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Analysis of human $Na_v1.8$ expressed in SH-SY5Y neuroblastoma cells

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Abstract

The tetrodotoxin-resistant voltage-gated sodium channel α-subunit $Na_v1.8$ is expressed in nociceptors and has been implicated in chronic pain. Difficulties of heterologous expression have so far precluded analysis of the pharmacological properties of human $Na_v1.8$. To address this we have introduced human $Na_v1.8$ in neuroblastoma SH-SY5Y cells. Voltage-clamp analysis showed that human $Na_v1.8$ generated an inward tetrodotoxin-resistant sodium current with an activating threshold around − 50 mV, half maximal activation at − 11 ± 3 mV and a reversal potential of 67 ± 4 mV. These properties closely match those of the endogenous rat tetrodotoxin-resistant sodium current in dorsal root ganglia suggesting that the expressed human channel is in a near physiological conformation. Human $Na_v1.8$ was resistant to tetrodotoxin and activated by the pyrethroid toxin deltamethrin. Both voltage-activated and deltamethrin-activated human $Na_v1.8$ were inhibited by the sodium channel blockers BIII 890 CL, NW-1029, and mexiletine. Inhibition of $Na_v1.8$ by these compounds may underlie their known analgesic effects in animal models.

Keywords: Pain; Dorsal root ganglion; Nociceptor; Sodium channel; Deltamethrin

1. Introduction

Voltage-gated sodium channels are responsible for the initiation and propagation of the action potential in excitable cells. They are multimeric proteins, consisting of a pore forming α subunit and accessory β subunits. A large number of α-subunit isoforms have been identified, which differ in primary structure, tissue distribution and pharmacological characteristics (Anderson and Greenberg, 2001; Catterall, 1999; Baker and Wood, 2001).

Small diameter sensory neurones express at least five different sodium channel α subunits, the combined action of which mediates action potential generation at the nerve ending and its subsequent conduction along the axon (Baker and Wood, 2001). Whilst a number of these α subunits are also expressed in other parts of the nervous system, at least one, $Na_v1.8$, is exclusively present in small diameter nociceptive neurones suggesting a role for this subunit in transmission of painful stimuli (Akopian et al., 1996; Djouhri et al., 2003; Dekker and Cronk, 2005). Genetic deletion studies and antisense oligonucleotide interference experiments have revealed a role for $Na_v1.8$ in the pathophysiological function of these neurones in pain signalling during inflammation and after nerve injury (Akopian et al., 1999; Lai et al., 2000, 2002; Laird et al., 2002; Roza et al., 2003). This has generated considerable interest in this subunit as a potential target for novel analgesic drugs.

When expressed in *Xenopus laevis* oocytes, $Na_v1.8$ generates a slow-inactivating Na^+ current which is resistant to tetrodotoxin (Akopian et al., 1996; Sivilotti et al., 1997). A similar tetrodotoxin-resistant sodium current can also be recorded in small diameter Dorsal root ganglion (DRG) neurones and is lacking from small diameter sensory neurones obtained from mice in which $Na_v1.8$ has been knocked out by genetic deletion (Akopian et al., 1999). The current can be restored by injecting a $Na_v1.8$ construct into sensory neurones from the $Na_v1.8$ −/− animals (Akopian et al., 1999). Hence, $Na_v1.8$ underlies a tetrodotoxin-resistant sodium current.

Cell lines expressing $Na_v1.8$ are highly desirable both for further characterisation of the fundamental pharmacological and biophysical properties of the channel and to provide a well defined template against which to screen chemical libraries with

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the aim of identifying selective inhibitors. This is pertinent in particular for human $Na_v1.8$ since unlike the rat channel, human $Na_v1.8$ can not be assayed in its endogenous context. So far, attempts to express $Na_v1.8$ in heterologous cell systems, mainly focusing on the rat orthologue, have had mixed success. After introduction of rat $Na_v1.8$ cDNA in COS, CHO or HEK-293, cells expressed either no or low levels of functional current or, if detected, the properties of the expressed channel differed from the endogenous channel in rat DRG neurones (John et al., 2004; Okuse et al., 2002). Recent studies have shown that it is possible to express rat $Na_v1.8$ in a native conformation in ND7.23 cells, an N18Tg2xDRG fusion cell line (Wood et al, 1990; John et al., 2004). Expression of human $Na_v1.8$ has been reported in *Xenopus laevis* oocytes (Rabert et al., 1998) and (cloned from human heart cDNA) was recently achieved in HEK-293 cells (Akiba et al., 2003). However the biophysical properties were different from endogenous currents measured in rat nociceptive neurones. One explanation for this observation is that intrinsic differences exist between rat and human $Na_v1.8$. Alternatively, the observed differences could be a result of the background in which the channel has been tested. The latter would be compatible with the data on rat $Na_v1.8$ and highlights the difficulty of expressing human $Na_v1.8$ in heterologous systems in a native conformation.

Here we report the use of SH-SY5Y neuroblastoma cells as a vehicle for functional expression of human $Na_v1.8$ and present a pharmacological and biophysical characterisation of the human channel in this cell background.

2. Materials and methods

2.1. Materials

NW1029 and BIII 890 CL were synthesized as published (Pevarello et al., 1998; Carter et al., 2000; Veneroni et al., 2003). Tetrodotoxin was obtained from Caltag Medsystems, UK or from Alomone Labs (TCS Biologicals, Botolph Claydon, Bucks, UK). Tetracaine, lidocaine, lamotrigine, carbamezapine and mexiletine were from Sigma, UK and deltamethrin was obtained from Calbiochem, UK. Standard chemicals were either AnalaR (BDH Merck Ltd, Lutterworth, Leistershire, UK) or Sigma (Poole, Dorset, UK).

2.2. Rat and human $Na_v1.8$ expression constructs

Rat $Na_v1.8$ ($rNa_v1.8$) cDNA was obtained from John Wood (Akopian et al., 1996) and transferred into pcDNA3.1 (InVitrogen, UK) or pIRESneo2 (BD Clontech, UK) using standard subcloning procedures. Human $Na_v1.8$ ($hNa_v1.8$) cDNA was obtained using human DRG RNA (Analytical Biological Services) as template for SmartTM cDNA synthesis (BD Clontech, UK) followed by specific amplification of $hNa_v1.8$ using Advantage Taq Polymerase (BD Clontech, UK), based upon the sequence information in the public database. PCR fragments were cloned into the vector pCRII (InVitrogen, UK), and spliced into the mammalian expression vector pIRES-neo2 for expression studies.

2.3. Cell maintenance and cell transfection

All media and media components were obtained from InVitrogen-Gibco. Neuroblastoma cell lines cells were obtained from ECACC (Salisbury, UK) and were maintained as advised by ECACC. Prior to transfection, cells were seeded at 90% confluence and left overnight. Cells were transfected using Lipofectamine 2000 (InVitrogen) following the instructions by the manufacturer. For antibiotic selection, cells were maintained in their individual media supplemented with 600 μg/ml G418 (Geneticin; Gibco). Clonal cells were picked by disk trypsinisation and clonal lines were routinely maintained in G418.

2.4. Reverse transcription PCR (RT-PCR) assay

Cells were washed in Phosphate Buffered Saline, harvested and lysed in RNeasy lysis buffer (Qiagen). Total cellular RNA was obtained using the RNeasy system (Qiagen) following the manufacturers instructions. One microgram of total RNA was reverse transcribed using AMV reverse transcriptase (Promega) in a final reaction volume of 20 μl. mRNA levels were determined by PCR using 30 cycles consisting of three temperature steps (30 s at 94 °C, 30 s at 58 °C and 3 min at 72 °C) followed by a single 10-min extension at 72 °C. P11 primers were 5′GTCACATATGCCATCTCAAATGGAACAC3′ and 5′TGACTGACGGATCCTTCTATGGGGGAAGCTGTGG3′; p36 primers were 5′GGGTCTGTCAAAGCCTATAC3′ and 5′CGGAAGTCACCAGATGTGTC3′; SCN1B primers were 5′GTGTATGGGATGACCTTCAA3′ and 5′ACATCATGATCTCAGACAC3′; cyclophilin primers were 5′ACCCCACCGTGTTCTTCGAC3′ and 5′CATTTGCCATGGACAAGATG3′. PCR products were analysed by agarose gel electrophoresis using 100 bp or 1 kb markers as reference (both Promega, Southampton, UK).

2.5. Membrane potential fluorescence assay

Cells were seeded at 50,000 cells/well in 96-well Costar black clear bottom plates (tissue culture treated) in medium supplemented with 600 μg/ml G418 and incubated overnight at 37 °C, 5% CO_2. Sodium channel activity was measured using the Fluorometric Imaging Plate Reader membrane potential sensitive dye reagent (FLIPR-MPD; Molecular Devices Corp) in the presence of brilliant black as an extracellular quenching reagent. Briefly, cells were washed in buffer A (145 mM Tetramethylammoniumchloride (Sigma), 2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, 5 mM KCl) and then incubated in 100 μl 14% (v/v) MPD in buffer A, 0.3 mM brilliant black, deltamethrin and sodium channel inhibitors at concentrations indicated in the results section) for 30 min at room temperature. Sodium channels were activated by addition of 50 μl buffer B (210 mM NaCl, 2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, 5 mM KCl, 14% (v/v) FLIPR-MPD, 0.3 mM Brilliant black) to give a final concentration of 70 mM sodium. For tetrodotoxin-resistance experiments, tetrodotoxin was present at 250 nM final concentration. Fluorescence was read using either a Molecular Devices

Flexstation or a Perkin Elmer ImageTrak. For Flexstation readings the excitation wavelength was 530 nm and the emission wavelength 565 nm. On the ImageTrak a 535 nm excitation filter was used whilst monitoring emission at 580 nm (20 nm bandpass). Assays were run in kinetic format with baseline fluorescence monitored for a period of 12 s prior to the addition of buffer B. Fluorescence was monitored for a further 70 s to allow maximal response to be attained. The magnitude of response was expressed as % increase in fluorescence above baseline using the calculation 100 × ((maximum fluorescence value / average baseline fluorescence) − 1). The fluorescence signal before addition of buffer B was used as baseline reference and $Na_v1.8$ activity was extrapolated from the fluorescence signal reached after addition of buffer B.

2.6. Electrophysiology assay

Membrane currents were recorded using the whole-cell patch-clamp technique. Cells were plated onto 35 mm poly-L-lysine-coated petri dishes and subsequently incubated in a 5% CO_2 incubator at 37 °C. Cells were used 18–48 h after plating. The extracellular recording solution contained 140 mM NaCl, 20 mM tetraethylammoniumchloride, 10 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 100 μM $CdCl_2$, 3 mM KCl, 1 μM tetrodotoxin. The solution was buffered to pH 7.3 by addition of NaOH and the osmolality was adjusted to 320 mOsm by addition of sucrose. The intracellular solution contained 140 mM CsF, 1 mM EGTA-Na, 10 mM HEPES, 10 mM NaCl. The solution was buffered to pH 7.3 by addition of CsOH and the osmolality adjusted to 320 mOsm by addition of sucrose. Electrodes were fabricated from thin-walled glass capillaries (GC150TF-10; Harvard apparatus, Edenbridge, Kent, UK) and had resistance of 1.5–2 MΩ when filled with recording solution. Recordings were made at 20 °C using a HEKA patch amplifier (HEKA Electronik, Lambrecht, Germany). Pulse protocols were generated and data stored to disk using pulse software (HEKA) running on a G4 Mac computer. Cells were held at −80 mV. Cell capacitance and series resistance were compensated electronically. Any residual capacity current and linear leak were subtracted by a P/8 subtraction protocol. Series resistance was generally compensated by 50–75%. Data are acquired at 25 kHz. In inhibitor studies, compounds were applied directly to the cell via a Valvebank perfusion system (Automate Scientific Inc., USA) coupled to a modified micro-loader (Eppendorf, Germany) to focus solution flow.

3. Results

3.1. Expression of human $Na_v1.8$ in SH-SY5Y neuroblastoma cells

A cDNA representing human $Na_v1.8$ was obtained by reverse transcription of RNA from adult DRG tissue and subsequent PCR amplification of cDNA using specific primers. The resulting cDNA encoded a protein with identical predicted sequence to that reported (Rabert et al., 1998) with the exception of the alanine residue at position 1073 which is a valine residue in the current sequence. The cDNA was transferred into the mammalian expression vector pIRESneo2 and independent clonal lines were established of SH-SY5Y cells transfected with this construct. In order to measure functional expression of $Na_v1.8$, we measured the effect of high sodium on the cell membrane potential in the presence of deltamethrin, a pyrethroid toxin that is known to prolong the open state of tetrodotoxin-resistant sodium channels (Tabarean and Narahashi, 1998, 2001). In the absence of extracellular sodium, deltamethrin nor tetrodotoxin affected the membrane potential in either control or human $Na_v1.8$-expressing cells (Fig. 1A). Addition of high sodium revealed a deltamethrin-induced change in membrane potential in cells transfected with human $Na_v1.8$ but not in control cells transfected with vector alone (Fig. 1B). Therefore the change in membrane potential is a consequence of sodium ion flux through $Na_v1.8$ and a measure of its activity. The effect of deltamethrin on $Na_v1.8$ was concentration dependent with an EC_{50} of 620 nM (Fig. 2A). The

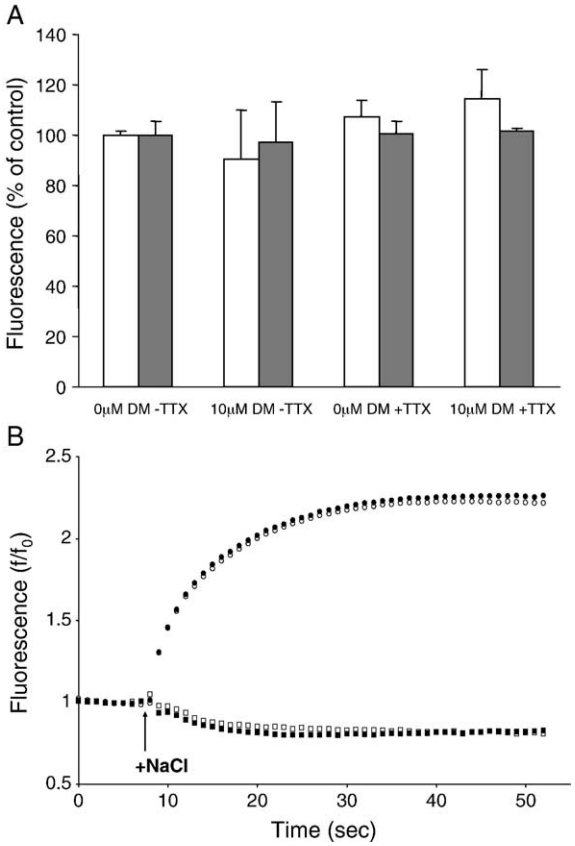


Fig. 1. Expression of human $Na_v1.8$ in SH-SY5Y cells. SH-SY5Y cells were transfected with human $Na_v1.8$, grown under G418 selection and clonal cell lines were derived. A) Fluorescence changes of a human $Na_v1.8$-expressing cell line (filled bars) or control cell line (open bars) in response to 10 μM deltametrin (DM) in the presence or absence of 250 nM tetrodotoxin (TTX). The data represent averages ± standard errors of 4 observations. B) A human $Na_v1.8$ expressing cell line (○●) or control cell line (□■) were equilibrated in low sodium buffer in the presence (●■) or absence (○□) of 250 nM tetrodotoxin after which the buffer was replaced by a high sodium buffer of otherwise identical composition. Changes in fluorescence were recorded over time and expressed as fraction of the equilibrated value. The data are a representative example of 4 observations.

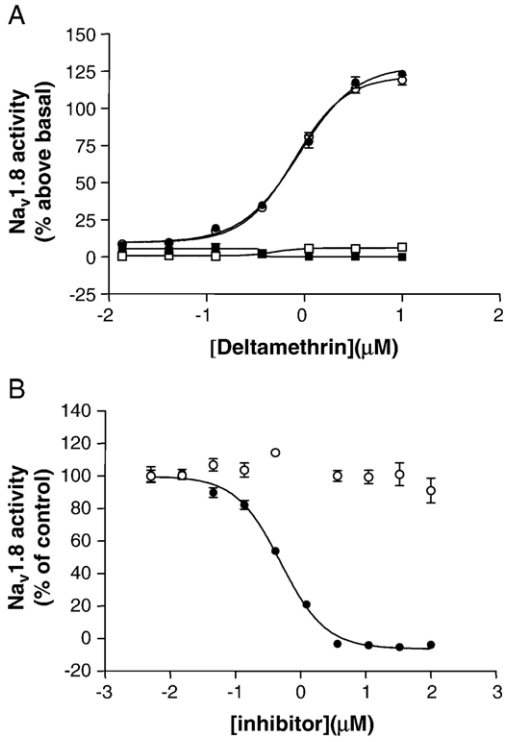


Fig. 2. Expression of human Na$_v$1.8 in SH-SY5Y cells. SH-SY5Y cells were transfected with human Na$_v$1.8, grown under G418 selection and clonal cell lines were derived. A) Response of a human Na$_v$1.8-expressing cell line (●○) or a control cell line (■□) to increasing concentrations of deltamethrin in the presence (●■) or absence (○□) of 250 nM tetrodotoxin. The data represent averages±standard errors of four observations. B) The effect of tetrodotoxin (○) or BIII 890 CL (●) on depolarisation induced by 10 μM deltamethrin. The data represent averages±standard errors of 10 observations.

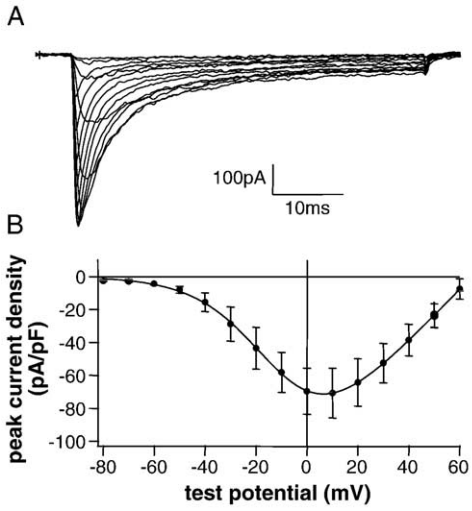
deltamethrin-induced change in membrane potential in Na$_v$1.8 expressing cells was not affected by the presence of tetrodotoxin in the incubation medium, indicating that the expressed channel was tetrodotoxin-resistant (Figs. 1B, 2B). However, it was inhibited by the recently described selective sodium channel blocker BIII 890 CL with an IC$_{50}$ value of 560 nM (Fig. 2B, Table 1). Thus, SH-SY5Y cells are capable of supporting functional expression of human Na$_v$1.8.

Table 1
Effect of sodium channel blockers on Na$_v$1.8 expressed in SH-SY5Y cells

	Human Na$_v$1.8 (pIC$_{50}$)	Rat Na$_v$1.8 (pIC$_{50}$)
BIII 890 CL	6.25±0.04	6.23±0.03
NW-1029	4.98±0.04	4.26±0.03
Tetracaine	5.51±0.07	4.31±0.08
Mexiletine	4.49±0.06	<4
Lamotrigine	<4	<4
Lidocaine	<4	<4
Carbamezapine	<4	<4
Tetrodotoxin	<4	<4

Cells were incubated with 10 μM deltamethrin in the presence of 250 nM tetrodotoxin and chosen inhibitor. Na$_v$1.8 activity was determined by increasing the extracellular Na^+ concentration and measuring the increase in fluorescence above baseline. Data were normalised to their fitted maximum for each inhibitor which was determined by linear regression using GraphPad Prism Software. Normalised data from three independent experiments (12–14 observations for each concentration) were combined to determine the overall pIC$_{50}$ for each inhibitor. Data are expressed as average pIC$_{50}$±the standard error of the fit.

In order to analyse the currents generated by human Na$_v$1.8, voltage-clamp experiments were performed. Currents generated in control cells were compared with those generated in clonal SH-SY5Y cells expressing human Na$_v$1.8. Cells were held at − 80 mV and currents were elicited by a voltage step to 0 mV. Control cells displayed tetrodotoxin-sensitive currents and were devoid of tetrodotoxin-resistant currents (Fig. 3C). The presence of human Na$_v$1.8 in SH-SY5Y cells is associated with the expression of a tetrodotoxin-resistant sodium current with average peak amplitude of 640±80 pA (mean±S.E.M.; n=10 cells). In order to determine the current–voltage relationship of human Na$_v$1.8, currents were evoked by step depolarisations from the holding potential to between − 80 and + 80 mV with 10 mV intervals (Fig. 3A) and the current density from 9 independent cells was plotted against the step potential (Fig. 3B). The peak current density was around 80 pA/pF with an activating threshold around −50 mV, half maximal activation at −11±3 mV and a



	g (nS)	V_{50} (mV)	E_{rev} (mV)	k (mV)
Human (Bather W)	-11.6±3	-10.7±3	67.5±4	11.7±1
rat TTX-r (Bather W)	-274±27	-27.6±2	55.9±2	-1.7±0.3

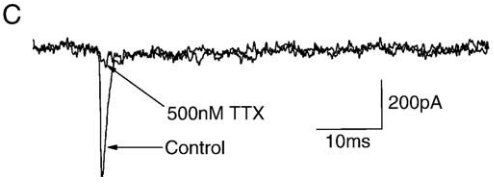


Fig. 3. Biophysical characterisation of human Na$_v$1.8. SH-SY5Y clonal cells expressing human Na$_v$1.8 were held at a holding potential of −80 mV and currents were evoked by stepwise depolarisation to between − 80 and 60 mV (30 ms 10 mV incrementing voltage steps). Recordings were made in the presence of 1 μM tetrodotoxin. A) Exemplary records from a single cell. B) Current/voltage relationship showing average current density (n=9 cells) and comparison with endogenous DRG tetrodotoxin-resistant current (table). The data was fit by a modified Boltzmann equation of the form: $I=(g(V_{rev}-V))/1+\exp((V_{50}-V)/k))$; where g is conductance (S), V_{rev} is the reversal potential (mV), V_{50} is the voltage for half activation (mV), and k is the slope (mV). C) Traces from non-transfected control cells in the presence and absence of tetrodotoxin.

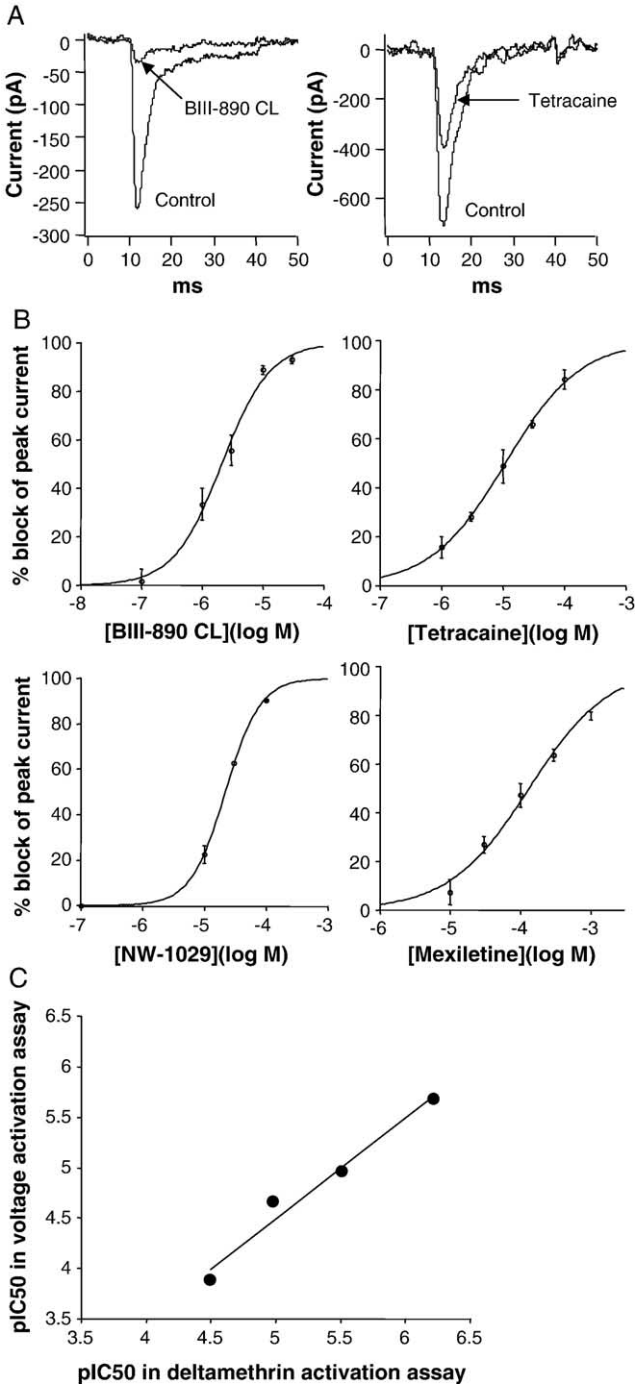


Fig. 4. Effect of sodium channel blockers on human $Na_v1.8$ currents SH-SY5Y cells stably expressing human $Na_v1.8$ was analysed by voltage clamp in the presence of 1 μM tetrodotoxin. Cells were held at − 80 mV and sodium current was elicited by 30 ms voltage step to 0 mV (0.33 Hz step frequency). A) Example current traces illustrating the block of human $Na_v1.8$ tetrodotoxin-resistant sodium currents seen in the presence of 10 μM BIII 890C or tetracaine. B) Concentration dependence of inhibition of human $Na_v1.8$ by BIII 890 CL, NW-1029, tetracaine and mexiletine. C) Correlation plot of inhibitor action on deltamethrin-activated and voltage-activated $hNa_v1.8$.

reversal potential of 67±4 mV. The V_{50} value was lower than that observed for human $Na_v1.8$ expressed in either *Xenopus* oocytes or HEK cells and closer to the value measured for the endogenous tetrodotoxin-resistant current in rat DRG neurones (Fig. 3B). Thus SH-SY5Y cells provide a cell vehicle for Nav1.8 allowing near native expression.

3.2. Effects of sodium channel inhibitors on human $Na_v1.8$

Using the membrane potential assay, we tested a range of sodium channel blockers, including local anaesthetics and recently developed sodium channel blockers, for their action on human $Na_v1.8$. Human $Na_v1.8$ was inhibited by BIII 890 CL, tetracaine, NW-1029 and mexiletine but not by lamotrigine, lidocaine, carbamezapine and tetrodotoxin (Table 1). BIII 890 CL showed the highest potency (Table 1) followed by tetracaine, NW-1029. Mexiletine has a low affinity for human $Na_v1.8$. Next we measured drug sensitivity under voltage control of membrane potentials. Cells were held at a membrane potential of − 80 mV and human $Na_v1.8$ currents were generated by a voltage step to 0 mV. The presence of BIII 890 CL, tetracaine, NW-1029 or mexiletine reduced the peak current (Fig. 4A, B). The action of these inhibitors was concentration dependent with IC_{50} values of 2.1±0.2, 11±0.4, 22±0.5 and 130±15 μM, respectively (Fig. 4B). These potencies are linear with those observed for deltamethrin-activated human $Na_v1.8$ indicating that the relative affinities of these compounds for $Na_v1.8$ are the same regardless of the assay (Fig. 4C).

3.3. Comparison with rat $Na_v1.8$

We compared the properties of human $Na_v1.8$ with those of the rat orthologue in the same cell background. Clonal cell lines of SH-SY5Y cells expressing rat $Na_v1.8$ were isolated. Voltage-clamp experiments showed that rat $Na_v1.8$-expressing cells displayed tetrodotoxin-resistant currents with average peak amplitude of 670±60 pA (mean±S.E.M.; n=7 cells). Rat $Na_v1.8$ showed a very similar pharmacological profile to human $Na_v1.8$ with BIII 890 CL (Fig. 5; Table 1), tetracaine and NW-1029 being inhibitory and the other blockers ineffective. For both human and rat $Na_v1.8$, BIII 890 CL showed the highest potency and the sensitivity to BIII 890 CL was identical for rat and human $Na_v1.8$ (Table 1). Differences in sensitivity of rat and human $Na_v1.8$ to tetracaine, NW-1029 and mexiletine were

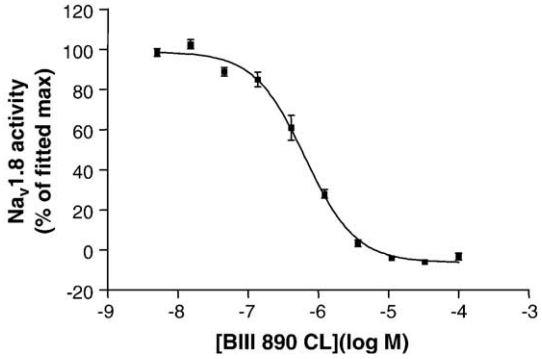


Fig. 5. Analysis of SH-SY5Y cell lines expressing rat $Na_v1.8$. Clonal cell lines expressing rat $Na_v1.8$ were activated with 10 μM deltamethrin and the effect of BIII 890 CL was determined as described in Fig. 2 for the human cell lines. The data represent averages±standard error of 14 observations.

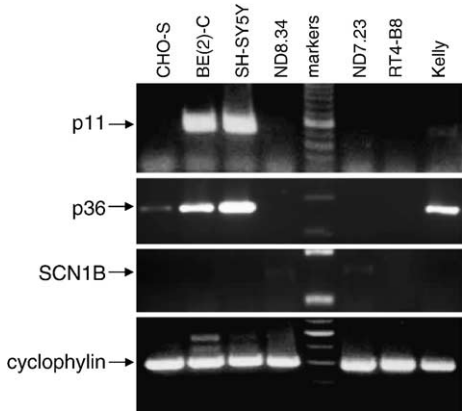


Fig. 6. Endogenous expression of sodium channel accessory proteins in neuroblastoma cells lines. RNA was isolated from neuroblastoma cell lines, reverse transcribed into cDNA and p11, p36, SCN1B or cyclophilin (positive control) were amplified by RT-PCR using specific oligonucleotide primers. Samples were analysed by agarose gel electrophoresis using either 100 bp (p11, cyclophilin) or 1 kb (p36, SCN1B) markers as reference.

observed whilst lidocaine, lamotrigine and carbamezapine did not inhibit either $Na_v1.8$ orthologue.

3.4. Expression of $Na_v1.8$ accessory proteins in SH-SY5Y cells

SH-SY5Y cells may support functional expression of $Na_v1.8$ by virtue of the presence of certain key accessory proteins. Recent studies have revealed that the small subunit of the annexin II complex, termed p11, is critical to the functional expression of $Na_v1.8$ in CHO cells and DRG neurones (Okuse et al., 2002). Furthermore, it has been reported that the sodium channel β1 subunit affects expression of $Na_v1.8$ in *Xenopus* oocytes (Rabert et al., 1998). In order to investigate if these potential accessory factors are endogenously expressed in SH-SY5Y cells, and in this way render these cells capable of supporting $Na_v1.8$ expression, we employed specific oligonucleotide primers and measured expression by RT-PCR. As shown in Fig. 6, SH-SY5Y cells express high levels of p11 mRNA. We also investigated the presence of p11 in other cell lines including other neuroblastoma cell lines and observed that BE(2)-C cells express p11 but other cell lines, including CHO cells, do not. As mentioned above p11 is part of a complex with the annexin heavy chain, p36. Interestingly, both SH-SY5Y cells and BE(2)-C cells expressed high levels of p36 (Fig. 6). CHO cells also showed p36 expression whilst other cell lines were negative for p36 (Fig. 6). High levels of expression of the sodium channel β1 subunit were noticed in HEK-293 cells (not shown) and low levels were present in N18Tg2xDRG fusion cell lines however no expression of this subunit was observed in other cell lines including SH-SY5Y cells (Fig. 6). Thus the presence of p11 either in complex with p36 or alone, may constitute a factor that renders SH-SY5Y cells capable of supporting $Na_v1.8$ expression.

4. Discussion

In this study we show that neuroblastoma SH-SY5Y cells support functional expression of human $Na_v1.8$ sodium channels. The basic biophysical properties of the expressed human current (threshold of activation −50 mV, V_{50} −11 mV) differed from those reported for the current from recombinant human $Na_v1.8$ expressed in *Xenopus laevis* oocytes (threshold of activation −20 mV, V_{50} 13 mV (Rabert et al., 1998)) and HEK293 cells (threshold −30 mV, V_{50} 2.5 mV (Akiba et al., 2003)). Human $Na_v1.8$ expressed in SH-SY5Y shares characteristics with the endogenous tetrodotoxin-resistant current in DRG neurones (threshold −50 mV, V_{50} −15 mV; (Roy and Narahashi, 1992); V_{50} −27 mV this study). Thus there is a progressive leftward shift in I/V relationship dictated by the expression system (oocytes > HEK > SH-SY5Y) resulting in a situation in SH-SY5Y cells that most closely resembles the endogenous current in DRG neurones.

The reason why SH-SY5Y cells are a suitable expression vehicle for $Na_v1.8$ is not clear at present. However SH-SY5Y cells may express critical accessory proteins required for optimal sodium channel expression. Several proteins determine the size and characteristics of the functional population of $Na_v1.8$. Sodium channel β_1 subunits but not β_2 subunits, affect the biophysical properties of $Na_v1.8$ in *Xenopus laevis* oocytes (Rabert et al., 1998) inducing a leftward shift in I/V relationship of the human current. However, β-subunit expression did not affect $Na_v1.8$ functional expression in CHO cells (Okuse et al., 2002) and HEK-293 cell lines containing sodium channel β_1 subunits reportedly expressed $Na_v1.8$ but kinetics and voltage dependence was different from those of the endogenous tetrodotoxin-resistant current in mouse DRG neurones (Liu et al., 2004). Our expression studies showed that SH-SY5Y cells did not express β_1 subunits confirming the notion that β_1 subunits themselves may not be sufficient to convey full functional characteristics to $Na_v1.8$. The annexin II light chain (p11) is a regulatory factor that facilitates the expression of $Na_v1.8$ in sensory neurones (Okuse et al., 2002). Sensory neurones express high endogenous levels of p11 and antisense downregulation of p11 expression results in a reduction of the $Na_v1.8$ current density in these cells. p11 binds directly to the amino terminus of $Na_v1.8$ and promotes the translocation of $Na_v1.8$ to the plasma membrane, producing functional channels (Okuse et al., 2002). p11 may be a factor in SH-SY5Y cells contributing to functional expression of $Na_v1.8$ since these cells express high levels of this subunit. p11 may act in conjunction with p36, the annexin II large chain since it too is expressed at high levels in SH-SY5Y cells.

Since SH-SY5Y cells support physiological expression of $Na_v1.8$, this expression system is appropriate for the screening for specific inhibitors of $Na_v1.8$. We have employed the pyrethroid insecticide deltamethrin as a proxy for $Na_v1.8$ activation in a cell based fluorescence assay system. The EC_{50} for channel modulation was within the range of that of the deltamethrin-induced modulation of endogenous tetrodotoxin-resistant sodium currents in rat DRG neurones (Tabarean and Narahashi, 1998, 2001). We tested a range of sodium channel blockers for their ability to modulate $Na_v1.8$. Tetrodotoxin did not inhibit human $Na_v1.8$ up to 100 μM which was the maximal concentration tested in this assay. This result differs from that observed by Akiba et al, who reported an IC_{50} value of 73.3 μM for tetrodotoxin inhibition of human $Na_v1.8$ expressed in HEK

cells. The difference between the two studies may be due to the difference in assay protocol, which involved deltamethrin activation in our protocol and voltage activation in the earlier study. Alternatively tetrodotoxin may affect human $Na_v1.8$ in a different manner depending on cell background.

Human $Na_v1.8$ was highly sensitive to BIII 890 CL. Tetracaine, NW-1029 and mexiletine also inhibited $Na_v1.8$ whilst lamotrigine, carbamezapine and lidocaine were inactive at the maximal concentration tested (100 μM). The inhibition of human $Na_v1.8$ by BIII 890 CL, tetracaine and NW-1029 has not been reported before however mexiletine and lidocaine affected human $Na_v1.8$ expressed in HEK293 cells with IC_{50} values of 345 and 332 μM, respectively. Human $Na_v1.8$ expressed in SH-SY5Y cells appears to be more sensitive to mexiletine which as for tetrodotoxin may be due to the different cell background in which the inhibitor was tested. The pattern of inhibition of $Na_v1.8$ is of interest in the light of the known effects of these compounds on pain-related behaviours in the intact animal and the proposed role of $Na_v1.8$ in pain signalling (Dekker and Cronk, 2005). NW-1029 reverses the mechanical allodynia induced by chronic inflammation or by chronic constriction of the sciatic nerve (Veneroni et al., 2003) and BIII 890 CL reverses mechanical joint hyperalgesia (Laird et al., 2001). Mexiletine is more effective in reversing the mechanical allodynia associated with nerve ischaemia than either lidocaine or lamotrigine (Erichsen et al., 2003). Although inhibition of $Na_v1.8$ may underlie the action of these compounds in vivo it should be noticed that the above inhibitors lack specificity and effects through other sodium channels may contribute to their anti-nociceptive action.

In summary, we have presented data indicating that SH-SY5Y neuroblastoma cell lines are capable of supporting physiological expression of human $Na_v1.8$ sodium channels allowing pharmacological assessment of human $Na_v1.8$ in a native conformation. Differences between rat and human $Na_v1.8$ observed before most likely relate to the differences in cell background in which the channels have been tested. Our cell system is important for further structure-function and pharmacological analysis of this sodium channel subunit and for the identification of novel chemical entities acting on this channel.

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