BIOPHYSICAL AND PHARMACOLOGICAL CHARACTERISATION OF NATIVE HUMAN $Na_V1.8$ CHANNELS FROM ISOLATED DORSAL ROOT GANGLIA (DRG).

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Introduction

The Nav1.8 TTX resistant (TTX-r) voltage-gated sodium channel is expressed exclusively in the nociceptor sub-population of primary afferent neurones and has slow inactivation kinetics and rapid recovery from inactivation (Cummins & Waxman 1997; Akopian *et al.,* 1996). These biophysical properties mean that Nav1.8 contributes to both electrogenesis and the maintenance of repetitive firing of action potentials (Blair & Bean 2002; Renganathan *et al.,* 2001; Waxman *et al.,* 2001). The expression and biophysical properties of Nav1.8 can be modulated by pro inflammatory mediators and findings in the literature support a key role for Nav1.8 in pain signalling (England *et al.,* 1996; Roza *et al.,* 2003; Kerr *et al.,* 2001; Coward *et al.,* 2000; Akopian *et al.,* 1999). Although the biophysical properties and pharmacology of native Nav1.8 currents has been investigated extensively in rodent DRGs, there is little reported data for human DRGs. Here we have characterised Nav1.8 current from TTX-r channels in human DRG and compared the biophysical properties with those of recombinantly expressed hNav1.8 channels. This work shows that the properties of the recombinantly expressed hNav1.8 channels closely match those of native TTX-r channels recorded from human DRG and that Nav1.8 has a functional role in these neurones.

Materials and Methods

Electrophysiological recordings were made in voltage clamp mode from HEK cells expressing human recombinant Nav1.8+β1 and in also from human DRGs within 12-24 hours of isolation, containing native TTX-r channels. For the latter, recordings were also made in current clamp mode. The biophysical properties and pharmacology of the channels was determined using a variety of protocols and commercially available compounds. In order to eliminate contamination by Nav1.9 currents recordings from human DRG were made from a holding potential of -80 mV at which there is little Nav1.9 conductance available for activation.

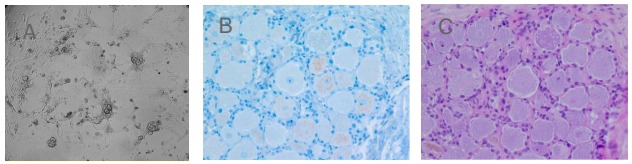

Figure 1. *A.* An image of cultured human DRGs. Scale bar = 100 µm. *B.* CGRP is a marker or peptidergic nocicepive primary afferent fibres. CGRP immunoreactivity was observed in human dorsal root ganglia (regions of red staining). *C.* H&E staining of the same section as in *B*.

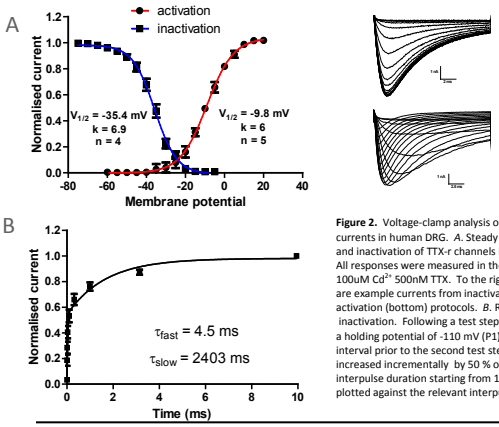

Figure 2. Voltage-clamp analysis of TTX-resistant currents in human DRG. *A.* Steady state activation and inactivation of TTX-r channels in human DRG. All responses were measured in the presence of 100uM Cd^{2+} 500nM TTX. To the right hand side are example currents from inactivation (top) and activation (bottom) protocols. *B.* Recovery from inactivation. Following a test step to 10 mV form a holding potential of -110 mV (P1), the recovery interval prior to the second test step (P1) was increased incrementally by 50 % of the previous interpulse duration starting from 1 ms. P2/P1 is plotted against the relevant interpulse duration.

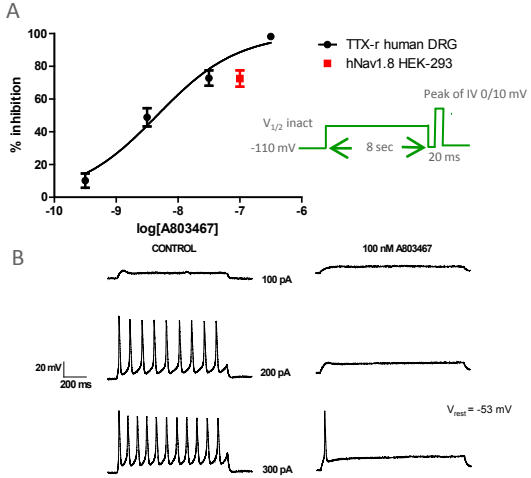

Figure 3. *A.* Potency determination of a selective Nav1.8 blocker (A803467) on TTX-r currents from human DRG. Inset is a diagrammatic representation of the voltage protocol used to evoke currents. IC_{50} = 5.05 nM; Hill slope 0.7. *B.* Current clamp analysis of the effect of a selective Nav1.8 blocker (A803467) on excitability of human DRG. Representative action potential (AP) traces from human DRG in control (LHS) and in the presence of 100 nM A803467 (RHS) at increasing step current injections.

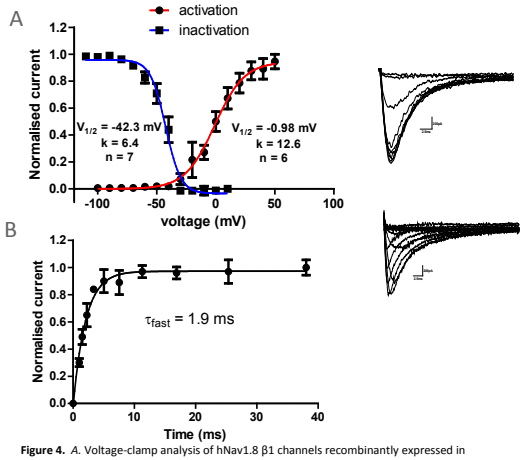

Figure 4. *A.* Voltage-clamp analysis of hNav1.8 β1 channels recombinantly expressed in HEK 283 cells. Steady state activation and inactivation of hNav1.8 channels. To the right hand side are example currents from inactivation (top) and activation (bottom) protocols. *B.* Recovery from inactivation; protocol same as in Fig. 2.

Results & Conclusion.

In this poster we have characterised the rapidly inactivating TTX-r current recorded from human DRG and proven pharmacologically using a selective blocker that the current recorded is Nav1.8. TTX-r currents have previously been reported in human DRGs, however, as the dataset was small, the currents were not fully characterised (Waxman *et al.,* 1999).

The biophysical properties of recombinantly expressed hNav1.8 channels in a variety of neuronal and non-neuronal backgrounds closely match those of the TTX-r current from human DRG. The $V_{1/2}$ activation for human TTX-r is –9.8 mV, which is approximately 9 mV more hyperpolarised than $V_{1/2}$ activation of recombinantly expressed hNav1.8+β1 in HEK-293 cells ($V_{1/2}$ activation -0.98 mV). $V_{1/2}$ activation of human TTX-r more closely matches $V_{1/2}$ activation values reported for hNav1.8 expressed in neuronal background (e.g. SH-SY5Y : -11 mV, Dekker *et al.,* 2005; ND7-23: -2.7 & -9.4 mV, Browne *et al.,* 2009, John *et al.,* 2004). The $V_{1/2}$ activation of human TTX-r is also similar to values reported for TTX-r in rat DRG (-17 mV, John *et al.,* 2004; -16 mV, Rush *et al.,* 1998). The $V_{1/2}$ fast inactivation of human TTX-r was -35.4 mV compared to -42.3 mV for hNav1.8+β1 in HEK-293 cells. This is within the range of published values for hNav1.8 expressed in various neuronal backgrounds (SH-SY5Y : -50 mV, Dekker *et al.,* 2005; ND7-23: -30.8 mV, John *et al.,* 2004). $V_{1/2}$ fast inactivation values reported for rat TTX-r are clustered around -25 and -46 mV (Rush *et al.,* 1998). Recovery from inactivation was fitted with a single exponential for the recombinantly expressed hNav1.8 (τ_{fast} for hNav1.8 β1 HEK-293 = 1.9 ms), whereas there was two phases of recovery for the human TTX-r channel (τ_{fast} = 4.5 ms and τ_{slow}= 2403 ms). Two exponential components have also been reported for recombinantly expressed hNav1.8 and rat TTX-r; HEK-293: τ_{fast} = 5.9 ms and τ_{slow} = 83.7 ms, ND7-23: τ_{fast} = 12.5 and 2271 ms and TTX-r rDRG: τ_{fast} = 15.7 ms and τ_{slow} =139.7 ms (Browne *et al.,* 2009; John *et al.,* 2004).

Application of a selective Nav1.8 blocker A-803467, inhibited the TTX-r current in human DRG with an IC_{50} of 5 nM. Application of 100 nM A803467 inhibited 76 % of the current in hNav1.8 +β1 in HEK-293 cells, which suggests that A803467 has similar potency against both human TTX-r and heterologously expressed Nav1.8. This is further demonstrated by previous data from recombinantly expressed human Nav1.8 using an identical voltage protocol (A-803467 IC_{50} = 8 nM, Jarvis et al., 2007). Using current-clamp recordings from human DRGs, 100 nM A-803467 reduced firing in response to increasing current injections as seen previously in rat DRG neurones (Jarvis *et al.,* 2007).

Therefore, under these recording conditions we have shown both biophysically and pharmacologically that Nav1.8 is present and functional in human DRG and that the properties of recombinant hNav1.8 channels expressed in a variety of neuronal and non-neuronal backgrounds, closely resemble those of the native channel.

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