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Differential modulation of $Na_v1.7$ and $Na_v1.8$ peripheral nerve sodium channels by the local anesthetic lidocaine

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1 Voltage-gated Na^+ channels are transmembrane proteins that are essential for the propagation of action potentials in excitable cells. $Na_v1.7$ and $Na_v1.8$ dorsal root ganglion Na^+ channels exhibit different kinetics and sensitivities to tetrodotoxin (TTX). We investigated the properties of both channels in the presence of lidocaine, a local anesthetic (LA) and class I anti-arrhythmic drug.

2 $Na_v1.7$ and $Na_v1.8$ Na^+ channels were coexpressed with the β_1-subunit in *Xenopus* oocytes. Na^+ currents were recorded using the two-microelectrode voltage-clamp technique.

3 Dose–response curves for both channels had different EC_{50} (dose producing 50% maximum current inhibition) (450 μM for $Na_v1.7$ and 104 μM for $Na_v1.8$). Lidocaine enhanced current decrease in a frequency-dependent manner. Steady-state inactivation of both channels was also affected by lidocaine, $Na_v1.7$ being the most sensitive. Only the steady-state activation of $Na_v1.8$ was affected while the entry of both channels into slow inactivation was affected by lidocaine, $Na_v1.8$ being affected to a larger degree.

4 Although the channels share homology at DIV S6, the LA binding site, they differ in their sensitivity to lidocaine. Recent studies suggest that other residues on DI and DII known to influence lidocaine binding may explain the differences in affinities between $Na_v1.7$ and $Na_v1.8$ Na^+ channels.

5 Understanding the properties of these channels and their pharmacology is of critical importance to developing drugs and finding effective therapies to treat chronic pain.
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Keywords: $Na_v1.7$; $Na_v1.8$; sodium channels; lidocaine; local anesthetics; pain; peripheral nerve; dorsal root ganglion; *Xenopus* oocytes

Abbreviations: D, domain; DRG, dorsal root ganglions; EC_{50}, dose producing 50% maximum current inhibition; LA, local anesthetics; Na^+, sodium; S, transmembrane segment; TTX, tetrodotoxin; TTX-S, tetrodotoxin-sensitive TTX-R, tetrodotoxin-resistant

Introduction

Voltage-gated sodium (Na^+) channels are key in regulating neuronal excitability and the generation and propagation of action potentials (Hille, 2001). They thus play an important role in transmitting nociceptive information throughout the peripheral and central nervous systems. At least 10 different isoforms of Na^+ channels have been identified in the brain, neurons and striated muscles. They differ in their gating properties, pharmacology (tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R)) and permeation (Goldin, 1999; Yu & Catterall, 2003). Dorsal root ganglion (DRG) neurons express at least six distinct isoforms of Na^+ channels (Rush *et al*., 1998). While the roles of the individual isoforms are unclear, recent studies have suggested that the TTX-S channels in DRG neurons play an important role in the early part of the action potential, while TTX-R channels are thought to be crucial throughout the whole time course of the action potential (Blair & Bean, 2002).

The major component of the Na^+ channels from different tissues is the 260 kDa α-subunit, which forms the pore of the channel (Goldin *et al*., 1986). The α-subunit is composed of four homologous domains (DI–DIV), each of which is composed of six transmembrane segments (S1–S6) (Catterall, 1992; Fozzard & Hanck, 1996). *In vivo*, most Na^+ channels associate with auxiliary β-subunits (β_1–β_4), which have an average molecular weight of 30 kDa. β-Subunits modulate the level of expression and gating of these channels (Isom *et al*., 1992; Morgan *et al*., 2000; Yu *et al*., 2003). For instance, β_1-subunits increase the expression of $Na_v1.8$ and accelerate the time constant of inactivation and recovery from inactivation of $Na_v1.7$ and $Na_v1.8$ (Vijayaragavan *et al*., 2001).

Na^+ channels are the target of multiple drugs that alter their activity (Chen *et al*., 2000; O'Leary & Chahine, 2002; Wang & Wang, 2003). For instance, a widely used local anesthetic (LA) and class I anti-arrhythmic, lidocaine, suppresses Na^+ currents by binding not only to DIV–S6 but also to S6 of DI and DII, blocking the channels in a use-dependent (frequency-dependent) and voltage-dependent manner (Ragsdale *et al*., 1994; Kondratiev & Tomaselli, 2003). A major effect of LAs is to further decrease Na^+ currents, thereby suppressing cellular excitability. It has been proposed that LA, in its charged form, preferentially binds inactivated channels (Hille, 1977; Hille, 2001). While most of the effect

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of lidocaine appears to develop gradually during depolarization, a significant proportion occurs rapidly. Lidocaine is thus believed to also block open channels (Matsubara *et al*., 1987).

The study presented here examined the effect of lidocaine on two DRG-specific Na^+ channel isoforms, namely $Na_v1.7$ and $Na_v1.8$. Both channels appear to accumulate in painful human neuromas and are responsible for ectopic axonal hyper excitability, resulting in abnormal sensory phenomena such as pain and paresthesias (Kretschmer *et al*., 2002). The channels were expressed in *Xenopus* oocytes in the presence of the auxiliary β_1-subunit. Their sensitivity to different concentrations of lidocaine as well as the effect on different gating properties were examined. We observed that $Na_v1.8$ was more sensitive to blocking by lidocaine. Understanding the modulation of these channels by LAs is important to understanding LA mechanisms of action in anesthesia and pain management.

Methods

Molecular biology

The rat $Na_v1.7$ α-subunit Na^+ channel cloned into the pCDNA3a vector was provided by Gail Mandel (Department of Neurobiology, State University of New York, NY, U.S.A.). The rat $Na_v1.8$ α-subunit was cloned from rat DRG neurons and inserted in the pSP64T vector (Vijayaragavan *et al*., 2001). The cRNA was prepared by the T7 (pCDNA3a) or SP6 (pSP64T) mMessage mMachine kit (Ambion, TX, U.S.A.).

Expression and electrophysiology

Xenopus laevis females were anesthetized by immersion in 1.5 mg ml^{-1} tricaine (Sigma, Oakville, ON, Canada), and two or three ovarian lobes were removed surgically under semisterile conditions. Follicular cells surrounding the oocytes

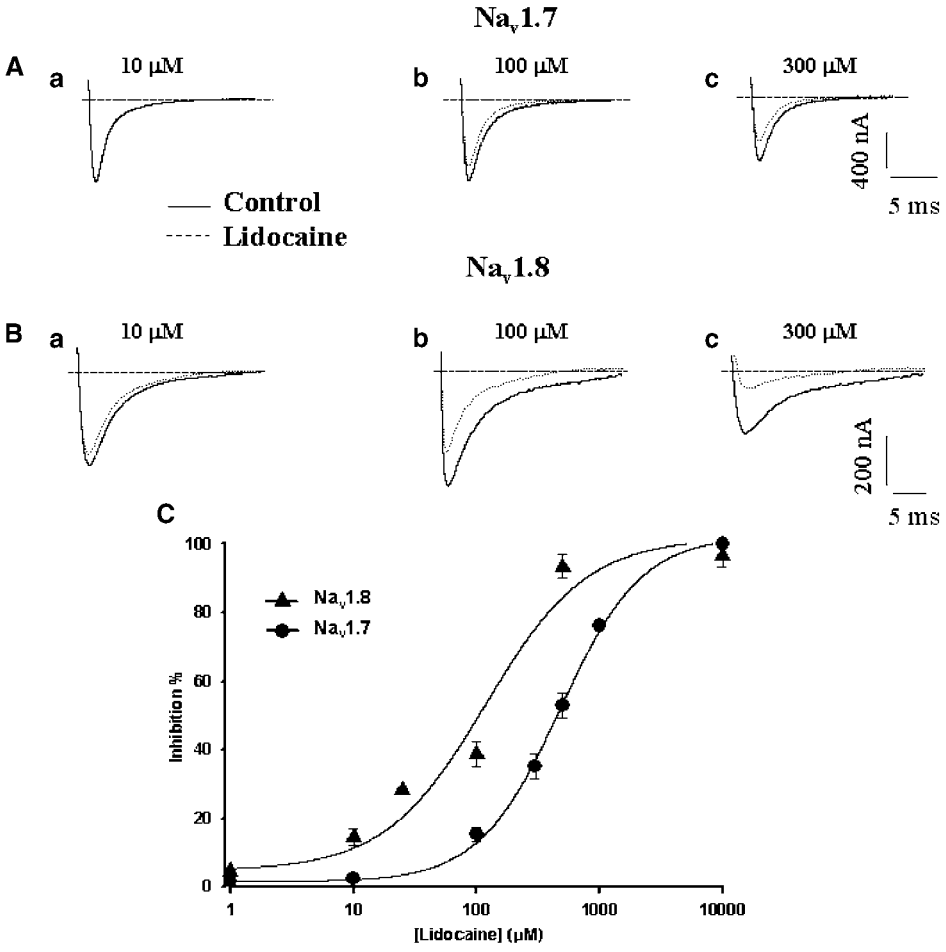

Figure 1 Dose-dependent block of $Na_v1.7$ and $Na_v1.8$ currents by different lidocaine concentrations. Whole-cell Na^+ currents were evoked every 5 s by 40 ms pulses to −15 mV from a holding potential of −100 mV until current stabilized ($Na_v1.7$). Solid lines represent control currents while dotted lines represent the current amplitude after superfusion with lidocaine to produce a steady-state lidocaine effect. For $Na_v1.8$, whole-cell Na^+ currents were evoked every 20 s by 40 ms pulses to +20 mV from a holding potential of −100 mV until current stability was obtained. Solid lines represent currents under control conditions while dotted lines represent currents after lidocaine superfusion to produce a steady-state lidocaine effect. (a) 10 μM , 100 μM (b) and 300 μM (c). $Na_v1.7$ (A) and Na_v 1.8 (B). (C) Dose–response curves for both $Na_v1.7$ and $Na_v1.8$ channels ($n=5$) were obtained from fits of a four parameters Hill's equation described in the Methods section. The values of the Hill coefficients for $Na_v1.7$ were: $a=100.79$, $b=1.31$, $c=477.10$ and $y_0=1.52$. For $Na_v1.8$, the values were: $a=96.98$, $b=1.06$, $c=118.31$ and $y_0=4.78$. Filled circles represent $Na_v1.7$ and filled triangles represent $Na_v1.8$. $Na_v1.8$ exhibits greater sensitivity to lidocaine than $Na_v1.7$ (~4.4-fold), with EC_{50} values of 104 and 450 μM, respectively.

were removed by incubation at 22°C for 2.5 h in calcium-free oocyte medium containing 82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES (pH 7.6) and 2 mg ml^{-1} collagenase (Sigma). The oocytes were washed first in calcium-free medium and then with 50% Leibovitz's L-15 medium (Life Technologies, Burlington, ON, Canada) supplemented with 15 mM HEPES (pH 7.6), 5 mM L-glutamine and 10 mg ml^{-1} gentamycin. The oocytes were stored in this medium until used. Stage IV–V oocytes were selected and microinjected with 50 nl of cRNA coding for the α-subunit of $Na_v1.7$ and the β_1-subunit or the $Na_v1.8$ α-subunit and the β_1-subunit (the β_1 subunit was included in all experiments since it was previously shown to improve the expression levels of $Na_v1.8$ and modulate its gating properties) (Vijayaragavan *et al*., 2001). The oocytes were stored at 18°C and used for experiments a few days later, depending on the level of expression of each channel type. The animals were treated in accordance with Canadian Institutes of Health Research guidelines. Whole-cell Na^+ currents in cRNA-injected oocytes were measured using a two-microelectrode voltage clamp at room temperature (22°C). The oocytes were impaled with 2 MΩ electrodes filled with 3 M KCl and were voltage-clamped with an OC-725 oocyte clamp (Warner Instruments, Hamden, CT, U.S.A.). Currents were filtered at 1.5 kHz with an eight-pole Bessel filter and were sampled at 10 kHz. Data were acquired and analyzed with pClamp software v7 (Axon Instruments, Foster City, CA, U.S.A.). Oocytes were held at −100 mV and pClamp software was used to generate pulses that depended on the electrophysiological protocol.

Current activation curves of the channels were plotted using the following Boltzmann equation: $G_{Na}/G_{Na,max} = 1/(1 + \exp((V + V_{1/2})/k_v))$, for which the G_{Na} (conductance) value for each clamped oocyte was determined by dividing the peak Na^+ current by the driving force ($V_m - E_{Na}$). The reversal potential (E_{Na}) for each oocyte expressing either channel was estimated by extrapolating the linear ascending segment of the current voltage relationship (I/V) curve to the voltage axis between 0 and +20 mV for $Na_v1.7$ and between +20 and +40 mV for $Na_v1.8$. V is the voltage test, $V_{1/2}$ is the voltage at which the channels are half-maximally activated and k_v is the slope factor. Steady-state inactivation *versus* voltage was also plotted using a similar but decaying Boltzmann equation.

Solutions and reagents

The Ringer's bath solution contained 90 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$ and 5 mM HEPES (pH 7.6).

Lidocaine (Sigma) was diluted at room temperature in the Ringer's solution to produce a 5 mM stock solution. Different concentrations were then applied to the oocytes by continuous superfusion during the course of the experiments.

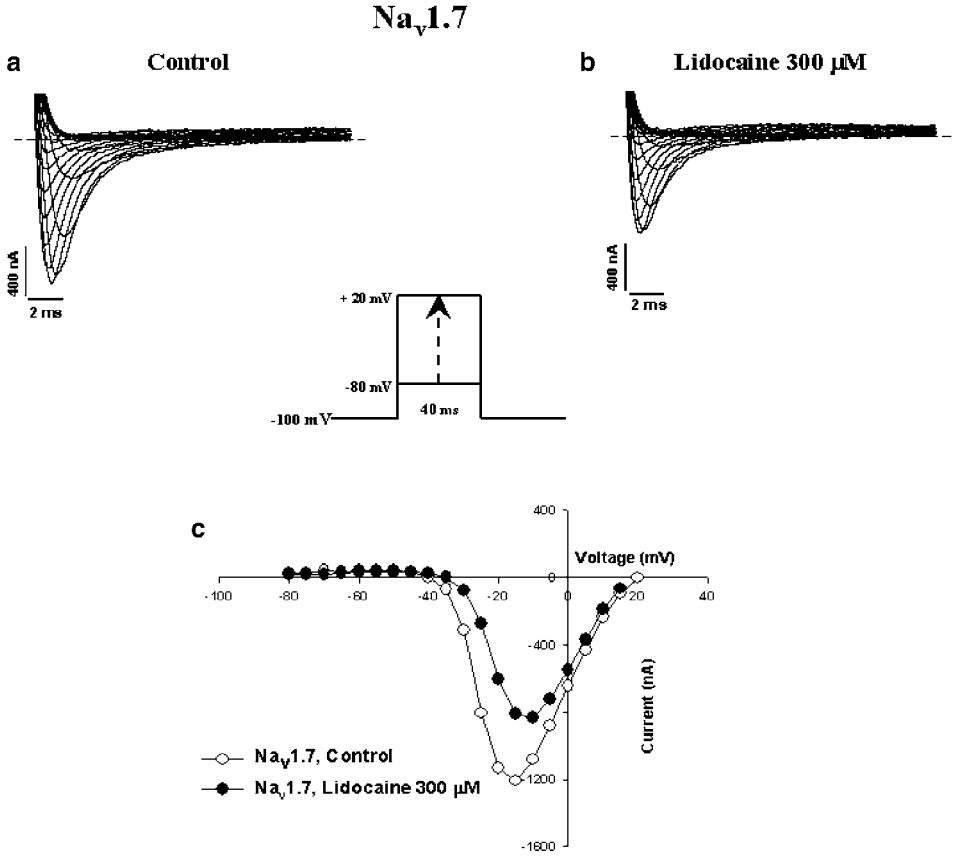

Figure 2 Effect of lidocaine on $Na_v1.7$ Na^+ channels heterologously expressed in *Xenopus* oocytes. Whole-cell Na^+ current traces of oocytes expressing $Na_v1.7$ before (a) and after (b) superfusion with 300 μM of lidocaine. Also shown in (c) are the effects of lidocaine (300 μM) on the current–voltage relationship (I/V curves) in control conditions (open circles) and in the presence of the anesthetic (filled circles). Currents were elicited by depolarizing steps between −80 and +20 mV in 5 mV increments from a holding potential of −100 mV (see figure inset for protocol). Dashed lines are zero current. I/V curves were obtained by plotting the current amplitude *versus* the voltage for the currents shown in (a) and (b).

Statistical analysis

Results of representative measures are expressed as means ± s.e.m. Data and graphs were analyzed using Sigmaplot 2001 for Windows version 7.0 (SPSS Inc., Chicago, IL, U.S.A.). The results were considered significant if P-values were <0.05. The fittings of the dose–response curves (Figure 1) were carried out with Sigmaplot 2001 for Windows version 7.0, using a four parameters Hill's equation: $y = y_0 + a\ x^b/(c^b + x^b)$.

Results

$Na_v1.7$ and $Na_v1.8$ Na^+ channels have different sensitivities to lidocaine

$Na_v1.7$ and $Na_v1.8$ exhibited different sensitivities to lidocaine. Figures 1A and B show representative current traces before and after superfusion of different concentrations of lidocaine for $Na_v1.7$ (10, 100 and 300 μM, Figures 1Aa, Ab and Ac, respectively) and $Na_v1.8$ (10, 100 and 300 μM, Figures 1Ba, Bb and Bc, respectively). The resulting dose–response curve (Figure 1C) shows a difference in sensitivity between the channels. $Na_v1.8$ has an EC_{50} (concentration of drug necessary to inhibit 50% of Na^+ currents) of 104 μM, while $Na_v1.7$ has an EC_{50} of 450 μM. Figures 2a and b show representative whole-cell Na^+ current traces recorded from oocytes expressing $Na_v1.7$ under control conditions (a) and in the presence of 300 μM lidocaine (b). Inward Na^+ currents were evoked by applying a series of depolarizing voltage steps between −80 to +20 mV in 5 mV increments (see figure inset for protocol). Figure 2C shows representative I/V curves for $Na_v1.7$ with and without the drug. The channels activated at −40 mV and peaked at −10 mV in both the absence and presence of 300 μM lidocaine. Figures 3A and B show representative whole-cell traces of Na^+ currents recorded from oocytes expressing $Na_v1.8$, under control conditions (a) and in the presence of 300 μM lidocaine (b). Na^+ currents were evoked by applying a series of depolarizing steps between −80 to +40 mV in 5 mV increments (see figure inset for protocol). Figure 3c shows representative I/V curves of $Na_v1.8$ in the presence and absence of 300 μM lidocaine. Lidocaine (300 μM) added to the bath solution produced a ~10 mV depolarized shift.

Effect of lidocaine on the gating of $Na_v1.7$ and $Na_v1.8$

The availability of Na^+ channels upon depolarization is dependent on a number of factors, one of them being the cell membrane resting potential. Fewer channels become available as the resting membrane potential progressively moves towards more depolarized voltages. This effect is due to the accumulation of channels in the nonconducting inactivated state. Experimentally, this phenomenon was measured using

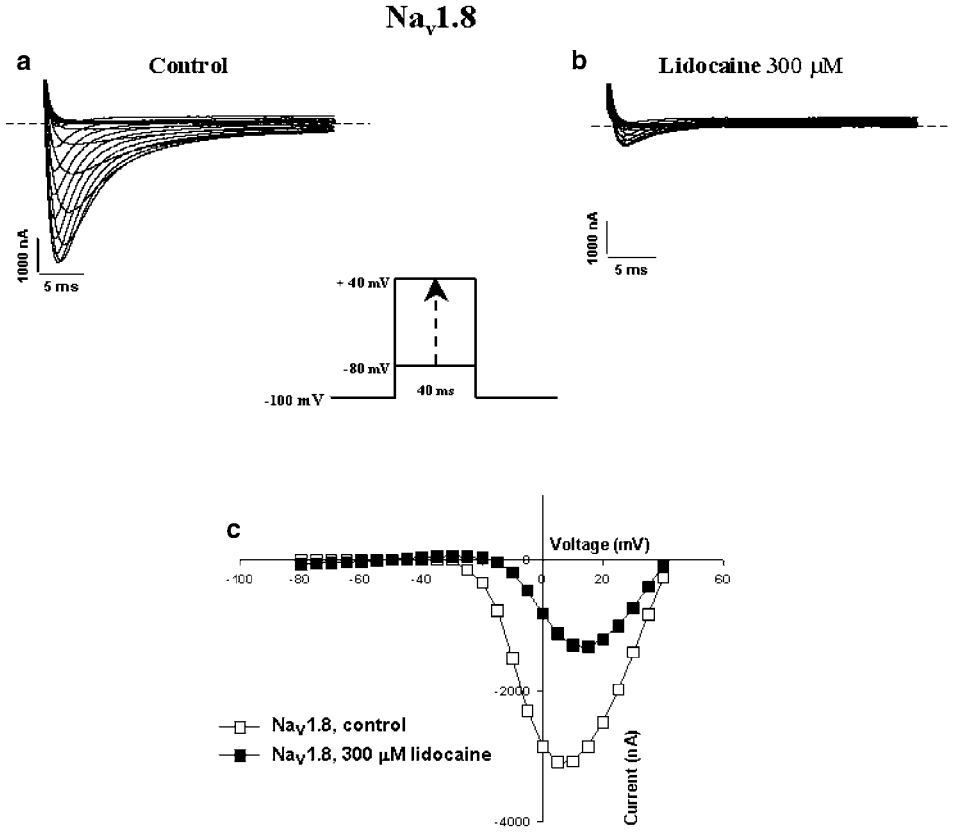

Figure 3 Effect of lidocaine on $Na_v1.8$ Na^+ channels heterologously expressed in *Xenopus* oocytes. Whole-cell Na^+ current traces of oocytes expressing $Na_v1.8$ before (a) and after (b) superfusion with 300 μM of lidocaine. Also shown in (c) are the effects of lidocaine (300 μM) on the current–voltage relationship (I/V curves) in control conditions (open circles) and in the presence of the anesthetic (filled circles). Currents were elicited by depolarizing steps between −80 and +40 mV in 5 mV increments from a holding potential of −100 mV (see figure inset for protocol). Dashed lines are zero current. I/V curves were obtained by plotting the current amplitude *versus* the voltage for the currents shown in (a) and (b).

constant 500 ms conditioning pulses to voltages between −110 and +30 mV. The fraction of available current left was measured by standard test pulses (–10 mV for $Na_v1.7$ and +15 mV for $Na_v1.8$). The normalized currents were then plotted against the conditioning voltage (Figure 4). Lidocaine (100 μM) significantly shifted the $V_{1/2}$ of inactivation for $Na_v1.7$ by 10.6 mV ($P<0.05$) towards more hyperpolarized values but did not significantly shift the slope factor of the curve (Table 1 and Figure 4a). Figure 4b shows that lidocaine had less effect on $Na_v1.8$. Lidocaine significantly shifted the $V_{1/2}$ towards more hyperpolarized voltages by 4 mV ($P<0.05$) and had a nonsignificant effect on the slope factor (k_v) (Table 1).

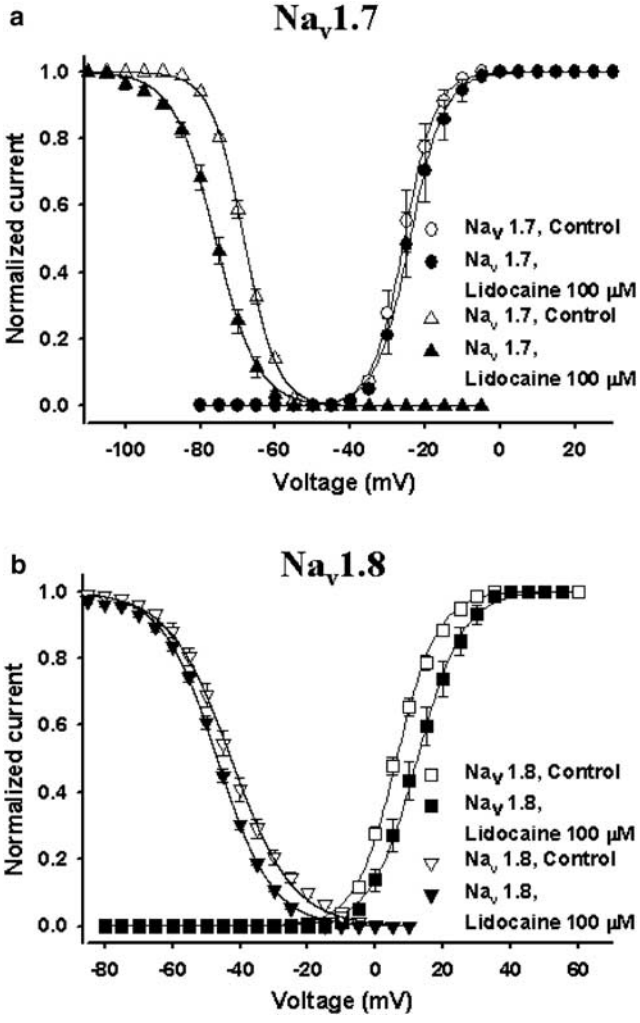

Figure 4 Effect of lidocaine on the steady-state inactivation and steady-state activation curves of $Na_v1.7$ (a) and $Na_v1.8$ (b). Steady-state activation curves were derived from the same family of currents used for the I/V curves (Figures 2c and 3c) using the standard procedure (see Methods). Steady-state inactivation were determined using 500 ms conditioning pulses to voltages between −110 and +30 mV and a standard test pulse to −20 mV for $Na_v1.7$ or +15 mV for $Na_v1.8$. Test currents were normalized and plotted against the conditioning voltage. The steady-state properties for $Na_v1.7$ (a, open circles and open triangles, respectively) and $Na_v1.8$ (b, open squares and open reversed triangles, respectively) in the absence of lidocaine are shown on the same graph as in the presence of lidocaine 100 μM (filled circles and filled triangles (a) and filled squares and filled reversed triangles (b)). The smooth curves are Boltzmann fits (the equations are shown in Methods). See Table 1 for $V_{1/2}$ and k_v values for both activation and inactivation.

The effect of lidocaine on the steady-state activation of the two channels was also investigated. The activation curves were derived from the I/V curves (see Methods). The activation curves of $Na_v1.7$ and $Na_v1.8$ in the absence and presence of lidocaine 100 μM were plotted against voltage (Figures 4a and b). For $Na_v1.7$, lidocaine did not shift the midpoint of steady-state activation or the slope factor significantly. For $Na_v1.8$, lidocaine caused a significant 6.1 mV depolarized shift of the midpoint of steady-sate activation ($P<0.05$). Lidocaine did not change significantly the slope factor.

Overall, lidocaine caused a significant hyperpolarizing shift in the steady-state inactivation of $Na_v1.7$ channels and affected the voltage-dependence of $Na_v1.8$ inactivation to a lesser extent. However, lidocaine significantly shifted the steady-state activation curve of $Na_v1.8$ toward more depolarized potentials but did not affect the voltage-dependent activation of $Na_v1.7$.

Lidocaine produces a use-dependent inhibition of both $Na_v1.7$ and $Na_v1.8$ channels

During step depolarization, Na^+ channels are induced to cycle through activated, inactivated and resting states. However, when they are subjected to a train of depolarizing pulses, the number of channels available to open is reduced and they progressively accumulate in the inactivated state. This phenomenon is referred to as use-dependence or 'frequency-dependent' blocking. In the presence of a LA, the further decrease in currents could be attributed to the accumulation of channels in a drug-modified state. This effect of rapid pulsing on $Na_v1.7$ and $Na_v1.8$ was tested by applying a series of 50 short 8 ms depolarizing pulses (−10 mV for $Na_v1.7$ and +15 mV for $Na_v1.8$). We observed a dramatic difference in the sensitivities of both $Na_v1.7$ and $Na_v1.8$ at different frequencies when they were superfused with the same concentrations of lidocaine (300 μM, Figure 5). As shown in Figure 5a, there was little change in $Na_v1.7$ channel availability when they were stimulated at frequencies between 0.5 to 5 Hz since the currents remained above 90% of their initial value. However, in the presence of 300 μM lidocaine, $Na_v1.7$ channel availability was reduced to slightly less than 80% of their maximal peak when stimulated at 5 Hz (Figure 5a). On the other hand, $Na_v1.8$ was more sensitive to the frequencies used when superfused with the same concentration of lidocaine (300 μM in Figure 5b). While little reduction in current was observed when the channels were pulsed at 0.5 Hz under control conditions, superfusion with lidocaine induced a 20% reduction in Na^+ currents. Pulsing under control conditions at 2 Hz also led to a decrease of approximately 20%, but, in the presence of lidocaine, the currents were reduced to 50% of their initial amplitude. At 5 Hz without the drug, a decrease to 70% of the initial current value was observed, while, in the presence of lidocaine, the decrease stabilized at 20% of the normalized initial current. The reduction in currents observed for $Na_v1.8$ increased with the frequency used.

$Na_v1.8$'s decrease in currents during use-dependent block was more pronounced in the presence of lidocaine with a higher frequency causing a more important blocking effect (Figure 6). This again pointed to a difference in channel sensitivity and shows that $Na_v1.8$ was more affected by the presence of lidocaine. Figure 6 shows a comparison of the

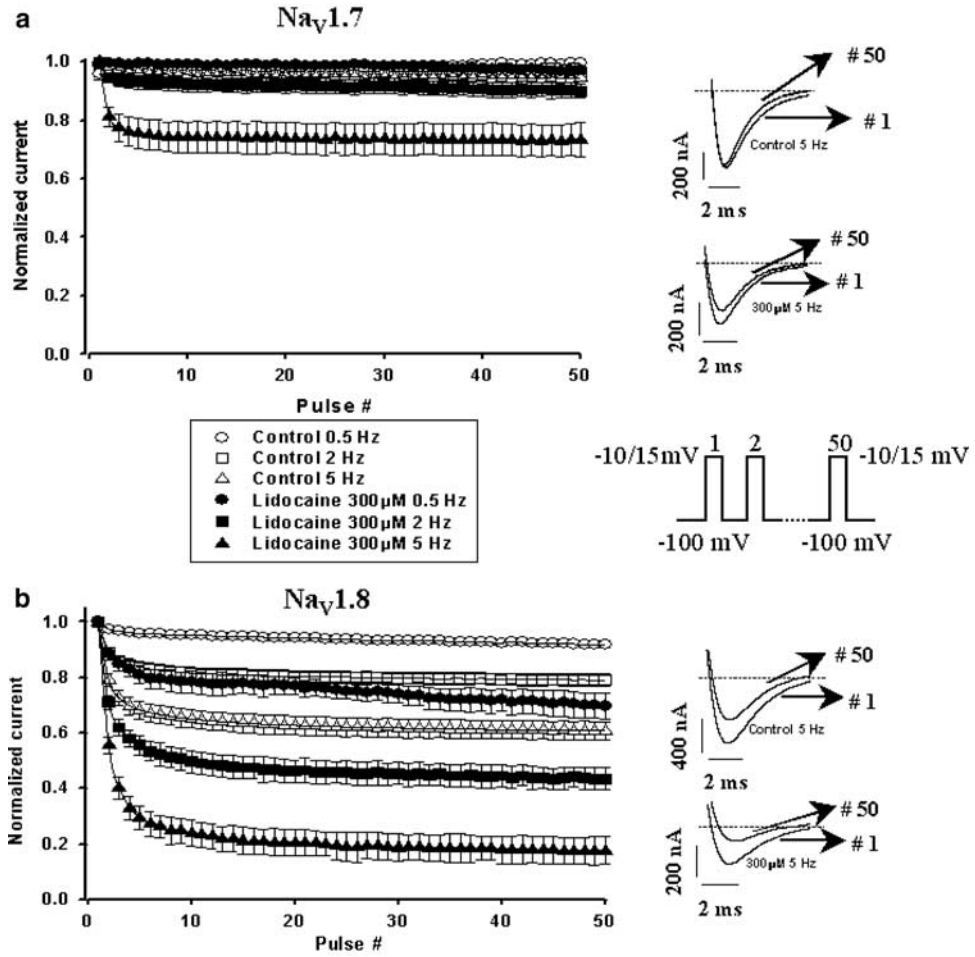

Figure 5 Frequency-dependent inhibition of $Na_v1.7$ (a) and $Na_v1.8$ (b) Na^+ currents in the presence and absence of lidocaine 300 μM. Oocytes were held at −100 mV and a train of fifty 8 ms pulses was applied to −10 mV ($Na_v1.7$) or +15 mV ($Na_v1.8$) at three different frequencies (0.5, 2 and 5 Hz), with the interpulse potential also set at −100 mV. The peak currents elicited by each pulse were normalized to the current of the first pulse ($P_n - P_1$, where $n = 1$–50) and were then plotted *versus* pulse number. Different open symbols represent control conditions while filled symbols represent the protocol in the presence of 300 μM lidocaine for the different frequencies (circles represent 0.5 Hz, squares represent 2 Hz and triangles represent 5 Hz). Examples of current traces at the 1st and 50th pulse of the protocol for each channel in the presence and absence of lidocaine are shown in the right panel. The central panel shows a schematic representation of the electrical protocol used.

current amplitudes at the 50th pulse of the use-dependent protocol at different concentrations of lidocaine (100 and 300 μM). No obvious differences were noted between the two concentrations for $Na_v1.7$, while a drastic decrease in the amplitudes of $Na_v1.8$ currents occurred with the increase in drug concentration when current amplitudes at the same frequencies were compared.

Development of slow inactivation for $Na_v1.7$ and $Na_v1.8$ in the presence of lidocaine

Na^+ channels have different inactivation states: fast, intermediate and slow. $Na_v1.7$ and $Na_v1.8$ expressed with the β_1-subunit exhibit different slow inactivation development kinetics (Vijayaragavan *et al.*, 2001). It therefore seemed appropriate to determine whether lidocaine affects the slow inactivation development of both channels. We therefore decided to compare the effects of lidocaine on $Na_v1.7$ and $Na_v1.8$. The onset of slow inactivation was measured experimentally by depolarizing the oocytes to either −20 mV ($Na_v1.7$) or +20 mV ($Na_v1.8$) for an interval that varied from 0 ms to 10 s to induce channel inactivation. After the depolarization step, the voltage was returned to −100 mV for 150 ms to allow recovery of fast-inactivated channels, before a standard 8 ms test pulse was applied to measure the amount of available Na^+ currents (Vijayaragavan *et al.*, 2001). The amplitudes of the Na^+ currents measured by the test pulse were then normalized *versus* the control currents and plotted against the duration of the conditioning-pulse interval. Using this protocol, the progressive decrease in currents with the increase of the prepulse duration was representative of channel entry into the slow-inactivated state from which channels do not recover during the short depolarization that precedes the test pulse (100 mV for 20 ms) (Figure 7 inset). The onset of slow inactivation for $Na_v1.7$ was best fitted with the sum of three exponentials (See Table 1 and Figure 7a). For $Na_v1.8$, the onset of slow inactivation was also best fitted with the sum of three exponentials (See Table 1 and Figure 7b). The time constants of slow inactivation of $Na_v1.7$ and $Na_v1.8$ are different in control conditions (Vijayaragavan *et al.*, 2001). We

showed that lidocaine enhanced entry into slow inactivation. The time constants in the presence of 100 μM lidocaine were significantly accelerated by lidocaine based on their response to slow inactivation, $Na_v1.8$ was the most affected (Table 1).

Discussion

In this study, we examined the effects of the widely used LA, lidocaine, on $Na_v1.7$ and $Na_v1.8$ channels and showed that they had different sensitivities and were differentially affected by lidocaine. $Na_v1.8$ was 4.4-fold more sensitive to lidocaine than $Na_v1.7$. Similar findings have been observed with native DRG neurons, where the TTX-R currents is more sensitive to lidocaine than the TTX-S currents (Roy & Narahashi, 1992). The effect of LA on Na^+ channels is generally characterized by a tonic block at low-frequency stimulations, a phasic block and a use-dependent block at high-frequency stimulations. Lidocaine decreased current in both tonic (0.5 Hz) and use-dependent blocking (2–5 Hz) of $Na_v1.7$ and $Na_v1.8$. However, during low-frequency stimulations, $Na_v1.8$ was four-fold more sensitive to the same concentration of drug.

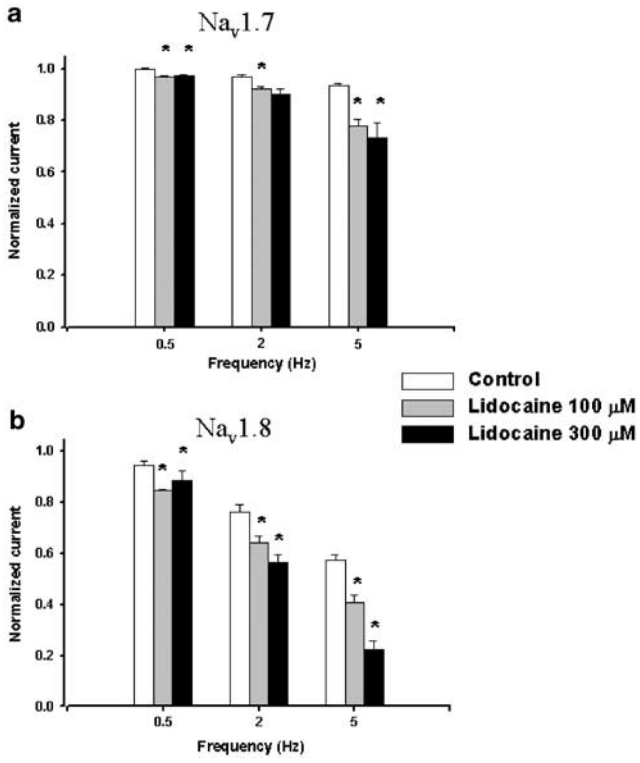

Figure 6 Bar plot representation of the relative amplitudes at the 50th sweep of the frequency dependence protocol used in Figure 5 for each frequency. The amplitudes of the last step were normalized *versus* the first step of the protocol. White columns are control currents, gray columns are currents' amplitudes after perfusion with 100 μM lidocaine and black columns are currents' amplitudes in the presence of 300 μM lidocaine. (* = $P<0.05$).

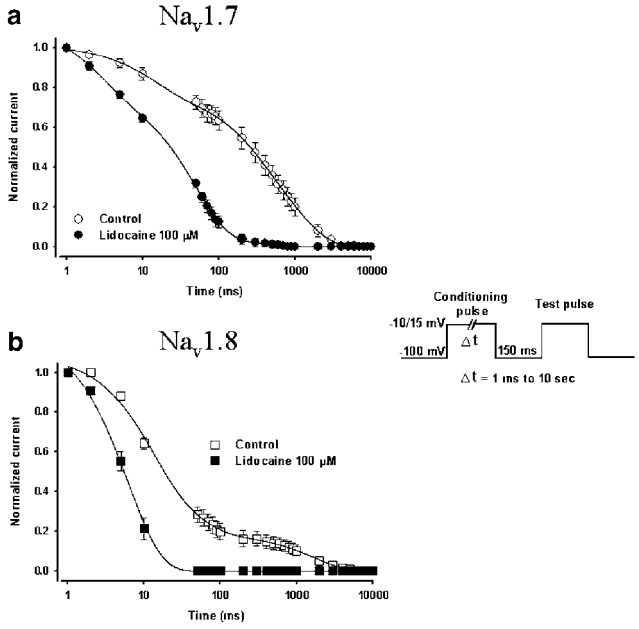

Figure 7 Development of slow inactivation by both $Na_v1.7$ (a) and $Na_v1.8$ (b) channels with and without lidocaine. Control conditions are open circles ($Na_v1.7$) and open squares ($Na_v1.8$) while experiments with the drug are represented by filled circles and squares. The entry into slow inactivation was measured using a double-pulse protocol consisting of a conditioning pulse of variable duration (1 ms to 10 s) to −10 mV ($Na_v1.7$) or 15 mV ($Na_v1.8$) to inactivate the channels. A 150 ms pulse to −100 mV was then applied to allow rapid recovery and a standard test pulse was used to measure the amount of available channels (see inset). The measured currents were then normalized and plotted against the duration of the conditioning pulse. The decrease in currents was best fitted in all cases with the sum of three exponentials (solid lines). See Table 1 for the time-constant values.

Table 1 Effects of lidocaine on fast activation, inactivation and slow inactivation parameters for Na_v 1.7 and Na_v 1.8

	Activation			*Inactivation*			*Slow inactivation*			
	$V_{1/2}$ (mV)	k_v	n	$V_{1/2}$ (mV)	k_v	n	τ_F (ms)	τ_I (ms)	τ_S (ms)	n
Na_v 1.7, control	−25.56±1.53	−3.75±0.17	5	−68.38±0.42	4.37±0.08	5	14.42±2.95	428.88±92.83	989.50±165.88	4
Na_v 1.7, lidocaine (100 μM)	−23.92±1.85	−3.89±0.22	5	−79.02±1.49*	5.52±0.24*	5	2.40±0.40*	47.00±3.62*	107.60±59.88*	4
Na_v 1.8, control	6.24±0.68	−5.73±0.21	5	−42.72±1.23	9.05±0.08	5	11.27±0.22	31.54±8.42	1656.34±278.81	5
Na_v 1.8, lidocaine (100 μM)	12.32±1.78*	−6.63±0.26	5	−46.81±0.58*	8.07±0.20*	5	6.52±0.85*	6.65±0.73*	6.49±0.86*	5

τ_F = fast inactivation time constant; τ_I = intermediate inactivation time constant; τ_S = slow inactivation time constant; n = number of experiments. * = $P<0.05$.

Voltage-gated Na^+ channels cycle through several states but principally go through the resting, open and inactivated states. In this study, we observed that repetitive pulsing caused a major current reduction for $Na_v1.8$ but had little effect on $Na_v1.7$. Lidocaine produced an even greater decrease in Na^+ currents for both channels (Figures 5 and 6). The enhancement of the inhibition of Na^+ currents by lidocaine was more significant for $Na_v1.8$ than for $Na_v1.7$ channels. This suggests that the drug binds more effectively to $Na_v1.8$ channels.

Lidocaine also differentially modified the gating properties of $Na_v1.7$ and $Na_v1.8$ channels. Lidocaine did not shift the steady-state activation curve significantly for $Na_v1.7$, but did for $Na_v1.8$. This may explain in part the greater affinity of lidocaine for $Na_v1.8$. Lidocaine shifted the steady-state inactivation curve of $Na_v1.7$ by 10.6 mV towards more hyperpolarized values. In contrast, lidocaine caused a hyperpolarizing shift of only 4.1 mV for $Na_v1.8$. The 10.6 mV shift of steady-state inactivation observed with $Na_v1.7$ could explain the decrease in Na^+ currents observed at low-frequency stimulations. This suggests that different mechanisms are involved in the blocking of the two channels by lidocaine.

Since lidocaine is an open channel blocker that interacts with the channel pore and since previous studies have suggested that it induces $Na_v1.4$ occupancy in the slow inactivated state, we investigated the effect of lidocaine on the slow inactivation of both channels. The onset of slow inactivation for $Na_v1.8$ under control conditions was much faster than for $Na_v1.7$. This tendency of $Na_v1.8$ to enter the slow inactivated state more rapidly could explain the greater sensitivity of this channel to slow repetitive stimulations (Figures 5 and 6) since the channels, once in the slow inactivated state, do not recover in the short interval between the pulses of the use-dependence protocol. While lidocaine greatly affected the slow inactivation of both channels, $Na_v1.8$ was affected the most. While $Na_v1.7$ was affected, it tended to be much more resistant, which explains why we observed a larger decrease in Na^+ currents during repetitive stimulations at the highest frequency and in the presence of lidocaine for $Na_v1.8$ (Figures 5 and 6). The high-affinity binding of lidocaine to the slow inactivated state of $Na_v1.8$ could be important in blocking nociceptor firing, as previously suggested (Blair & Bean, 2002).

Although Na^+ channels' amino-acid sequences appear to be very conserved, some differences have been observed among the members of the Na^+ channel family. While the DIV S6 segment is regarded by most as the high affinity-binding site for local anesthetics (the region conserved by both $Na_v1.7$ and $Na_v1.8$), mutations of residues on other S6 regions can affect the binding of LAs. For instance, three residues (I436, I782 and V787) on the S6 segments of DI and DII in $Na_v1.4$ were suggested to play a role in the binding of LAs (Kondratiev & Tomaselli, 2003). Amino-acid sequence comparisons have shown that these three residues are not conserved between $Na_v1.7$ and $Na_v1.8$. The residue equivalent to I436 (DI) is conserved in $Na_v1.7$, while it is a valine in $Na_v1.8$. A mutation of the I436 residue in $Na_v1.4$ increases channel sensitivity to lidocaine. Furthermore, I782 on DII of $Na_v1.4$, which is not conserved in $Na_v1.8$, affects use-dependent lidocaine blocking of the channel. When mutated, this residue causes the channel to become much more resistant to use-dependent blocking by lidocaine (Kondratiev & Tomaselli, 2003). A mutation of the V787 residue on the DII of $Na_v1.4$ increases the affinity of the channel for lidocaine. The valine residue is present on $Na_v1.7$ but the corresponding amino acid on $Na_v1.8$ is a leucine. The residue is in position 831 in $Na_v1.8$ but the corresponding amino acid is different in $Na_v1.7$ (methionine). Changes to all these residues could explain the differential affinity of $Na_v1.8$ and $Na_v1.7$ for lidocaine. Further studies will be required to test the role of these residues in the differential modulation of Nav1.7 and Nav1.8 by lidocaine.

$Na_v1.8$, the TTX-R Na^+ channel, plays a key role in neuropathic pain (Gold, 1999; Gold *et al*., 2003). Its high sensitivity to LA drugs observed in the present study makes it an ideal target for the development of drugs to treat neuropathic pain.

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