Evaluation of hNa$_V$1.9 Screening Cascade for Analgesic Drug Discovery

Thomas D. M. Hill, Graham D. Smith, Katie L. Puddefoot, Sergiy Tokar, Alexander S. Haworth, Catherine M. Hodgson, Ayesha Jinat, Anthony M. Rush, Edward B. Stevens, Gary S. Clark

Metrion Biosciences Ltd, Building 2, Granta Centre, Granta Park, Cambridge, CB21 6AL, UK

Introduction

Encoded by the *SCN11A* gene, Na$_V$1.9 is a voltage-gated sodium (Na$_V$) channel highly expressed in trigeminal ganglion neurons and small-diameter nociceptors in the dorsal root ganglion. Na$_V$1.9 acts as a threshold channel with a lower activation threshold, slower biophysical properties and a large window current compared to the other Na$_V$ isoforms[1]. These characteristics are important for its role in the regulation of neuronal excitability and the modulation of inflammatory and neuropathic pain. Clinically, Na$_V$1.9 dysfunction has been implicated in altered pain perception in humans (Figure 1), evidencing its potential as a non-opioid pain target[2-4].

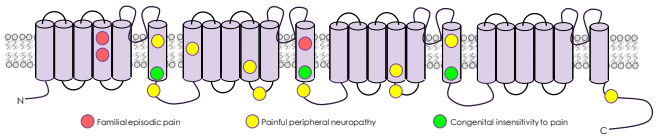


Figure 1 – Schematic of pain-related mutations in hNa$_V$1.9 adapted from Kabata *et al.*[5]

High-throughput Na$_V$1.9 drug discovery programmes have been hindered to date by the lack of the cellular tools and screening assays. Hence, the generation of a robust Nav1.9 screening cascade would greatly accelerate the development of selective Na$_V$1.9 modulators without the side-effects associated with current pain treatment options.

Methods

- **Cell culture** - A stably-expressing monoclonal CHO-hNa$_V$1.9 cell line was generated in-house.
- **Manual patch clamp electrophysiology** - CHO-hNa$_V$1.9 cells were stimulated from -100 mV to +50 mV (50 ms, 10 mV steps) from a holding potential of -140 mV, at 0.05 Hz
- **Automated patch clamp electrophysiology** - For compound screening, cells stepped to -40 mV (50 ms) from -140 mV (0.05 Hz). IV stimulation was the same as manual patch, except 100 ms steps. Recordings were made using multi-hole on a Qube 384 (Sophion Bioscience).

Results

1. hNa$_V$1.9 properties recorded using manual patch clamp

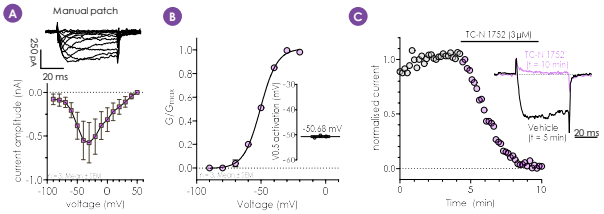


Figure 1 - Representative current traces and IV analysis of hNa$_V$1.9, recorded using the whole-cell manual patch clamp technique (A). Conductance/voltage plot for hNa$_V$1.9 (B). hNa$_V$1.9 currents were inhibited using the Na$_V$ channel blocker, TC-N 1752, at 3 µM (C).

2. Biophysical assessment of hNa$_V$1.9 using automated patch clamp technology

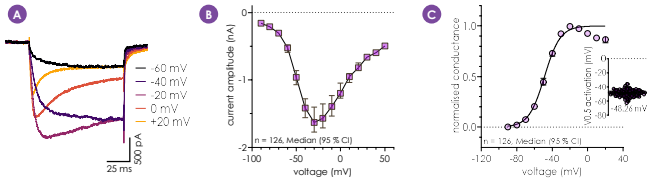
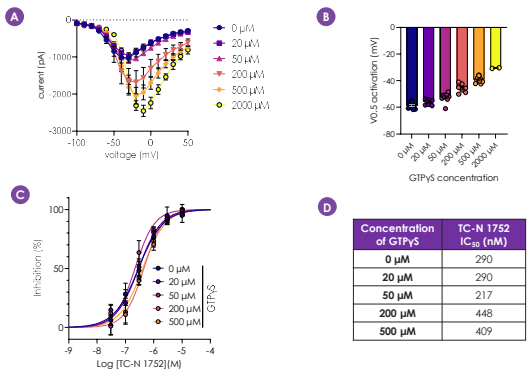


Figure 2 - Na$_V$1.9 channels have distinct biophysical properties compared to the other Na$_V$ isoforms[1] (A). The IV relationship (B) and conductance (C) of hNa$_V$1.9 currents recorded from 126 Qube 384 multi-hole wells were consistent with the known characteristics of native hNa$_V$1.9 and data obtained using the manual patch clamp technique.

3. Effects of GTPγS on hNa$_V$1.9 on biophysics/pharmacology



D

Concentration of GTPγS	TC-N 1752 IC$_{50}$ (nM)
0 µM	290
20 µM	290
50 µM	217
200 µM	448
500 µM	409

Figure 3 - Enhanced G-protein signalling has been shown to potentiate Na$_V$1.9 current amplitudes[6]. Addition of up to 500 µM intracellular GTPγS resulted in larger hNa$_V$1.9 currents, with a depolarising shift V$_{0.5}$ of activation (A,B). Importantly, GTPγS concentration did not alter hNa$_V$1.9 pharmacology (C,D). A concentration of 200 µM was used for routine screening.

4. Pharmacological assessment of hNa$_V$1.9 using automated patch clamp

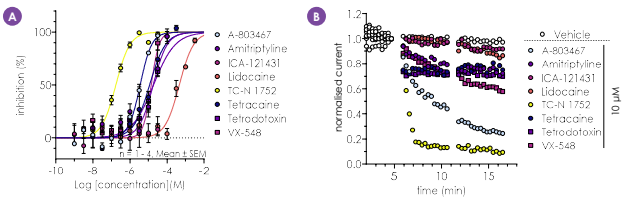


Figure 4 - A Qube 384 assay validated using a selection of Na$_V$ inhibitors with a range of potencies and isoform selectivity against hNa$_V$1.9. Calculated IC$_{50}$ values (µM): A-803467 – 3.5, Amitriptyline – 18, ICA-121431 >30, Lidocaine – 460, TC-N 1752 – 0.2, Tetracaine – 11.5, TTX – 18.7, VX-548 – 14.1 (A). Representative I-t plots of vehicle or compound (at 10 µM) are shown in B.

5. Blinded assessment of hNa$_V$1.9 pharmacology using spiked plated approach

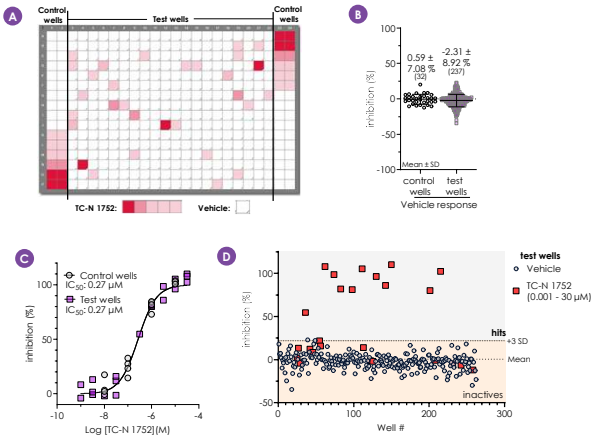


Figure 5 - The robustness of the Qube 384 assay was further validated by assessing the potency of TC-N 1752, using a randomised spiked plate approach (plate map - A). Vehicle response and TC-N 1752 potency correlated well between control and test wells (B, C). In test wells, the vehicle response displayed low variability with the TC-N 1752 response (at >0.1 µM) easily discernible above the mean vehicle response + 3 SD threshold (D).

6. Na$_V$1.9 screening cascade

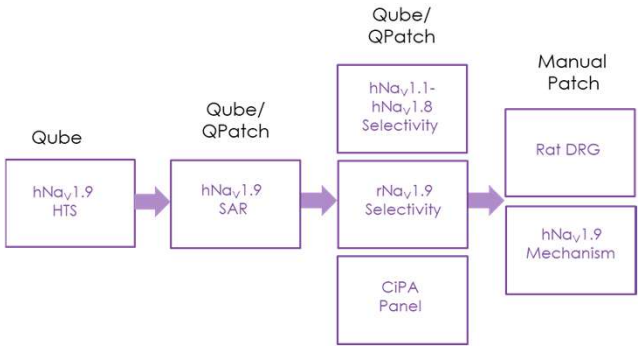


Figure 6 – The Nav1.9 screening cascade consists of: an HTS assay using Qube; SAR support using Qube or QPatch; Nav isoform selectivity, rat Nav1.9 selectivity, and cardiac channel liability (CiPA) using Qube or QPatch; and validation against endogenous rat Nav1.9, as well as mechanism of action studies for compound inhibition using manual patch clamp

Conclusions

- hNa$_V$1.9 biophysics from this cell line match the characteristics of native hNa$_V$1.9
- A robust screen sequence has been developed based around hNa$_V$1.9 Qube 384 automated patch clamp assay to accelerate the development of selective Na$_V$1.9 modulators for utility in the treatment of pain

References

1. Dib-Hajj S. *et al.* Trends Neurosci. 2002;25(5):253-259
2. Leipold E. *et al.* Nat. Genet. 2013;45(11):1399-1404
3. Zhang X. Y. *et al.* Am. J. Hum. Genet. 2013;93(5):957-966
4. Huang J. *et al.* Am. J. Hum. Genet. 2014;137(6):1627-1642
5. Kabata R. *et al.* PLoS One 2018;13(12):e0208516
6. Vanoye C. G. *et al.* J. Gen. Physiol. 2013;141(2):193-202