV with a hNa1.8 activation of 2.7 mV (Vr=338). In order to study hNa1.8 involved in pain pathways in a more physiological environment, we used stem cell-derived neurons, more specifically with an overexpression of hNa1.8. In these cells, endogenous hNa1.8-mediated currents were recorded with an action potential amplitude consistent with hNa1.7.

Our results demonstrate that hNa1.8 channels can be successfully studied on high throughput electrophysiological systems, facilitating the discovery of novel pain therapeutics.

State-Dependence of Tetracaine on hNa1,7 Channels

Current-voltage (IV) plot for hNa1,7 recorded on the SyncroPatch 384E showing current activation from a single cell line as shown.

-120 to 2 mV

Screenshot of the PatchControl 384E software showing an experiment using CHO cells expressing hNa1,7. hNa1,7 current amplitudes were higher for the second peak (black trace) than for the first peak (blue trace) and the second peak (black trace) is shifted again to the right. For control solution without tetrodotoxin was added (white), and then increasing concentrations of tetrodotoxin is different shades of blue (cumulative concentration-response). Bar graph showing mean ± SEM of the peak current amplitude on the resting state (left panel) and on the first (middle panel) and on the second (right panel) pulses for hNa1,7 and for the two seconds (right panel). Data from Obergrossberger et al., 2016. (A) 2,1.77 -793.

Effect of Protoxin II on hNa1,7 channels

Protoxin II reduces both tetrodotoxin-sensitive and -resistant voltage-gated sodium channels, efficiently reducing the current. hNa1,7 channel was sensitive to this action, whereas hNa1,8 channel was not reduced in SyncroPatch 384E. Currents were evoked by depolarizing pulses from holding potential of -100 mV to step potentials varying from -40 mV to 150 mV. The IC50 value for current inhibition by Protoxin II was 1.4 µM.

hNa1,8 Current-Voltage (IV) Relationship Recorded with Multihole Substrate

Compound Affinity and Assay Stability for the Slow Inactivated State of hNa1,8 Channels

High Throughput Automated Patch Clamp of hNa1,9

hNa1,9 is TXR-INSENSITIVE

Incubation at 32°C Increases Channel Expression

Concentration-Dependent Block of hNa1,9 by Lidocaine

The IC50 matches the literature value of 238µM exactly.

10% Success Rate

QCs: R >100 MΩ– Ω

Study course of current levels from recording on the left, applying the two state pulse protocol. A 5 point CFC curve of tetrodotoxin was measured at 0, 5, 7, 9 and 12.5 µM. Right panel shows a concentration of 1 µM TTX, followed by a block of lidocaine (full block).

hNa1,9 Current-Voltage (IV) Relationship Recorded with Multihole Substrate

Current response to a voltage step protocol of Cell Neurons (left) and the corresponding IV plot for an average of 54 cells (right). Vr = -40 mV when calculated with a Boltzmann resolution.

Stem Cell-Derived Neurons Recorded on the Patchliner

Adherent Cell Neurons [left] and a suspension for use on the Patchliner (right).

Current response to a voltage step protocol of Cell Neurons (left) and the corresponding IV plot for an average of 54 cells (right). Vr = -40 mV when calculated with a Boltzmann resolution.

Data from hippenmeyer et al., 2012. (A) 2,15.9 12.4 2 1057.

Data from hippenmeyer et al., 2012. (B) 2,17.9 12.4 2 1057.

Current responses to a voltage step protocol of Ompataputin neurons (left) and the corresponding IV plot for an average of 54 cells (right). Vr = -30 mV when calculated with a Boltzmann resolution.

Action potential elicited using a 1 ms current pulse of 80 µA. Action potentials were elicited by 10 µA steps and returned upon washout.

Data from Feix et al., 2007. (A) 2,19.9 12.4 2 1067.

Data from Feix et al., 2007. (B) 2,19.9 12.4 2 1067.

Summary

hNa1,7, 1.8 and 1.9 were recorded on the SyncroPatch 384E with high success rates for rFast, rCurrent and composite currents.

Current-voltage relationships and values for hNa1,7 and 1.9 were obtained using a variety of patch clamp protocols and presented in the literature.

The IC50 values for tetrodotoxin and lidocaine were as expected. Tetrodotoxin exerted state dependence on hNa,7 and 1,8.

Stem cell-derived neurons were used on the automated patch clamp device. The Patchliner, where TXR-sensitive hNa-mediated currents were recorded. In current clamp mode, action potentials were abolished by lidocaine.