

Validation of Na_v1.7 sodium channel function in iCell[®] human induced pluripotent stem cell-derived sensory neurons

Introduction

Voltage-gated sodium channels of the subtype Na_v1.7 (SCN9A) are essential for initiation and propagation of pain signals in the peripheral nervous system¹. Loss of function mutations in the *SCN9A* gene cause congenital insensitivity to pain², while gain of function mutations that increase Na_v1.7 activity cause severe pain syndromes (e.g. primary erythromelgia or paroxysmal extreme pain disorder)³. In nociceptive dorsal root ganglia (DRG) neurons, Na_v1.7 channels are activated by small membrane depolarizations and facilitate the generation of action potentials. Na_v1.7 is therefore a key target for the development of novel, non-opioid painkillers.

Human induced pluripotent stem cell-derived sensory neurons (hiPSC-SNs) are increasingly being utilized in biopharma for pain drug discovery, because of their biological-relevance, high purity, and accessibility – mitigating the limited availability of human DRG neurons. While these attributes position hiPSC-SNs as the next-generation in vitro pain model, continued verification of sensory function and pharmacology is needed.

Here, we present patch clamp data demonstrating the presence and inhibition of functional Na_v1.7 in hiPSC-derived sensory neurons using iCell Sensory Neurons (iCell-SNs) from FUJIFILM Cellular Dynamics. These data verify the biological relevance and utility of patch clamp recording and iCell-SNs for non-opioid drug discovery and compound screening.

Results

Na_v1.7 belongs to the group of tetrodotoxin- (TTX-) sensitive sodium channels⁴. Fig. 1 illustrates inhibition of the sodium current by 0.5 μM TTX. Current inhibition occurred rapidly and could be reversed by subsequent application of control solution (data not shown).

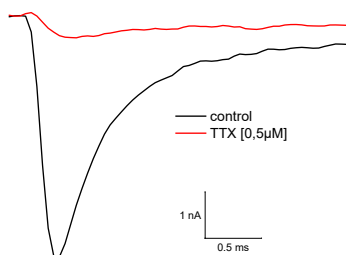


Figure 1: Representative inward sodium currents in iCell Sensory Neurons recorded under control conditions (vehicle) and in the presence of 0.5 μM TTX (voltage protocol 1, see Methods).

More evidence for the expression of Na_v1.7 was obtained by using selective inhibitors. Figs. 2, and 3 illustrate concentration-dependent inhibition of the inward sodium currents in iCell-SNs by the selective Na_v1.7 inhibitors GDC-0276 and GDC-0310⁵.

In the first series of experiments, current inhibition in the neurons was studied at a holding potential of -100 mV, at which the channels are primarily in a resting closed state. After a stabilization phase of 5 minutes, where the inward current amplitudes reached a steady state, the peak current amplitudes were estimated under control conditions (vehicle) and after a 3-minute incubation with GDC-0276 and GDC-0310.

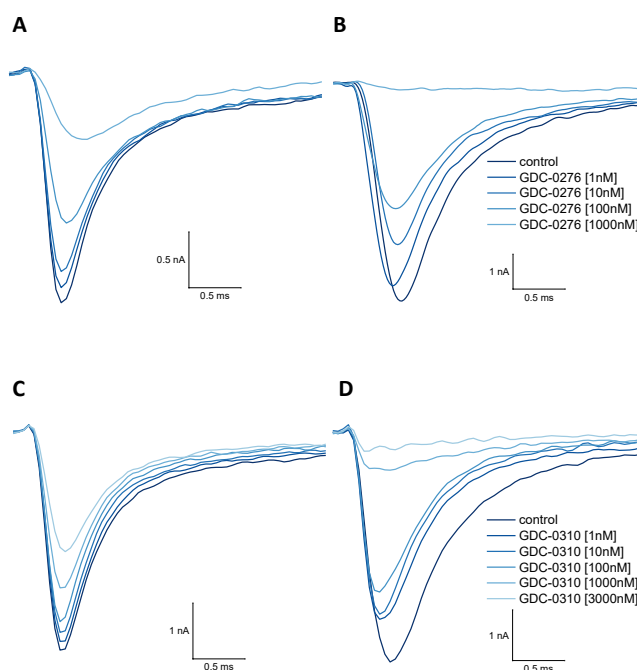


Figure 2: Representative inward sodium currents in iCell Sensory Neurons recorded under control conditions (vehicle) and in the presence of increasing concentrations of the selective Na_v1.7 inhibitors GDC-0276 (A-B) and GDC-0310 (C-D). Current traces on the left were obtained using voltage protocol 1 (see Methods) to study resting-state inhibition, and the traces on the right using voltage protocol 2 (see Methods) to study inactivated-state inhibition.

Application Note

The corresponding concentration – response curves are illustrated in Fig. 3A, and B. Substantially stronger current inhibition was observed when the test pulses were administered after a 5.5-second conditioning pulse (protocol 2). The conditioning depolarization to -40 mV induced 45% inactivation. Under these conditions, 1 μM of GDC-0276 and 3 μM GDC-0310 induced near-complete inhibition of the inward current (Fig.3, red traces), compared to only 59% (GDC-0276) and 48% (GDC-0310) resting state block, respectively (Fig.3, black traces), in iCell-SNs.

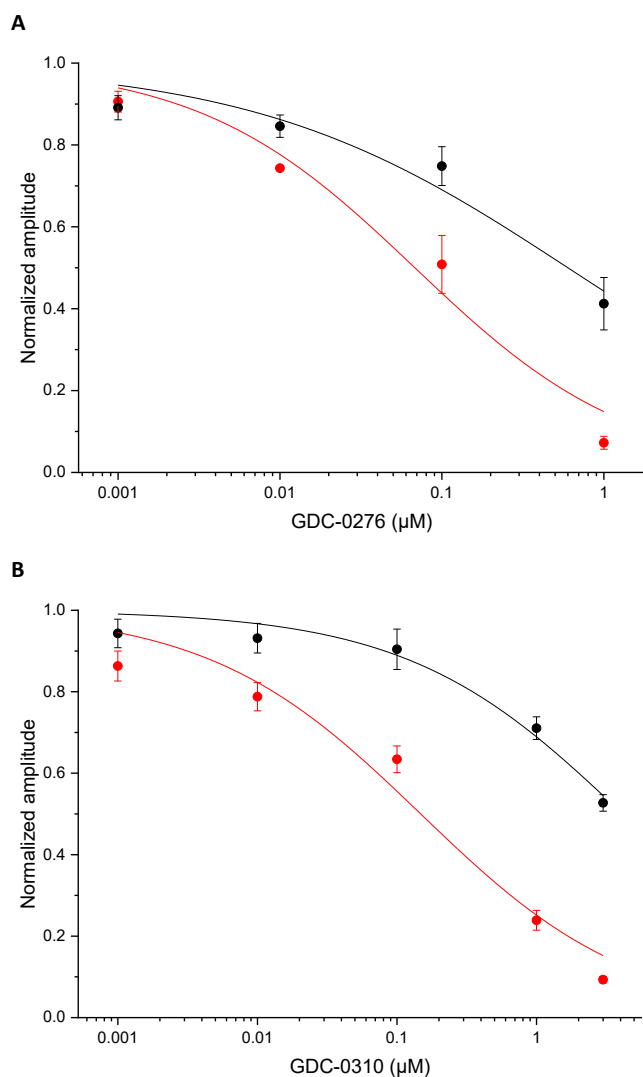


Figure 3: Concentration–response curves for the inhibitory effects of GDC-0276 and GDC-0310 **(A)** GDC-0276 exhibited IC_{50} s of $0.068 \pm 0.025 \mu\text{M}$ ($n = 3$) and $0.600 \pm 0.272 \mu\text{M}$ ($n=3$) for inhibition of the inactivated (red) and resting (black) states of the channel respectively. **(B)** GDC-0310 exhibited IC_{50} s of $0.148 \pm 0.054 \mu\text{M}$ ($n=5$) and $4.147 \pm 1.185 \mu\text{M}$ ($n=4$) for inhibition of the inactivated (red) and resting (black) states of the channel.

Summary

We present several lines of evidence supporting $\text{Na}_v1.7$ expression in iCell Sensory Neurons. Sodium inward currents were reversibly inhibited by TTX as well as by the selective $\text{Na}_v1.7$ inhibitors GDC-0276 and GDC-0310. Furthermore, inhibition was substantially enhanced when a conditioning (inactivating) pulse preceded the test pulse. This is in line with previous studies in $\text{Na}_v1.7$ expressing HEK cell line indicating a high affinity interaction of both compounds with the inactivated conformation of $\text{Na}_v1.7$ channel⁵. Our data suggest that iCell Sensory Neurons represent a valuable model for studies on $\text{Na}_v1.7$.

Methods

Cells

Human induced pluripotent stem cell derived sensory neurons (iCell Sensory Neurons, 21527; Catalog # R1252) were obtained from FUJIFILM Cellular Dynamics, <https://www.fujifilmcdi.com>.

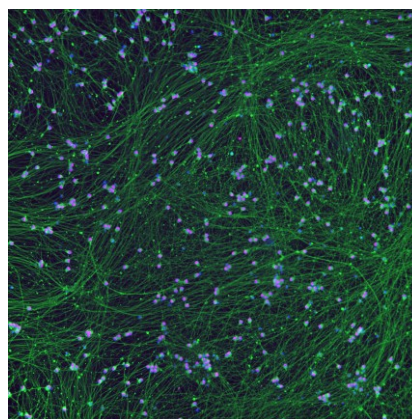


Figure 4: Representative image of iCell Sensory Neurons at 21 days in culture. Cells express Beta-III Tubulin (green), BRN3A (magenta), and DAPI (blue). Image provided by FUJIFILM Cellular Dynamics.

Cell Culture

Cells were cultured according to recommended FUJIFILM iCell Sensory Neurons User's Guide and used 14-21 days post-thawing the frozen vials.

Application Note

Electrophysiology

Inhibition of sodium currents by GDC-0276 and GDC-0310 in iCell-SNs was studied using two voltage protocols. With protocol 1, the sodium channels sojourn predominantly in the resting state while a depolarising conditioning pulse applied during protocol 2 enabled drug interaction with channels in the inactivated state (Fig. 5A, and B).

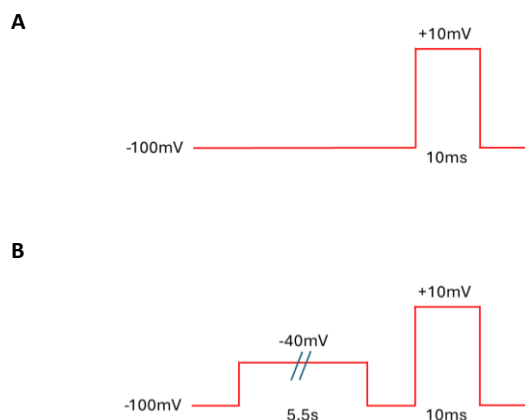


Figure 5: Applied voltage-clamp protocols. **(A)** Protocol 1 for studying resting state inhibition: iCell-SNs were depolarised for 10 ms from a holding potential of -100 mV to test potential of +10 mV. **(B)** Protocol 2 for studying the role of inactivation: A 5.5-s conditioning voltage step to -40 mV and a subsequent 10 ms step to -100 mV preceded the test pulse to +10 mV. During protocol 1 the channels sojourn predominantly in the resting channel conformation while drug interaction with the inactivated state was enabled by a conditioning depolarising pulse.

Prior to recording, cells were incubated with test compounds for 3 minutes. All recordings were conducted in an external solution containing TEA-Cl to block voltage-gated potassium channels.

Analysis

Maximum peak current amplitudes were quantified, normalized to vehicle control, and used to construct the concentration-response curves.

References

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