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# Lidocaine block of neonatal $Na_v1.3$ is differentially modulated by co-expression of β1 and β3 subunits

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**Abstract**

The effects of lidocaine on neonatal $Na_v1.3$ ($Na_v1.3n$) expressed alone and in combination with β1 and β3 subunits in *Xenopus* oocytes were examined. Lidocaine reversibly inhibited the peak $Na_v1.3n$ current, shifted the steady-state inactivation curve to hyperpolarized potentials and delayed recovery from inactivation. These effects were attenuated by the co-expression of the β subunits, with greater attenuating effects being observed in oocytes co-expressing β1 compared to those co-expressing β3. Use-dependent block by lidocaine was assessed at 1 Hz train frequency for 60 pulses. Lidocaine caused similar use-dependent block of current amplitude at pulse 60 for $Na_v1.3n$ and $Na_v1.3n + β3$. In oocytes co-expressing β1, these use-dependent actions were reduced. In conclusion, the effects of lidocaine on $Na_v1.3n$ are differentially modulated by β1 and β3 subunits. Since these subunits exhibit a complementary distribution, this finding may have importance in our understanding of lidocaine action.


*Keywords:* $Na_v1.3$ $Na^+$ channel; Neonatal; β-Subunit; Lidocaine; Oocyte

## 1. Introduction

Lidocaine exhibits both local anaesthetic and antiarrhythmic actions attributable to its inhibitory actions upon voltage-gated $Na^+$ channels. Although lidocaine has a class I antiarrhythmic properties, it is infrequently used in the treatment of cardiac arrhythmias. However, the use of lidocaine in the treatment of chronic neuropathic pain such as diabetic neuropathic pain is extensive (Kastrup et al., 1987; Mao and Chen, 2000).

Lidocaine produces a tonic (resting) block and a phasic use-dependent block of $Na^+$ channels (Hille, 1977; Hondeghem and Katzung, 1977). Tonic block results from binding of lidocaine to the resting state of the channel with low affinity, whilst during phasic block, lidocaine binds to the inactivated state of the channel with a higher affinity (Bean et al., 1983). Recent studies have proposed that lidocaine may affect transition states within the activation process rather than mediate its effects by stabilizing or accumulating the inactivated state (Hanck et al., 1994, 2000; Vedantham and Cannon, 1999). Although the mechanisms by which lidocaine mediates its actions are controversial, it is clear that lidocaine has different affinities for the different $Na^+$ channel isoforms. For example, neuronal $Na^+$ channel alpha subunits have been shown to exhibit different affinities for lidocaine (Bottaro et al., 1986; Roy and Narahashi, 1992). The cardiac specific isoform $Na_v1.5$ has been shown to have higher affinity for lidocaine in comparison to the skeletal muscle specific isoform $Na_v1.4$ (Makielski et al., 1999). Such differences in affinities are probably due to differences in channel-gating kinetics rather than to lidocaine-binding site differences (Nuss et al., 2000).

Voltage-gated $Na^+$ channels are composed of a pore-forming α subunit and auxiliary subunits β1 (Isom et al., 1992), β2 (Isom et al., 1995) and more recently, β3 (Morgan et al., 2000). Although ion permeation and voltage sensing are primarily determined by the α subunit, β subunits are important modulators of $Na^+$ channel function (Patton et al., 1994) and have been shown to affect lidocaine action. For example, β1 has been shown to modulate lidocaine affinity

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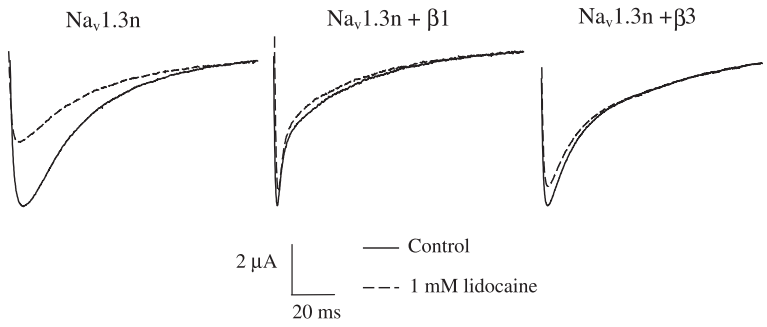


Fig. 1. Tonic lidocaine block of Na$_v$1.3n alone and co-expressed with β1 or β3 in oocytes ($n=7-9$). Cells were depolarised from a holding potential of −100 to −10 mV for 150 ms before returning to −100 mV. Control traces are shown as solid lines; effects of lidocaine are shown as broken lines. Lidocaine (1 mM) was perfused for 3–5 min before the protocol was repeated. Under control drug-free conditions, current amplitude and decay were unchanged (data not shown).

of Na$_v$1.4 and Na$_v$1.5 channels expressed in *Xenopus* oocytes. Co-expression of β1 with Na$_v$1.5 was found to decrease tonic block by lidocaine and to increase rate of recovery from block (Makielski et al., 1996, 1999), whilst β1 modestly increased tonic block for Na$_v$1.4 (Makielski et al., 1999). Thus, the auxiliary subunit β1 can differentially modulate the pharmacology of $Na^+$ channel function. As both β1 and β3 have been shown to affect $Na^+$ channel kinetics, we hypothesised that β3 may also affect lidocaine block.

An increase in Na$_v$1.3 messenger ribonucleic acid (mRNA) has been detected in small diameter dorsal root ganglion neurones (c-fibres) following the chronic constriction model of neuropathic pain (Dib-Hajj et al., 1999; Kim et al., 2001). Although it is not clear whether the increase is a switching on of the neonatal splice variant, it has been hypothesised by others that this may indeed be the case (Waxman et al., 1994). In other diseases such as epilepsy, seizure activity was found to reactivate the neonatal splicing event, inducing the expression of the neonatal forms of both Na$_v$1.3 and Na$_v$1.2 (Aronica et al., 2001). In addition, β3 mRNA (Shah et al., 2000) has been shown to increase in small diameter dorsal root ganglion neurones (c-fibres) following the chronic constriction model of neuropathic pain. Interestingly, β1 mRNA, which is expressed mainly in large Aβ fibres, remains unchanged. In view of this, we have examined the effect of lidocaine on neonatal Na$_v$1.3 (Na$_v$1.3n), with particular interest in the effects of co-expressing the β3 subunit. For comparison, we also determined the effects of β1 subunit co-expression, assessing both tonic and phasic block by lidocaine.

## 2. Materials and methods

Rat neonatal Na$_v$1.3 subunit complimentary deoxyribonucleic acid (cDNA) was engineered into the pCI vector (Promega, Southampton, UK.) and linearized using Not1. Rat β1 and β3 cDNAs were engineered into the

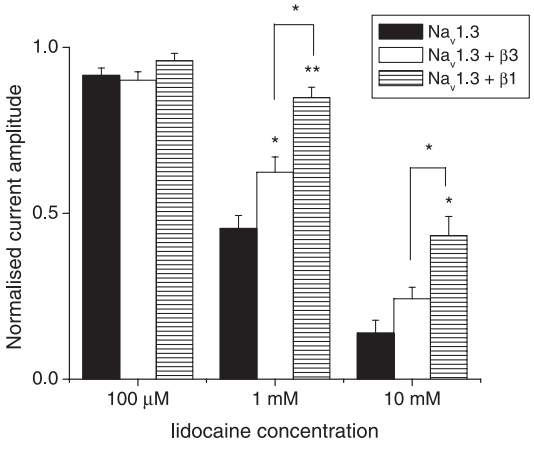


Fig. 2. Concentration-dependent tonic block by lidocaine on Na$_v$1.3n alone or co-expressed with β1 or β3. Currents were elicited by a depolarising step from a holding potential of −100 to −10 mV for 150 ms Current amplitudes were normalised to peak currents recorded under drug-free conditions. Values represent normalised means ± S.E.M. $^{**}P<0.001$; $^{*}P<0.05$; unpaired *t*-test, compared with control $n=4$ for each.

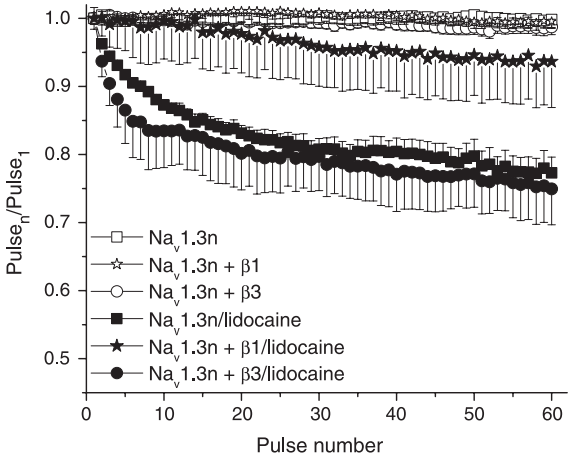


Fig. 3. Use-dependent block of Na$_v$1.3n alone or co-expressed with β1 or β3. Currents were elicited by a 100-ms pulse from −100 to +10 mV at 1 Hz frequency for 60 pulses. Each peak $Na^+$ current was normalised to current amplitude at pulse 1. Values represent means ± S.E.M., Na$_v$1.3n ($n=5$); β1 ($n=8$) and β3 ($n=9$).

pBG7.2 vector and were linearized using Nde1. Capped mRNA was transcribed in vitro from linearized cDNAs using T7 MessageMachine (Ambion, Abingdon, Oxfordshire, UK).

*Xenopus laevis* were anaesthetised by immersion in 0.3% (w/v) 3-amino benzoic acid (Sigma), and ovarian lobes were removed. Oocytes were dissociated enzymatically using 0.3% (w/v) collagenase (Sigma) in $Ca^{2+}$-free solution (82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM, HEPES, pH 7.6). Prepared oocytes were microinjected with 50 nl of mRNAs dissolved in water. A 10:1 ratio of β1 and β3 mRNA to $Na_v1.3n$ mRNA was used. Oocytes were incubated at 18 °C in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6).

Two-electrode voltage clamp recordings were performed 3–6 days after microinjection of mRNAs using

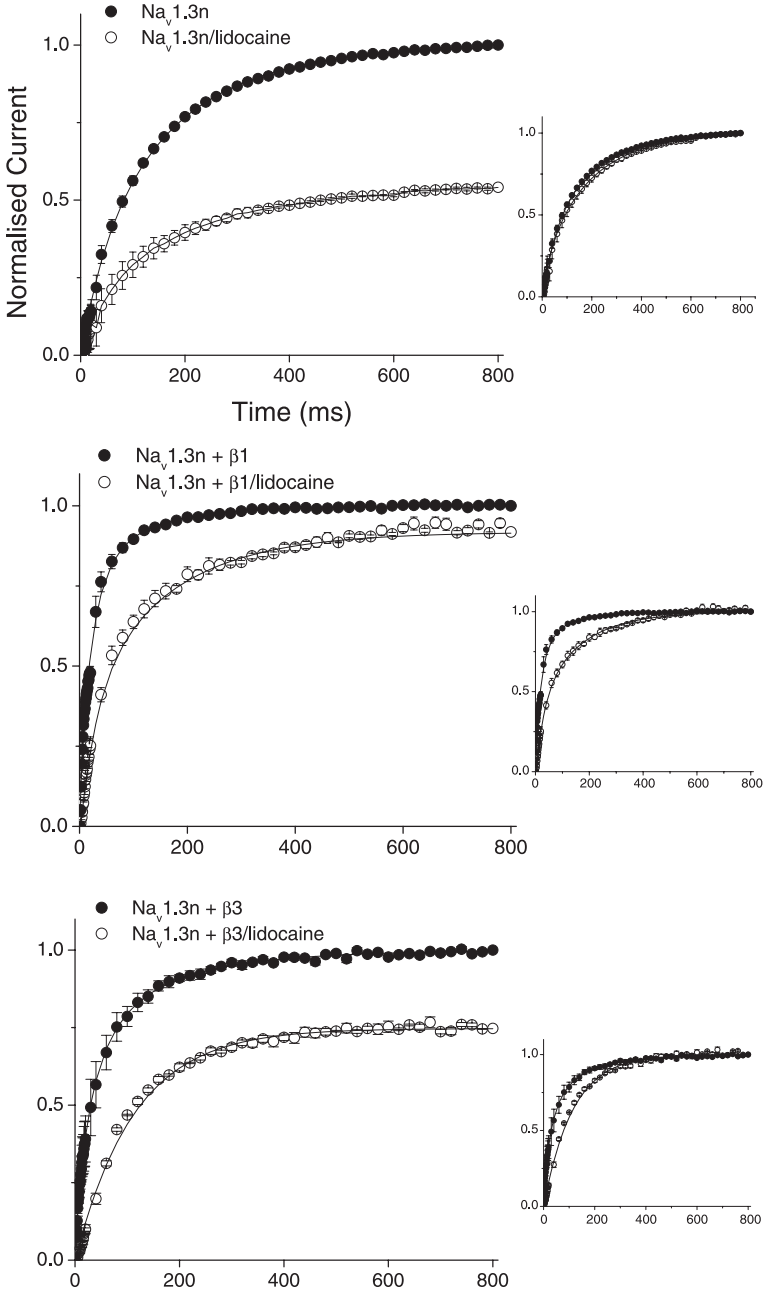


Fig. 4. Effects of lidocaine on recovery from inactivation at −100 mV. Recovery from inactivation in control (closed symbols) and from 1 mM lidocaine block (open symbols) was studied by a two-pulse protocol consisting of a 500-ms conditioning prepulse from −100 to −10 mV followed by a variable (1–800 ms at −100 mV) recovery time after which a test pulse to −10 mV for 200 ms was applied. The peak current at each test pulse was expressed as a fraction of maximum peak current under drug-free conditions. To demonstrate the effects of lidocaine on recovery kinetics, the inset shows recovery from inactivation normalised to the respective peak currents for control and after lidocaine exposure. Recovery from inactivation data was fitted with a single or double exponential equation. See Table 1 for fitting data. Values represent means ± S.E.M.

a GeneClamp 500 amplifier (Axon Instruments, CA, USA) or Warner oocyte clamp (OC725C) interfaced to a Digidata 1200 A/D board with Clampex software (v6, Axon Instruments). Oocytes were continually perfused with ND96 solution. Microelectrodes filled with 3 M KCl had resistances between 0.5 and 1 MΩ. Capacitative, leak and endogenous currents were removed by subtracting currents recorded in the presence of 1 μM tetrodotoxin from those recorded in the absence of tetrodotoxin. All recordings were performed at room temperature (23 °C).

Details of voltage protocols are provided with the data. Voltage pulse protocols were applied from a holding potential of −100 mV. Currents were filtered at 5 kHz and digitised at 20 kHz. Lidocaine (Sigma) was dissolved in ND96 and applied by continuous perfusion. Data analysis was performed using Clampfit software (v6, Axon Instruments) and Origin (v5, Microcal Software, MA, USA). Statistical analyses were performed using a *t*-test for normally distributed data as determined by the Kolmogorov-Smirnov test, or the Rank Sum test for non-normalised data (Sigma Stat, Jandel). Averaged data are presented as means ± S.E.M. Consistent with prior studies of $Na^+$ currents in RNA-injected oocytes, current decay was described as either a sum of a single $y = A_1[1 - \exp(-t/\tau_1)]$ or double exponential function $y = A_1[1 - \exp(-t/\tau_1)] + A_2[1 - \exp(-t/\tau_2)]$. The percentage of the current represented by the fast time constant was calculated from the equation $A_1/(A_1 + A_2)$, where $A_1$ and $A_2$ are the amplitudes of the fast and slow gating modes. Steady-state inactivation curves were fitted with a double Boltzmann function:

$$g/g_{max} = A_1/(1 + \exp((V - V_{1/2})/k)) + A_2/(1 + \exp((V - V_{1/2})/k))$$

where $g/g_{max}$ is the ratio of conductance to maximum conductance, $V_{1/2}$ is voltage of half-maximal inactivation and $k$ is the slope factor. The percentage of the current represented by component 1 was calculated from the equation $A_1/(A_1 + A_2)$, where $A_1$ and $A_2$ are the amplitudes of the first and second Boltzmann function, respectively.

## 3. Results

### 3.1. Co-expression of β subunits with $Na_v1.3n$ accelerates current decay

Sodium currents were elicited by step depolarisations from a holding potential of −100 to −10 mV for 150 ms (see closed lines in Fig. 1). Current decay was fit to a single exponential for $Na_v1.3n$ alone ($\tau_1$:18.6 ± 1.3 ms; $n = 9$). β1 subunit co-expression has been shown to reduce the proportion of slow gating (Balser et al., 1996). Co-expression with β1 and β3 subunits accelerated the current decay of $Na_v1.3n$, consistent with a reduction in slow gating. Time constants were as follows: $Na_v1.3n + \beta1$ $\tau_1$: 1.5 ± 0.1 ms, $\tau_2$: 27.2 ± 1.7 ms ($n = 9$); $Na_v1.3n + \beta3$ $\tau_1$: 2.8 ± 1.1 ms, $\tau_2$: 18.4 ± 2.9 ms ($n = 7$). The proportion of the current described by the fast time constant for β1 co-expression was significantly greater than those recorded for β3 co-expression (β1 = 71 ± 5%; β3 = 48 ± 10%; $P < 0.05$).

### 3.2. Tonic block by lidocaine is attenuated by β subunit co-expression

Lidocaine (1 mM) effect on tonic block was most pronounced in oocytes expressing $Na_v1.3n$ alone (Fig. 1). Current amplitude was depressed by 50 ± 5% and the decay time constant was accelerated ($\tau$ from 19.7 ± 1.2 to 16.4 ± 0.8 ms ($P < 0.05$; $n = 9$)). These effects were attenuated by β subunit co-expression. Lidocaine decreased peak current amplitude of $Na_v1.3n + \beta3$ expressing oocytes by 31 ± 4% whilst having even less of an effect on oocytes expressing $Na_v1.3n + \beta1$ (11 ± 1%). Current decay time constants were not changed ($Na_v1.3n + \beta1$ $\tau_1$: 1.5 ± 0.1 ms ($P = 0.7$), $\tau_2$: 26.9 ± 2.7 ms ($P = 0.8$; $n = 9$); $Na_v1.3n + \beta3$ $\tau_1$: 2.3 ± 0.5 ms ($P = 0.5$), $\tau_2$: 16.9 ± 1.2 ms ($P = 0.7$; $n = 7$)). These effects of lidocaine were fully reversible on washout for all conditions. Concentration-dependent tonic block by lidocaine (0.1–10 mM) is shown in Fig. 2. Co-expression of either β1 or β3 significantly attenuated the block by 1 mM lidocaine compared to $Na_v1.3n$ alone. In addition, this attenuation of block by β1 was significantly greater than that by β3.

Table 1
Effects of lidocaine and β subunit co-expression on recovery from inactivation kinetics

| Subunit | Control | | | | Lidocaine (1 mM) | | | | *n* |
|---|---|---|---|---|---|---|---|---|---|
| | $A_1$ | $\tau_1$ | $A_2$ | $\tau_2$ | $A_1$ | $\tau_1$ | $A_2$ | $\tau_2$ | |
| $Na_v1.3n$ | 0.36 ± 0.08 | 55.5 ± 10.7 | 0.64 ± 0.07 | 188.5 ± 12.9 | 0.33 ± 0.08 | 40.3 ± 20.1 | 0.67 ± 0.08 | 197.2 ± 1.7 | 4 |
| $Na_v1.3n \pm \beta1$ | 0.66 ± 0.07 | 16.8 ± 3.5 | 0.34 ± 0.11 | 123.4 ± 39.9 | 0.46 ± 0.03[a] | 31.4 ± 4.4[b] | 0.54 ± 0.03[a] | 163.7 ± 11.8 | 4 |
| $Na_v1.3n \pm \beta3$ | 0.67 ± 0.10 | 33.8 ± 6.3 | 0.33 ± 0.08 | 183.0 ± 20.1 | 1[a] | 106.8 ± 1.6[a] | | | 5 |

Effects of lidocaine on the recovery from inactivation of $Na_v1.3n$ alone and co-expressing β1 or β3. Recovery from inactivation was determined under control conditions and after 3 min of lidocaine (1 mM) perfusion. Parameters were calculated by fitting either a single exponential function in the form $y = A_1(1 - \exp(-t/\tau_1))$ or double exponential function: $y = A_1(1 - \exp(-t/\tau_1)) + A_2(1 - \exp(-t/\tau_2))$ to data shown in Fig. 4. Values represent means ± S.E.M.

[a] $P < 0.001$; paired *t*-test compared with control.

[b] $P < 0.05$; paired *t*-test compared with control.

### 3.3. Use-dependent block by lidocaine is reduced by β1 but not β3 co-expression

Use-dependent block by lidocaine (1 mM) is shown in Fig. 3. Currents were elicited by a 100-ms pulse from −100 to +10 mV at 1 Hz frequency for 60 pulses. Each peak $Na^+$ current was normalised to the peak current during the first pulse. Under control conditions, there was no reduction in peak current as a result of the protocol (Fig. 3, open symbols). Normalised current amplitude at pulse 60 for each of the conditions were as follows: $Na_v1.3n$: 0.99 ± 0.01 ($n=5$); $Na_v1.3n+\beta1$: 0.99 ± 0.01 ($n=8$); $Na_v1.3n+\beta3$: 0.98 ± 0.01 ($n=9$). In the presence of lidocaine, use-dependent block for both $Na_v1.3n$ alone and $Na_v1.3n+\beta3$ were similar (current reduced at pulse 60 by 23 ± 2% and 25 ± 5% ($P<0.001$), respectively). Co-expression of β1 greatly attenuated the use-dependent block by lidocaine. Current amplitude at pulse 60 was blocked by only 6 ± 4% and was not significantly different from control. These effects were greatly attenuated compared to $Na_v1.3n$ alone and even β3 co-expression ($P<0.05$).

### 3.4. β subunit co-expression attenuates lidocaines effects on recovery from inactivation

Since lidocaine has a higher affinity for the inactivated state of the channel, these differential effects of lidocaine could be explained by differences in recovery from inactivation as a result of co-expressing β subunits. To test this hypothesis, we determined whether lidocaine increased the time required for recovery from inactivation.

Recovery from inactivation was determined using a two pulse protocol consisting of a 500-ms conditioning prepulse from −100 to −10 mV followed by a variable (1–800 ms) recovery time at −100 mV, after which, a test pulse to −10 mV for 200 ms was applied (Fig. 4). Recovery from inactivation data was fitted with either a single exponential or the sum of two exponentials, and the fitting parameters are shown in Table 1. The actions of 1 mM lidocaine on recovery from inactivation were not only to reduce the number of channels recovering but also the rate at which they recovered. The most obvious result was the fact that lidocaine reduced the number of channels that recovered from inactivation compared to drug-free conditions. In oocytes expressing the channel alone, 46 ± 2% of channels failed to recover from inactivation after 800 ms and were therefore considered to be blocked by lidocaine (Fig. 4). In a similar manner to tonic block, the effects of co-expressing either β1 or β3 were to attenuate this block (β1 = 9 ± 1% and β3 = 26 ± 1%). Secondary to the blocking actions of lidocaine were the effects on recovery kinetics. Under control conditions, 36% of $Na^+$ channels recovered with the faster tau ($\tau_1$) for $Na_v1.3n$ alone. Co-expression of either the β1 or β3 subunits each significantly ($P<0.05$) increased the proportion of channels recovering with $\tau_1$ (66% and 67%, respectively; see Table 1). Interestingly, the effects of lidocaine (1 mM) on recovery kinetics were only observed in cells co-expressed with the β subunits. For clarity, recovery from inactivation was normalised to the respective peak current recorded at time point 800 ms for control and lidocaine and shown as an inset for each figure (Fig. 4). Lidocaine failed to affect the rate of recovery for $Na_v1.3n$ alone. Recovery kinetics and percentage fast gating were unchanged in the presence of lidocaine (Table 1). In

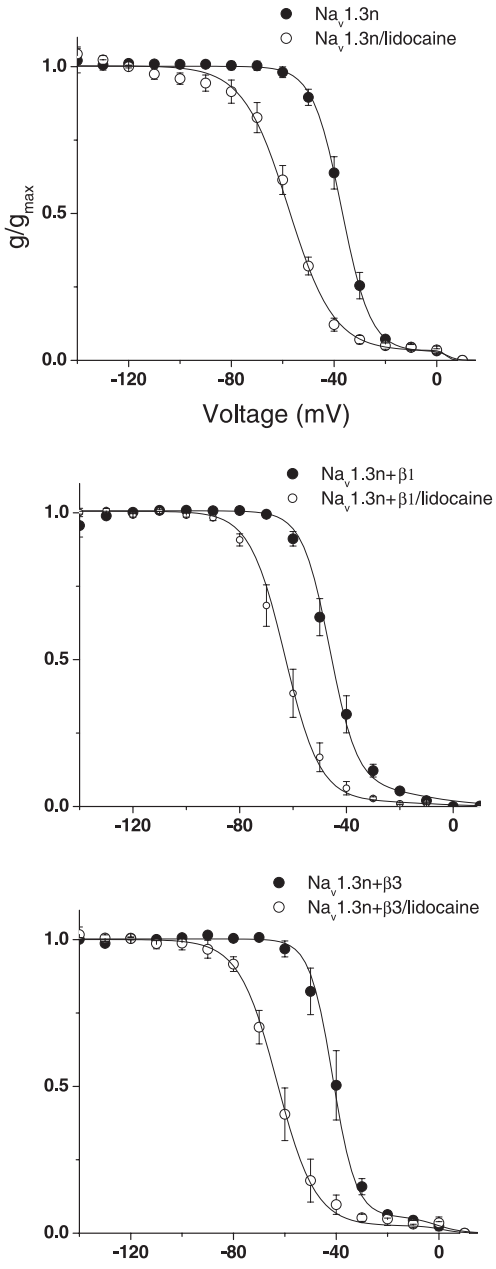


Fig. 5. Effects of lidocaine on steady-state inactivation of $Na_v1.3n$ alone and co-expressing β1 or β3. The protocol for steady-state inactivation consisted of a conditioning pulse of 500 ms ranging from −130 to +10 mV in 5 mV increments followed by a test pulse to +10 mV. Control data are represented by the filled circles; effects of lidocaine are shown by the open circles. Values represent means ± S.E.M. $Na_v1.3n$ ($n=9$); β1($n=9$) and β3 ($n=6$).

Table 2
Effects of lidocaine block on steady-state inactivation kinetics

| Subunit | Control | | | | | | Lidocaine (1 mM) | | | | | | n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Component 1 | | | Component 2 | | | Component 1 | | | Component 2 | | | |
| | $A_1$ | $V_{1/2}$ | $k$ | $A_2$ | $V_{1/2}$ | $k$ | $A_1$ | $V_{1/2}$ | $k$ | $A_2$ | $V_{1/2}$ | $k$ | |
| $Na_v1.3n$ | $0.97 \pm 0.01$ | $-37.0 \pm 1.4$ | $5.6 \pm 0.5$ | $0.03 \pm .01$ | $4.4 \pm 0.5$ | $1.9 \pm 0.07$ | $0.97 \pm 0.01$ | $-57.7 \pm 2.0^a$ | $8.8 \pm 1.5^b$ | $0.03 \pm 0.01$ | $3.6 \pm 1.5$ | $1.0 \pm 0.5^b$ | 9 |
| $Na_v1.3n + \beta1$ | $0.93 \pm 0.02$ | $-46.3 \pm 1.9$ | $5.1 \pm 0.2$ | $0.07 \pm 0.02$ | $-14.3 \pm 5.1$ | $13.0 \pm 2.4$ | $0.99 \pm 0.01$ | $-63.0 \pm 2.5^a$ | $6.6 \pm 0.3^a$ | $0.01 \pm 0.01$ | $-9.7 \pm 5.1$ | $11.6 \pm 4.8$ | 9 |
| $Na_v1.3n + \beta3$ | $0.95 \pm 0.2$ | $-41.3 \pm 2.7$ | $4.5 \pm 0.2$ | $0.05 \pm 0.02$ | $-1.0 \pm 2.8$ | $5.2 \pm 1.3$ | $0.98 \pm 0.01$ | $-63.0 \pm 2.9^a$ | $7.5 \pm 1.1^b$ | $0.02 \pm 0.01$ | $1.5 \pm 1.5$ | $3.8 \pm 0.4$ | 6 |

Parameters were calculated by fitting a double Boltzmann function $g/g_{max} = A_1/(1+\exp((V-V_{1/2})/k)) + A_2/(1+\exp((V-V_{1/2})/k))$, where $g/g_{max}$ is the ratio of conductance to maximum conductance, $V_{1/2}$ is the voltage of half-maximal inactivation and $k$ is the slope factor. Values represent means ± S.E.M.

[a] $P<0.001$; paired $t$-test compared with control.

[b] $P<0.05$; paired $t$-test compared with control.

contrast, lidocaine increased the proportion of slow recovery in oocytes co-expressing either β1 or β3 subunits. In the presence of lidocaine, $\tau_1$ for β1 was significantly increased and the percentage represented by the fast $\tau$ was significantly reduced by 20%. Recovery from inactivation for β3 in the presence of lidocaine was best fit to a single exponential with a $\tau$ of $106.8 \pm 1.6$ ms.

### 3.5. Lidocaine shifts the steady-state inactivation curve

Since lidocaine has a higher affinity for the inactivated state of the channel, the steady-state inactivation curve would be expected to shift to greater negative potentials as inactivated channels became blocked by lidocaine. We have previously shown that the steady-state inactivation curve of the *adult* isoform of $Na_v1.3$ was best fit by a double Boltzmann function with 35% being represented by the more negative $V_{1/2}$ value (Shah et al., 2001). In contrast, 97% of the steady-state inactivation curve of the *neonatal* isoform, $Na_v1.3n$ was represented by the component with the more negative $V_{1/2}$ value. Co-expression of β1, but not β3 subunit, caused a significant shift in the $V_{1/2}$ of component 1 of the steady-state inactivation curve (by $9.3 \pm 1.9$ mV; $P<0.001$). Lidocaine caused a significant shift in the $V_{1/2}$ in the hyperpolarizing direction for all the conditions when compared with drug-free conditions (Fig. 5 and Table 2). Interestingly, similar shifts were recorded for $Na_v1.3n$ (shift by $-20.7 \pm 2.0$ mV; $P<0.001$) and $Na_v1.3n + \beta3$ (shift by $-21.7 \pm 2.9$ mV; $P<0.001$). The shift in $V_{1/2}$ seen with β1 co-expression was attenuated, causing a shift of only $-16.7 \pm 1.6$ mV ($P<0.001$). In the presence of lidocaine slope, values were significantly increased for Nav1.3n, Nav1.3n + β1 and Nav1.3n + β3.

## 4. Discussion

To our knowledge, this is the first study to examine the effects of the auxiliary subunits β1 and β3 on lidocaine block of the embryonic $Na^+$ channel $Na_v1.3n$. The embryonic isoform occurs as a result of the splicing of exon 5 in adult $Na_v1.3$ causing a substitution of the negatively charged aspartic acid residue at position 209 for a neutrally charged serine residue (Gustafson et al., 1993). Since this residue is immediately before the voltage sensor (S4) of domain 1, this difference could account for discrepancies between this study and previous studies on the adult $Na_v1.3$ isoform (Shah et al., 2001).

In the present study, we have examined the effects of the β subunits on this neonatal channel, with particular interest in the β3 subunit since mRNA for the β3 and $Na_v1.3$ increase in small diameter (c-fibre) dorsal root ganglion neurones following the chronic constriction model of neuropathic pain (Cummins and Waxman, 1997; Dib-Hajj et al., 1999; Kim et al., 2001; Shah et al., 2000). Although no studies to date have directly demonstrated that the increased

expression of $Na_v1.3$ is in fact the neonatal splice variant, it has been suggested that this may be the case for at least some neurones (Waxman et al., 1994). Indeed, in other disease states such as epilepsy, neonatal splice variant forms of both $Na_v1.3$ and $Na_v1.2$ are increased and have been correlated with seizure activity (Aronica et al., 2001; Gastaldi et al., 1997; Whitaker et al., 2001).

Although β3 and β1 are highly homologous sharing 57% sequence identity, the effects of lidocaine on oocytes co-expressing the β3 subunit were less pronounced. In fact, under conditions of use-dependent block and steady-state inactivation, the effects of β3 co-expression were not different from the expression of $Na_v1.3n$ alone. These differences could be attributable to the acidic aspartic acid residue at position 6, which is replaced by a neutral proline in β3 (Morgan et al., 2000). This residue is one out of the three residues that are thought to be important in the modulation $Na^+$ channel gating by β subunits (McCormick et al., 1998).

The mechanisms by which lidocaine block is achieved are not completely understood but are known to involve tonic and use-dependent mechanisms. Studies have implicated the involvement of the fast inactivation gate in the use-dependent actions of lidocaine (Bennett et al., 1995; Hille, 1977). Lidocaine has also been shown to interact with residues Phe-1764 and Tyr-1771 in S6 of domain IV of $Na_v1.2$ (Bennett et al., 1995; Ragsdale et al., 1994). Other studies have demonstrated the importance of residues located in the SS1–SS2 regions (P loops) of each domain in stabilizing the interaction between lidocaine and the pore (Sunami et al., 1997, 2000). Since it has been proposed that β1 binds within this region, its interaction may destabilize this binding thereby attenuating block by lidocaine (Makita et al., 1996; McCormick et al., 1998; Qu et al., 1999). However, it is more likely that the attenuating effects of β subunit co-expression result from changes in inactivation gating. The β subunits reduce the proportion of slow gating whilst lidocaine has a higher affinity for slow inactivated channels (Balser et al., 1996; Chen et al., 2000; Kambouris et al., 1998; Nuss et al., 1995, 2000).

Systemic lidocaine has been used for neuropathic pain relief (Bach et al., 1990; Kastrup et al., 1987; Koppert et al., 2000; Mao and Chen, 2000). $Na_v1.3$ mRNA (Cummins and Waxman, 1997) and β3 mRNA increase whilst β1 mRNA, expressed mainly in large Aβ fibres, remains unchanged following the chronic constriction model of neuropathic pain (Shah et al., 2000). Therefore, β3 is likely to couple with $Na_v1.3$ in small dorsal root ganglion neurones in chronic pain states. Thus, the absence of abundant β1 in these cells may be a possible reason for the higher affinity of lidocaine to tetrodotoxin-sensitive channels during pain. Neuropathic pain is associated with repetitive firing of primary afferent fibres, and $Na^+$ channel blocking local anaesthetics have been shown to suppress neuropathic pain at concentrations well below those reported for channel inhibition (Strichartz et al., 2002). Interestingly, β3 co-expression did not attenuate use-dependent block by lidocaine and did reduce steady-state channel availability compared with β1 co-expression. These results could provide some insight as to why local anaesthetics are effective in relieving neuropathic pain.

In summary, we have shown that the effects of both tonic and use-dependent lidocaine block on neonatal $Na_v1.3$ in oocytes co-expressing the novel β3 subunit differ greatly from oocytes co-expressing the β1 subunit. This difference is likely a result of inactivation kinetic changes associated with β subunit co-expression. This study demonstrates the importance of β subunits in modulating drug effects and should therefore be considered in future mechanistic studies.

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