



Scottish
Biomedical

Electrophysiological properties of
recombinant human sodium channels
1.1-1.8

May 2011

Contents

1.	Methods	2
2.	Electrophysiological properties of recombinant human Na _v 1.1 channel	4
3.	Electrophysiological properties of human recombinant Na _v 1.3 channel	6
4.	Electrophysiological properties of human recombinant Na _v 1.4 channel	8
5.	Electrophysiological properties of human recombinant Na _v 1.5 channel	10
6.	Electrophysiological properties of human recombinant Na _v 1.6 channel	12
7.	Electrophysiological properties of human recombinant Na _v 1.7 channel	14
8.	Electrophysiological properties of human recombinant Na _v 1.8 channel	16

1 Methods

Cells were grown and prepared as outlined in the attached SOP for HEK-293 cell line. They were plated onto 15mm plastic dishes and incubated for 1-3 days in a 5% CO₂ incubator for conventional whole-cell patch clamp experiments. Internal (pipette) solution contained (in mM): Cs-aspartate (110), Cs-Cl (20), Na₂ATP (4), Na₂GTP (0.4), MgCl₂.6H₂O (1), EGTA (0.15), HEPES (5), pH 7.25 with CsOH, and was used to study sodium channels without the interference of potassium currents. Cells were superfused at room temperature with a standard physiological solution containing (mM): NaCl (140), KCl (4), CaCl₂ (2), MgCl₂ (1), glucose (10), HEPES (10), pH 7.35 with NaOH. Patch pipettes were pulled from borosilicate glass and had tip resistances of 1.5-5.0 MΩ when filled with the above solution. Manual patch clamp was carried out using an Axon 200B amplifier (Axon Instruments). The software program pClamp (version 10) from Axon Instruments was used to stimulate and record electrical activity. Capacitative transients were compensated electronically from the recordings, however the voltage drop across the series resistance and the liquid junction potential were not compensated. The series resistance was generally less than 10 MΩ, with a mean cell capacity of around 15-25 pF. Standard activation protocol was used to calculate voltage peak sodium, from a holding potential of -90 mV. To generate current-voltage (I-V) relations, voltage pulses from -90 to +80 mV over 50 ms were repetitively applied at 1 sec intervals, with the holding potential between pulses kept at -90 mV. To generate a steady-state voltage dependent inactivation curve a protocol with a series of two square voltage pulses was used. The pulse one (P₁=pre-pulse) was 500 ms long and was immediately followed by a second pulse (P₂=test pulse), which was 50 ms long. Pulse one varied between the holding potential of -120 mV to +50 mV and pulse two stepped from a holding potential of -120 to the peak (usually between -10 and 0 mV), before returning to the holding potential again. After pulse two there was a 1 second interval when the cell membrane was clamped to the holding potential of -120 mV. In subsequent series of pulses, pulse one increased in 10 mV intervals from -110 to +50 mV whereas pulse two remained constant at -10 mV. Activation/inactivation curves have been created by transforming I-V current into conductance and normalizing to the peak (G/G_{max}). Single Boltzmann equations were used to fit the data by varying the V₅₀ potential and slope (*k*) factors. Tetrodotoxin (TTX) was prepared as 10 mM stock solution in water and subsequently diluted in physiological solutions.

2 Electrophysiological properties of hNa_v 1.1 $\alpha+\beta_1$

2.1 Human Na_v 1.1 Biophysics

Detailed analysis of human Na_v 1.1 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (IV) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability and block of the resting state; and half-inactivation usually between -70 and -50 mV was used to ensure block of the inactivate state. The rapid kinetics and complete inactivation of hNa_v 1.1 currents were in line with published data. Currents began to activate at -30 mV and typically reached a peak during test pulses to 0 mV, before reversing positive to +60 mV. Inward currents decayed completely during test pulses, typical of hNa_v 1.1 channels. Average current was about -1250 pA (n=16) with expression levels at around 90%. An example of the hNa_v 1.1 currents evoked by the I-V protocol is shown in Figure 1 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

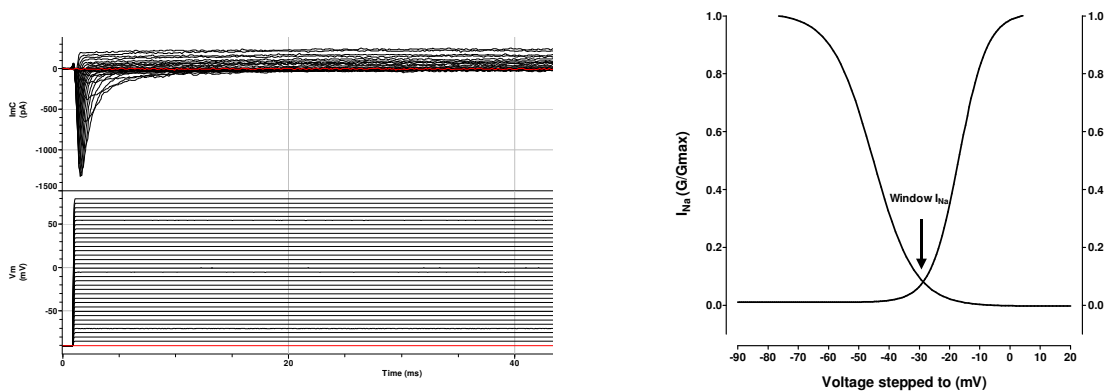


Figure 1: Voltage-dependent properties of hNa_v 1.1 HEK-293 cell line.

Left: example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 10 mV increments). The -90 mV voltage pulse is highlighted by the red trace.

Right: Normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h1/2}$ of -17 ± 3 mV and $V_{1/2}$ of -45 ± 5 mV; slope factors were 5 ± 2 and 7 ± 4 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNa_v 1.1 HEK-293 cell lines are similar to published data for heterologously expressed hNa_v 1.1 channels (see review by Catterall et al, J Neuroscience 2008).

2.2 Human Na_v 1.1 Pharmacology

Human Na_v 1.1 is part of the tetrodotoxin sensitive (TTX-S) sodium channels commonly found in the CNS and it is viewed as a target for antiepileptic drugs. Human Na_v 1.1 plays a major role for action potential initiation and repetitive firing in neurons.

The reference compound TTX was tested previously against hNa_v 1.1 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration–response curve. A calculated IC₅₀ of around 4 nM was obtained, with a full block of the sodium current with 100 nM TTX (Figure 2). This result confirmed that the expressed channel was a member of the TTX-sensitive family and complete block by 100 nM TTX rules out the presence of endogenous TTX-R channels in the parental HEK-293 cell line.

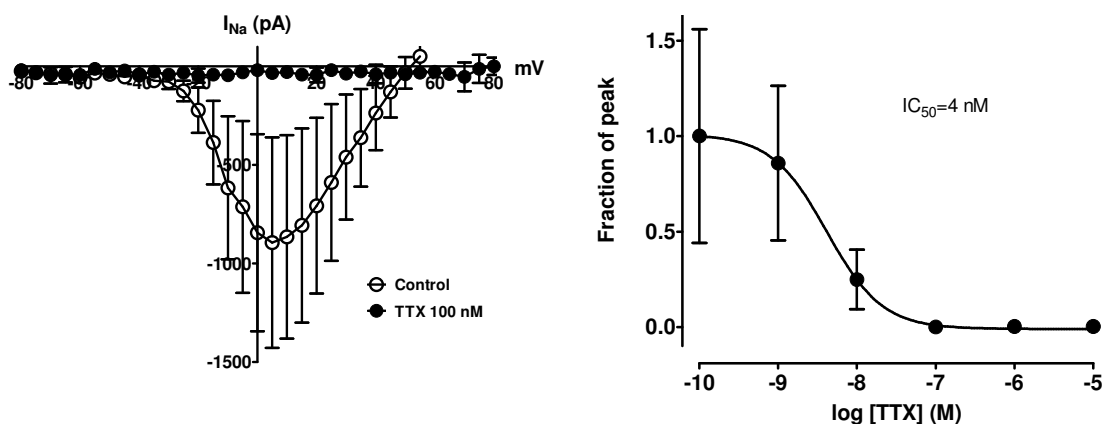


Figure 2: Block of hNa_v 1.1 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=3). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to +80 mV.

Right: Concentration-dependent effects of tetrodotoxin (0.1 nM-10 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNa_v 1.1 α+β₁. Cells were stimulated from a holding potential of -90 mV to +80 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

3 Electrophysiological properties of hNa_v 1.3 $\alpha+\beta_1$

3.1 Human Na_v 1.3 Biophysics

Detailed analysis of human Na_v 1.3 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (IV) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability and block of the resting state; and half-inactivation usually between -75 and -60 mV was used to ensure block of the inactivate state. The rapid kinetics and complete inactivation of hNa_v 1.3 currents were in line with published data. Currents began to activate at -40 mV and typically reached a peak during test pulses to 0 mV, before reversing positive to +60 mV. Average current was about -1300 pA ($n=17$) with expression levels at around 95%. Inward currents decayed completely during test pulses, typical of hNa_v 1.3 channels. An example of the hNa_v 1.3 currents evoked by the I-V protocol is shown in Figure 3 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

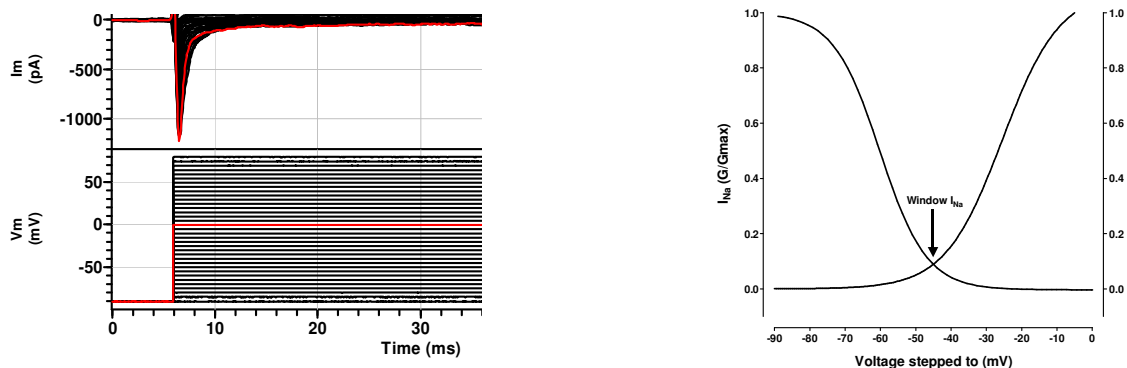


Figure 3: Voltage-dependent properties of hNa_v 1.3 HEK-293 cell line.

Left: Example of current traces during an I-V protocol (50 ms test pulses from -90 mV to +80mV (in 5 mV increments). The peak voltage pulse is highlighted by the red trace.

Right: Normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h_{1/2}}$ of -25 ± 2 mV and $V_{1/2}$ of -66 ± 1 mV; slope factors were 5 ± 2 and 8 ± 1 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNa_v 1.3 HEK-293 cell lines are similar to published data for heterologously expressed hNa_v 1.3 channels (see review by Momim & Wood, *Curr Opin Neurobiol*, 2008).

3.2 Human Na_v 1.3 Pharmacology

Human Na_v 1.3 is part of the tetrodotoxin sensitive (TTX-S) sodium channels commonly found in the CNS and it is viewed as a target for analgesic and antiepileptic drugs. Human Na_v 1.3 plays a major role for action potential initiation and conduction, and repetitive firing in neurons.

The reference compound TTX was tested previously against hNa_v 1.3 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 5 nM was obtained, with a full block of the sodium current with 100 nM TTX (Figure 4). This result confirmed that the expressed channel was a member of the TTX-sensitive family and complete block by 100 nM TTX rules out the presence of endogenous TTX-R channels in the parental HEK-293 cell line.

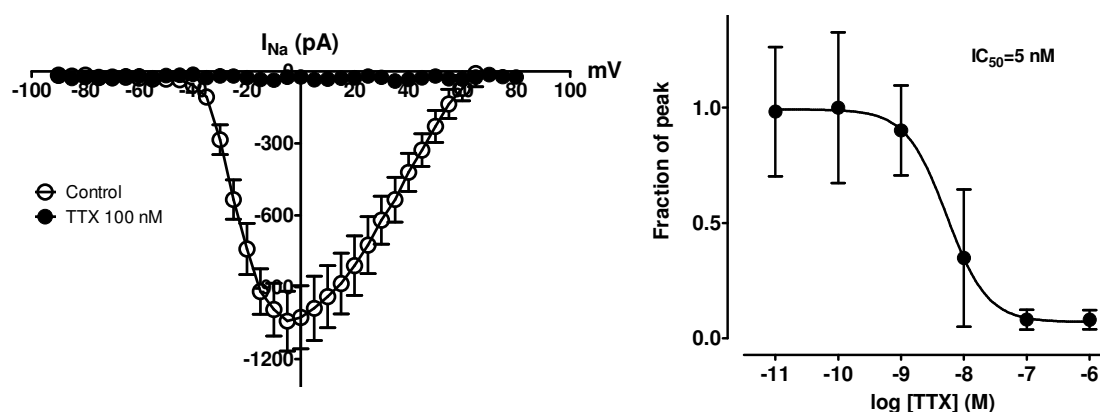


Figure 4: Block of hNa_v 1.3 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=9). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to +80 mV.

Right: Concentration-dependent effects of tetrodotoxin (0.01 nM-1 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNa_v 1.3 α+β₁. Cells were stimulated from a holding potential of -90 mV to +80 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

4 Electrophysiological properties of hNa_v 1.4 α + β ₁

4.1 Human Na_v 1.4 Biophysics

Detailed analysis of human Na_v 1.4 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (I-V) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel. The rapid kinetics and complete inactivation of hNa_v 1.4 currents were in line with published data. Currents began to activate at -40 mV and typically reached a peak during test pulses to -10 mV, before reversing positive to +60 mV. Inward currents decayed completely during test pulses, typical of hNa_v 1.4 channels. Average current was about -2000 pA (n=6) with expression levels at around 95%. An example of the hNa_v 1.4 currents evoked by the I-V protocol is shown in Figure 5 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were always adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

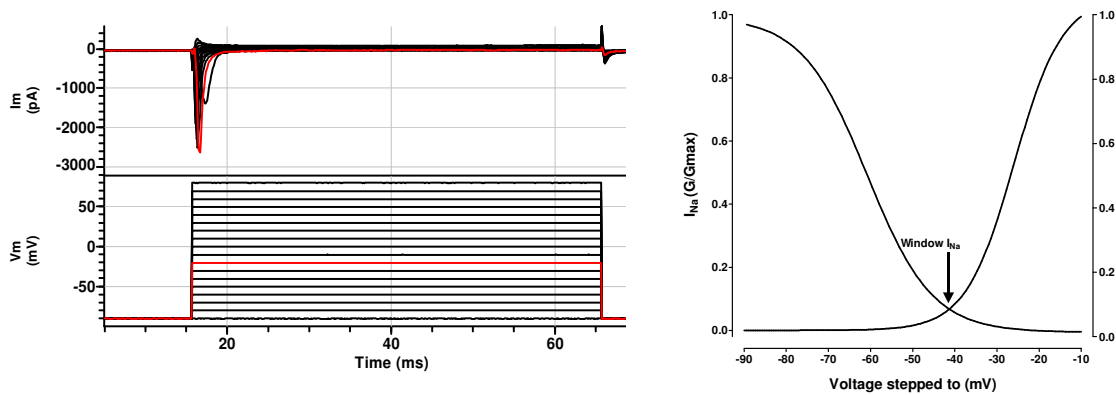


Figure 5: Voltage-dependent properties of hNa_v 1.4 HEK-293 cell line.

Left: example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 10 mV increments). The peak voltage pulse is highlighted by the red trace.

Right: normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h1/2}$ of -26 ± 2 mV and $V_{1/2}$ of -61 ± 2 mV; slope factors were 6 ± 2 and 8 ± 2 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNa_v 1.4 HEK-293 cell lines are similar to published data for heterologously expressed hNa_v 1.4 channels (see review by Catterall et al., Pharmacol Review 2005).

4.2 Human Na_v 1.4 Pharmacology

Human Na_v 1.4 is part of the tetrodotoxin sensitive (TTX-S) sodium channels commonly found in the skeletal muscle and it is viewed as a target for local anaesthetic drugs. Human Na_v 1.4 plays a major role for action potential initiation and transmission in skeletal muscle.

The reference compound TTX was tested previously against hNa_v 1.4 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 15 nM was obtained, with a full block of the sodium current with 100 nM TTX (Figure 6).

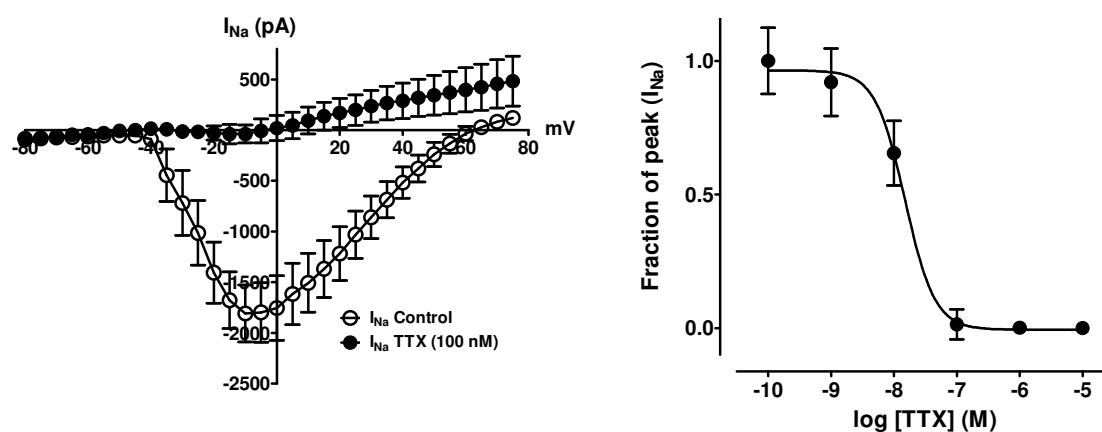


Figure 6: Block of hNa_v 1.4 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=6). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to + 80 mV.

Right: Concentration-dependent effects of tetrodotoxin (0.1 nM-10 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNa_v 1.4 α+β₁. Cells were stimulated from a holding potential of -90 mV to +80 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

5 Electrophysiological properties of hNa_v 1.5 $\alpha+\beta_1$

5.1 Human Na_v 1.5 Biophysics

Detailed analysis of human Na_v 1.5 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (IV) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_∞); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability and block of the resting state; and half-inactivation usually between -80 and -70 mV was used to ensure block of the inactivate state. The rapid kinetics and complete inactivation of hNa_v 1.5 currents were in line with published data. Currents began to activate at -40 mV and typically reached a peak during test pulses to -20 mV, before reversing positive to +60 mV. Inward currents decayed completely during test pulses, typical of hNa_v 1.5 channels. Average current was about -4600 pA (n=7) with expression levels at around 95%. An example of the hNa_v 1.5 currents evoked by the I-V protocol is shown in Figure 7 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

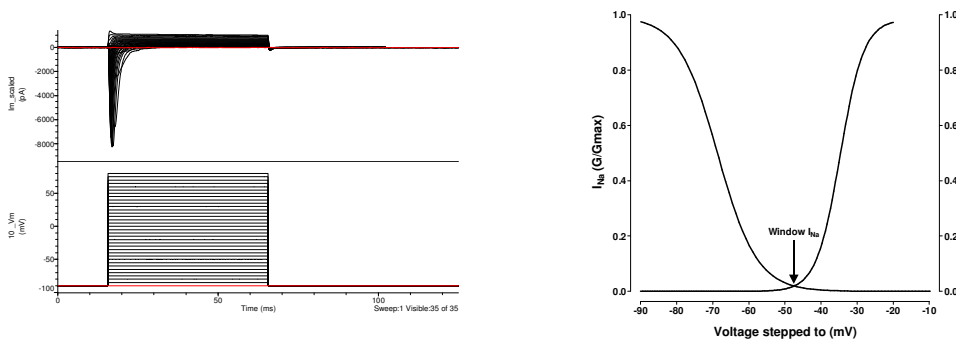


Figure 7: Voltage-dependent properties of hNa_v 1.5 HEK-293 cell line.

Left: Example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 10 mV increments). The -90 mV voltage pulse is highlighted by the red trace.

Right: Normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h1/2}$ of -35 ± 1 mV and $V_{1/2}$ of -69 ± 1 mV; slope factors were 3.3 ± 0.8 and 5.4 ± 0.8 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNa_v 1.5 HEK-293 cell lines are similar to published data for heterologously expressed hNa_v 1.5 channels (see review by Abriel H, Cardiovas Res 2007).

5.2 Human Na_v 1.5 Pharmacology

Human Na_v 1.5 is part of the tetrodotoxin resistant (TTX-R) sodium channels and is generally known as the cardiac sodium channel and it is viewed as a target for antiarrhythmic drugs. Human Na_v 1.5 is crucial for the initiation and propagation of the cardiac action potential.

The reference compound TTX was tested previously against hNa_v 1.5 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 5 μM was obtained, with a nearly full block of the sodium current with 100 μM TTX (Figure 8). This result confirmed that the expressed channel was a member of the TTX-resistant family and absence of block by 100 nM TTX rules out the presence of endogenous TTX-sensitive channels in the parental HEK-293 cell line.

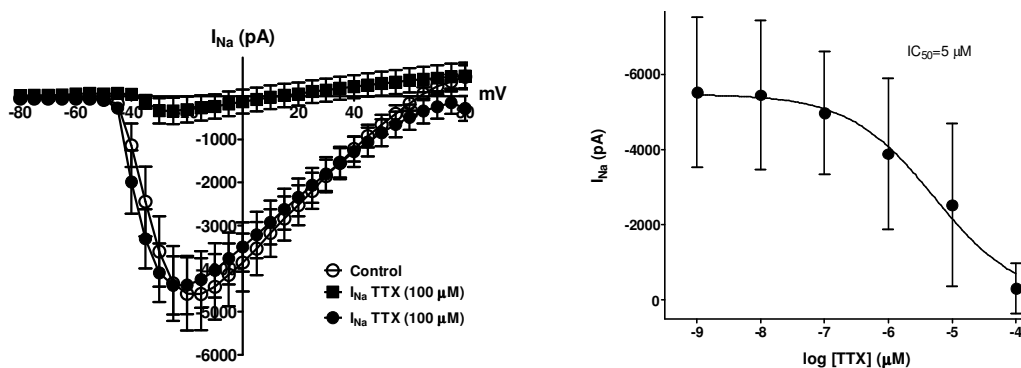


Figure 8: Block of hNa_v 1.5 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=7) and at 100 μM (solid squares; n=5). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to +80 mV.

Right: Concentration-dependent effects of tetrodotoxin (1 nM-100 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNa_v 1.5 α+β₁. Cells were stimulated from a holding potential of -90 mV to -10 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

6 Electrophysiological properties of hNa_v 1.6 α + β ₁

6.1 Human Na_v 1.6 Biophysics

Detailed analysis of human Na_v 1.6 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (IV) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability and block of the resting state; and half-inactivation usually between -80 and -60 mV was used to ensure block of the inactivate state. The rapid kinetics and complete inactivation of hNa_v 1.6 currents were in line with published data. Currents began to activate at -30 mV and typically reached a peak during test pulses to 0 mV, before reversing positive to +60 mV. Inward currents decayed completely during test pulses, typical of hNa_v 1.6 channels. Average current was about -1600 pA (n=5) with expression levels at around 95%. An example of the hNa_v 1.6 currents evoked by the I-V protocol is shown in Figure 9 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

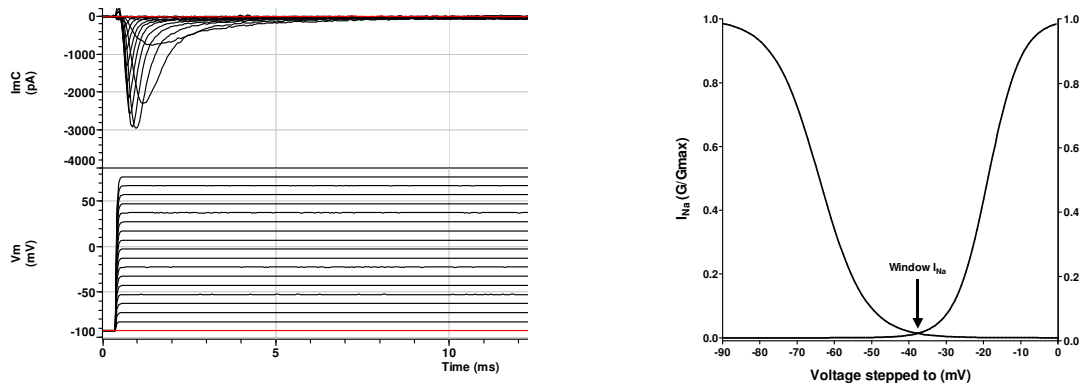


Figure 9: Voltage-dependent properties of hNa_v 1.6 HEK-293 cell line.

Left: example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 10 mV increments). The -90 mV voltage pulse is highlighted by the red trace.

Right: Normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h1/2}$ of -19 ± 1 mV and $V_{1/2}$ of -64 ± 1 mV; slope factors were 4 ± 2 and 6 ± 1 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNav 1.6 HEK-293 cell lines are similar to published data for heterologously expressed hNav_v 1.6 channels (see review by Catterall et al, Pharmacol Review 2005).

6.2 Human Na_v 1.6 Pharmacology

Human Na_v 1.6 is part of the tetrodotoxin sensitive (TTX-S) sodium channels commonly found in nodes of Ranvier of sensory and motor axons in the CNS and PNS and it is viewed as a target for antiepileptic, analgesic drugs. Human Na_v 1.6 plays a major role for action potential initiation and transmission in central neurons and their myelinated axons.

The reference compound TTX was tested against hNav_v 1.6 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 6 nM was obtained, with a full block of the sodium current with 100 nM TTX (Figure 10). This result confirmed that the expressed channel was a member of the TTX-sensitive family and complete block by 100 nM TTX rules out the presence of endogenous TTX-R channels in the parental HEK-293 cell line.

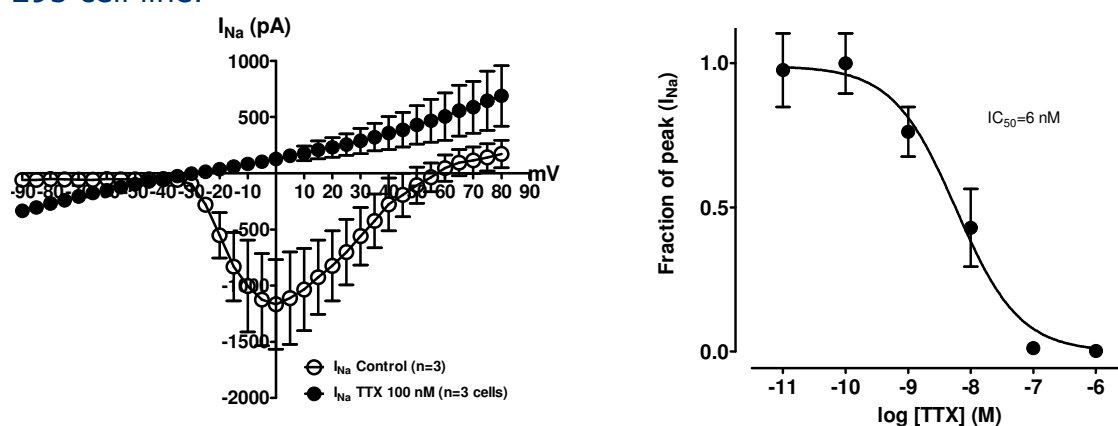


Figure 10: Block of hNav_v 1.6 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=3). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to +80 mV.

Right: Concentration-dependent effects of tetrodotoxin (0.01 nM-1 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNav_v 1.6 α+β₁. Cells were stimulated from a holding potential of -90 mV to -10 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

7 Electrophysiological properties of hNa_v 1.7 $\alpha+\beta_1$

7.1 Human Na_v 1.7 Biophysics

Detailed analysis of human Na_v 1.7 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (IV) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability and block of the resting state; and half-inactivation usually between -80 and -60 mV was used to ensure block of the inactivate state. The rapid kinetics and complete inactivation of hNa_v 1.7 currents were in line with published data. Currents began to activate at -30 mV and typically reached a peak during test pulses to 0 mV, before reversing positive to +60 mV. Average current was about -800 pA (n=12) with expression levels at around 90%. Inward currents decayed completely during test pulses, typical of hNa_v 1.7 channels. An example of the hNa_v 1.7 currents evoked by the I-V protocol is shown in Fig. 11 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

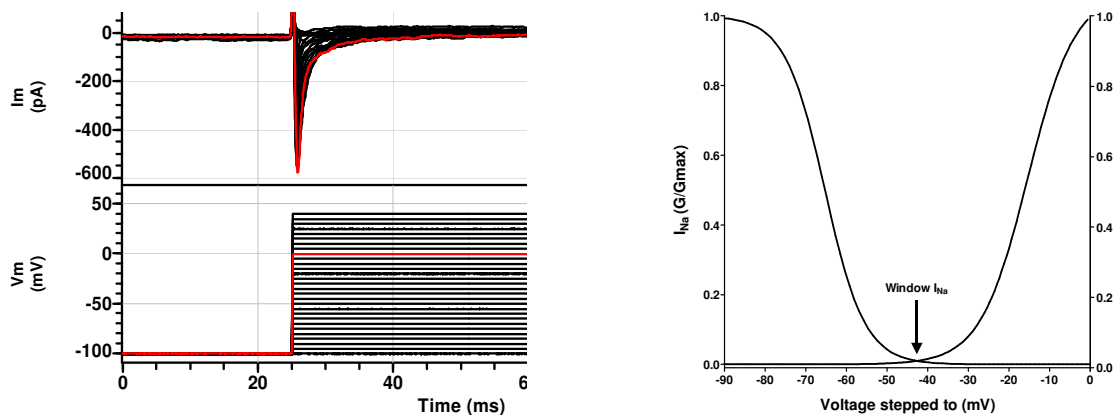


Figure 11: Voltage-dependent properties of hNa_v 1.7 HEK-293 cell line.

Left: example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 10 mV increments). The peak voltage pulse is highlighted by the red trace.

Right: averaged activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h_{1/2}}$ of -15 ± 2 mV and $V_{1/2}$ of -65 ± 2 mV; slope factors were 5 ± 2 and 5 ± 2 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNav_v 1.7 HEK-293 cell lines are similar to published data for heterologously expressed hNav_v 1.7 channels (see review by Drenth & Waxman, J Clin Invest 2007).

7.2 Human Na_v 1.7 Pharmacology

Human Na_v 1.7 is part of the tetrodotoxin sensitive (TTX-S) sodium channels and is commonly found in all types of DRG neurons, sympathetic neurons, Schwann cells, and neuroendocrine cells. Na_v 1.7 is generally viewed as a target for pain therapy. Human Na_v 1.7 is important for the initiation and transmission of the action potential in peripheral neurons.

The reference compound TTX was tested previously against hNav_v 1.7 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 8 nM was obtained, with a full block of the sodium current with 100 nM TTX (Figure 12). This result confirmed that the expressed channel was a member of the TTX-sensitive family and absence of block by 100 nM TTX rules out the presence of endogenous TTX-R channels in the parental HEK-293 cell line.

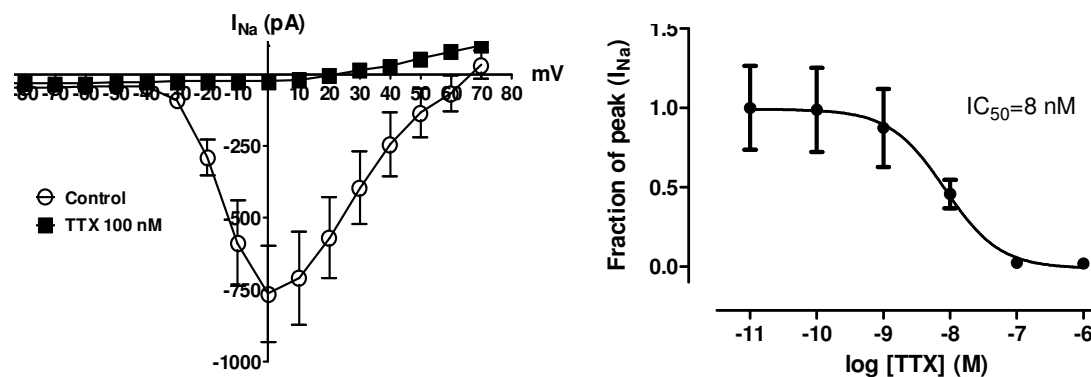


Figure 12: Block of hNav_v 1.7 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open circles) and in the presence of TTX at 100 nM (solid circles; n=4). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to + 80 mV.

Right: Concentration-dependent effects of tetrodotoxin (0.01 nM-1 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNav_v 1.7 α+β₁. Cells were stimulated from a holding potential of -90 mV to -10 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

8 Electrophysiological properties of hNa_v 1.8 α

8.1 Human Na_v 1.8 Biophysics

Detailed analysis of human Na_v 1.8 channel biophysics was previously carried out by manual patch clamp at Scottish Biomedical. The current-voltage (I-V) relationship was used to calculate half-activation potential, and 500 ms pre-pulses used to create inactivation curves for estimation of channel availability (h_{∞}); all experiments were conducted using a holding potential of -90 mV to ensure maximum channel availability. The rapid kinetics and complete inactivation of hNa_v 1.8 currents were in line with published data. Currents began to activate at -20 mV and typically reached a peak during test pulses to +10 mV, before reversing positive to +60 mV. Average current was about -600 pA (n=5) with expression levels at around 75%. An example of the hNa_v 1.8 currents evoked by the I-V protocol is shown in Figure 13 below, as well as the normalised I-V plot for both activation and inactivation protocols. Inactivation and activation curves were adequately fitted with single Boltzmann equations, indicating the presence of a single major sodium conductance.

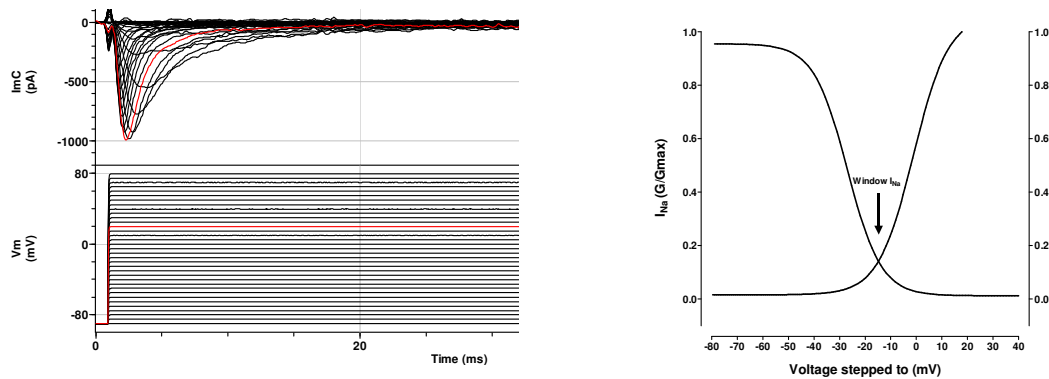


Figure 13: Voltage-dependent properties of hNa_v 1.8 HEK-293 cell line.

Left: example of current traces during an I-V protocol (50 ms test pulses from -90 mV in 5 mV increments). The peak voltage pulse is highlighted by the red trace.

Right: normalised activation and inactivation current data was used to plot the steady-state activation and inactivation curves, which were fitted with single Boltzmann equations with values for $V_{h_{1/2}}$ of -1 ± 4 mV and $V_{1/2}$ of -27 ± 6 mV; slope factors were 7 ± 3 and 7 ± 1 , respectively.

Overall, the voltage-dependent properties of the Scottish Biomedical hNav_v 1.8 HEK-293 cell lines are similar to published data for heterologously expressed hNav_v 1.8 channels (see review by Cummins et al., Pain 2007).

8.2 Human Na_v 1.8 Pharmacology

Human Na_v 1.8 is part of the tetrodotoxin resistant (TTX-R) sodium channels and is commonly found in small and medium-sized DRG neurons. Human Na_v 1.8 is viewed as a target for pain therapy and has been shown to contribute substantially to the sodium inward current underlying the action potential in DRG neurons.

The reference compound TTX was tested previously against hNav_v 1.8 using a 6-point IC₅₀ centered around its known affinity based on published electrophysiological data. Concentration-response data for the effect of TTX on peak I_{Na} were fitted iteratively (Graphpad Prism) using a variable slope sigmoidal concentration-response curve. A calculated IC₅₀ of around 45 μM was obtained, with around 70% block of the sodium current with 50 μM TTX (Figure 14). This result confirmed that the expressed channel was a member of the TTX-resistant family and absence of block by 100 nM TTX rules out the presence of endogenous TTX-S channels in the parental HEK-293 cell line.

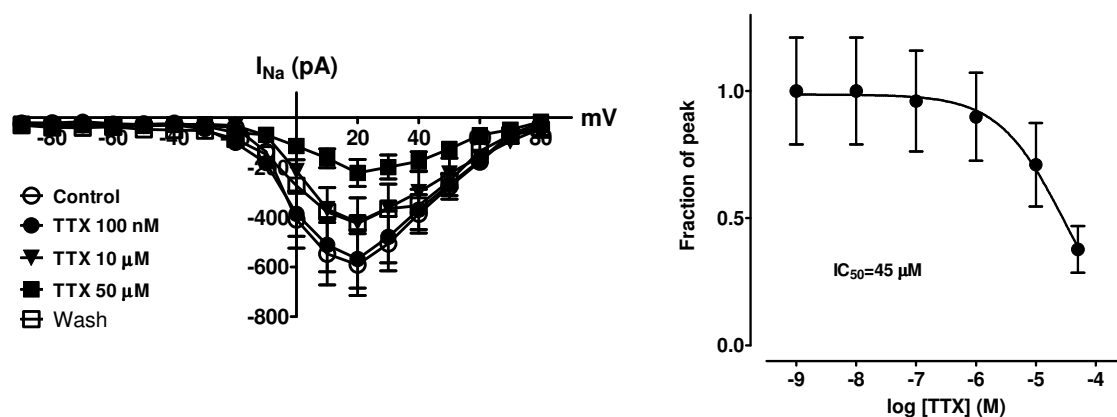


Figure 14: Block of hNav_v 1.8 by TTX on manual patch-clamp.

Left: Current-Voltage relationships of I_{Na} expressed in terms of absolute current (pA), in the absence (open symbols) and in the presence of TTX at 100 nM, 10 and 50 μM (solid symbols; n=3-4). Values are means, with error bars denoting SEM. The holding potential was -90 mV up to +80 mV.

Right: Concentration-dependent effects of tetrodotoxin (1 nM-50 μM) on I_{Na} currents (pA) obtained from HEK-293 cells stable transfected with hNav_v 1.8 α. Cells were stimulated from a holding potential of -90 mV to -10 mV with 50 ms pulses at 1 Hz. The mean data points were fitted by a variable slope sigmoidal curve using the Hill equation.

Scottish Biomedical

Todd Campus
West of Scotland Science Park
Todd Campus
Glasgow
G20 0XA

Tel: 44 (0) 141 587 6100

Fax: 44 (0) 141 587 6110

www.scottish-biomedical.com