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Human and Rat $Na_v1.3$ Voltage-Gated Sodium Channels Differ in Inactivation Properties and Sensitivity to the Pyrethroid Insecticide Tefluthrin

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Abstract

Voltage-gated sodium channels are important sites for the neurotoxic actions of pyrethroid insecticides in mammals. The pore-forming α subunits of mammalian sodium channels are encoded by a family of 9 genes, designated $Na_v1.1$ - $Na_v1.9$. Native sodium channels in the adult central nervous system (CNS) are heterotrimeric complexes of one of these 9 α subunits and two auxiliary (β) subunits. Here we compare the functional properties and pyrethroid sensitivity of the rat and human $Na_v1.3$ isoforms, which are abundantly expressed in the developing CNS. Coexpression of the rat $Na_v1.3$ and human $Na_v1.3$ α subunits in combination with their conspecific β1 and β2 subunits in *Xenopus laevis* oocytes gave channels with markedly different inactivation properties and sensitivities to the pyrethroid insecticide tefluthrin. Rat $Na_v1.3$ channels inactivated more slowly than human $Na_v1.3$ channels during a depolarizing pulse. The rat and human channels also differed in their voltage dependence of steady-state inactivation. Exposure of rat and human $Na_v1.3$ channels to 100 μM tefluthrin in the resting state produced populations of channels that activated, inactivated and deactivated more slowly than unmodified channels. For both rat and human channels, application of trains of depolarizing prepulses enhanced the extent of tefluthrin modification approximately twofold; this result implies that tefluthrin may bind to both the resting and open states of the channel. Modification of rat $Na_v1.3$ channels by 100 μM tefluthrin was four-fold greater than that measured in parallel assays with human $Na_v1.3$ channels. Human $Na_v1.3$ channels were also less sensitive to tefluthrin than rat $Na_v1.2$ channels, which are considered to be relatively insensitive to pyrethroids. These data provide the first direct comparison of the functional and pharmacological properties of orthologous rat and human sodium channels and demonstrate that orthologous channels with a high degree of amino acid sequence conservation differ in both their functional properties and their sensitivities to pyrethroid insecticides.

Keywords

$Na_v1.3$; oocyte; sodium channel; pyrethroid; tefluthrin; rat; human

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Introduction

Pyrethroid insecticides are an important class of environmental neurotoxicants. The nature and severity of pyrethroid neurotoxicity to mammals vary widely with chemical structure (Soderlund et al., 2002). Pyrethroids containing the α-cyano-3-phenoxybenzyl moiety (often termed Type II pyrethroids) typically exhibit higher acute toxicity and produce the CS (choreoathetosis with salivation) intoxication syndrome in mammals, whereas a wide structural variety of compounds lacking the α-cyano-3-phenoxybenzyl group (termed Type I compounds) are generally less toxic than Type II compounds and produce the T (coarse whole body tremor) syndrome of intoxication. The characteristic signs of pyrethroid intoxication are evident not only following oral dosing but also following direct introduction into the central nervous system (CNS) (Gray and Soderlund, 1985), thereby implicating central targets in systemic intoxication.

Voltage-gated sodium channels are the sites of insecticidal action of pyrethroids (Bloomquist, 1993; Soderlund, 1995) and are also important sites of neurotoxic action in mammals (Bloomquist, 1993; Narahashi, 1996; Soderlund et al., 2002). Native sodium channels in the CNS of adult mammals are heteromultimeric complexes comprised of one large (~260 kDa) α subunit and two smaller (33–36 kDa) auxiliary β subunits (Meadows and Isom, 2005). Sodium channel α subunits form the ion pore, confer the fundamental functional and pharmacological properties of the channel, and contain the binding site for pyrethroids (Catterall, 2000; Cestèle and Catterall, 2000; Trainer et al., 1997). Sodium channel β subunits modulate channel gating and kinetics, regulate channel expression at the level of the plasma membrane, and contribute to cell adhesion and cell-cell communication (Meadows and Isom, 2005).

Mammalian genomes contain nine genes that code for sodium channel α subunit isoforms, which are designated $Na_v1.1$ - $Na_v1.9$ (Goldin et al., 2000). The $Na_v1.1$, $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ isoforms are expressed in the CNS (Goldin, 2001) and represent potential targets for the systemic neurotoxic actions of pyrethroids. Overlapping patterns of sodium channel α subunit expression in the CNS (Felts et al., 1997; Whitaker et al., 2001) limit the utility of native neuronal preparations to identify isoform-dependent differences in pharmacology. This limitation can be overcome by the heterologous expression of cloned individual sodium channel isoforms in the unfertilized oocytes of the frog *Xenopus laevis* or in transfected mammalian cell lines. The available information on the sensitivity of individual mammalian sodium channel isoforms to pyrethroids is based primarily on expression studies using the *Xenopus* oocyte system. Rat $Na_v1.2$ sodium channels, which are abundantly expressed in the adult CNS, exhibit very low sensitivity to modification by deltamethrin and other pyrethroids (Smith and Soderlund, 1998; Vais et al., 2000). In contrast rat $Na_v1.8$ channels, which are resistant to block by tetrodotoxin and restricted in distribution to the peripheral nervous system, are sensitive to modification by a wide structural variety of pyrethroids (Choi and Soderlund, 2006; Smith and Soderlund, 2001; Soderlund and Lee, 2001). Recent studies show that the rat $Na_v1.3$ isoform is much more sensitive to modification by pyrethroids of the Type II structural class than the rat $Na_v1.2$ isoform (Meacham et al., 2008). The sensitivity of the $Na_v1.3$ isoform is of particular interest because it is preferentially expressed in the embryonic and early postnatal rodent CNS (Felts et al., 1997) and may therefore be an important target for developmental neurotoxic effects attributed to pyrethroids (Shafer et al., 2005).

Sodium channel α subunit genes are highly conserved, so that orthologous subunits in rats and humans are >95% identical at the level of amino acid sequence (Goldin, 2001). However, this degree of sequence conservation still results in 50–100 amino acid sequence differences between orthologous channel proteins. Studies of the functional impact of mutations in insect

sodium channels that are linked with pyrethroid resistance (Soderlund and Knipple, 2003) show that a single point mutation can profoundly alter pyrethroid sensitivity. It is therefore important to determine experimentally the relative pyrethroid sensitivity of orthologous rat and human sodium channels to improve the extrapolation from toxicological studies with rats to human risk assessments. This study was undertaken to compare directly the properties and pyrethroid sensitivity of rat and human $Na_v1.3$ sodium channels using the *Xenopus* oocyte expression system. Our results identify substantial species differences in channel inactivation and in sensitivity to tefluthrin, the registered pyrethroid having the highest acute oral toxicity to mammals (Soderlund et al., 2002).

Materials and Methods

Cloned voltage-sensitive sodium channel subunit cDNAs were obtained from the following sources: rat $Na_v1.2$ ($rNa_v1.2$; adult splice variant), rat β1 (rβ1) and rat β2 (rβ2) from W. A. Catterall (University of Washington, Seattle, WA); rat $Na_v1.3$ ($rNa_v1.3$; adult splice variant) from A. L. Goldin (University of California, Irvine, CA); and human $Na_v1.3$ ($hNa_v1.3$; adult splice variant), human β1 (hβ1) and human β2 (hβ2) from Origene Technologies (Rockville, MD). Plasmid cDNAs were digested with restriction enzymes to provide linear templates for cRNA synthesis *in vitro* using a commercial kit (mMessage mMachine, Ambion, Austin, TX). The integrity of synthesized cRNA was determined by electrophoresis in 1% agarose – formaldehyde gels.

Stage V–VI oocytes were removed from female *X. laevis* frogs (Nasco, Ft. Atkinson, WI) as described elsewhere (Smith and Soderlund, 2001). This procedure was performed in accordance with National Institutes of Health guidelines and followed a protocol that was approved by the Cornell University Animal Care and Use Committee. Oocytes were injected with 1:1:1 (mass ratio) mixtures of an α subunit cRNA and the conspecific β1 and β2 cRNAs (0.5 – 5 ng/oocyte); this mixture provided a ~9-fold molar excess of β1 and β2 cRNAs to ensure the preferential expression of α+β1+β2 complexes. Injected oocytes were incubated in ND-96 medium (in mM: 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES; adjusted to pH 7.6 with NaOH) supplemented with 5% horse serum (Sigma-Aldrich), 1% streptomycin/penicillin, and 1% sodium pyruvate (Goldin, 1992) at 19°C for 3–5 days until electrophysiological analysis of sodium currents.

Sodium currents were recorded from oocytes perfused with ND-96 at 19–21°C in the two-electrode voltage clamp configuration using an Axon Geneclamp 500B amplifier (Molecular Devices, Foster City, CA) and microelectrodes filled with 3 M KCl. To determine the voltage dependence of activation, oocytes were clamped at a membrane potential of −100 mV and currents were measured during a 40-ms depolarizing test pulse to potentials from −60 mV to 35 mV in 5-mV increments. Maximal peak transient currents were obtained upon depolarization to either −10 mV ($rNa_v1.3$) or −5 mV ($hNa_v1.3$, $rNa_v1.2$). To determine the voltage dependence of steady-state inactivation, oocytes were clamped at a membrane potential of −100 mV followed by a 100-ms conditioning prepulse to potentials from −100 mV to 10 mV in 5-mV increments and then a 40-ms test pulse to the either −10 mV ($rNa_v1.3$) or −5 mV ($hNa_v1.3$, $rNa_v1.2$). Currents were filtered at 2 kHz with a low-pass 4-pole Bessel filter and digitized at 50 kHz (Digidata 1320A; Molecular Devices). For determinations of use dependence, oocytes were given trains of 1 to 100 5-ms conditioning prepulses to 10 mV, separated by a 10-ms interpulse interval at the holding potential, followed by a 40-ms test pulse to −10 mV ($rNa_v1.3$) or −5 mV ($hNa_v1.3$, $rNa_v1.2$). Experiments in the presence of tefluthrin employed 10-s intervals between pulses or pulse trains to permit complete recovery from pyrethroid modification. Capacitive transients and leak currents were subtracted using the P/4 method (Bezanilla and Armstrong, 1977).

Tefluthrin (98.8% purity; Syngenta, Bracknell, Berks., UK) was prepared as a stock solution in dimethyl sulfoxide (DMSO) and diluted with ND-96 immediately before use to a final concentration of 100 μM. The final DMSO concentration in the bath did not exceed 0.1%, a concentration that had no effect on sodium currents. Oocytes were perfused at 0.45 ml/min with pyrethroid in ND-96. All experiments with pyrethroids employed a disposable capillary perfusion system (Tatebayashi and Narahashi, 1994) and custom-fabricated single-use recording chambers (~300 μl volume) (Smith and Soderlund, 2001) to prevent cross-contamination between oocytes.

Data were acquired and analyzed using pClamp 8.2 (Axon Instruments, Burlingame, CA) and Origin 7.0 (OriginLab Corp., Northampton, MA). The Boltzmann equation [$y = (A_1 - A_2)/(1 + e^{(x-x0)/dx}) + A_2$] was used to fit conductance-voltage and sodium current inactivation data. Time constants for fast sodium channel inactivation were obtained using the Chebyshev method in Origin 7.0 to fit the falling phase of the peak transient current to a double exponential decay model. Net tefluthrin-modified currents were reconstructed by subtracting a scaled control current obtained prior to pyrethroid exposure from the pyrethroid-modified current measured in the same oocyte. Time constants for the activation and inactivation of tefluthrin-modified channels were obtained using the Chebyshev method to fit the rising and falling phases, respectively, of these net currents to a single exponential decay model. Time constants for pyrethroid-induced sodium tail currents were obtained from fits of post-depolarization currents to a double exponential decay model. The conductance of the pyrethroid-induced sodium tail current, extrapolated to the moment of repolarization and normalized to the conductance of the peak current measured in the same oocyte in the absence of pyrethroid, was employed to calculate the fraction of sodium channels modified by each compound in each experiment as described previously (Tatebayashi and Narahashi, 1994). Statistical analyses were performed using the Prism software package (GraphPad Software, La Jolla, CA).

Results

Kinetic and gating properties of $rNa_v1.3$ and $hNa_v1.3$ sodium channels

We expressed the $rNa_v1.3$ and $hNa_v1.3$ α subunits in combination with their conspecific β1 and β2 subunits to give channels that reflect the presumed heterotrimeric structure of the majority of native $Na_v1.3$-containing channels in the adult CNS (Catterall, 2000). Typical sodium currents recorded from oocytes expressing $rNa_v1.3$ or $hNa_v1.3$ sodium channels are shown in Fig. 1A. Rat $Na_v1.3$ channels gave currents that activated rapidly but inactivated incompletely during a 40-ms depolarizing pulse. In contrast, $hNa_v1.3$ channels activated and inactivated rapidly. The decay of the peak transient currents was fitted best by a two-component, single exponential decay model that yielded first-order decay constants for the fast initial phase (τ_{fast}) and slower secondary phase (τ_{slow}) of inactivation. The fast component of inactivation was similar for both channels (Fig. 1B), whereas the slow component of inactivation of $rNa_v1.3$ channels was slower and exhibited a stronger voltage dependence than that of $hNa_v1.3$ channels (Fig. 1C). We used the proportionality constants of the double exponential fits of peak current decay to calculate the fraction of the current carried by $rNa_v1.3$ and $hNa_v1.3$ channels that represented the slower component of inactivation (Fig. 1D). The distribution of channels into the two kinetic populations was only slightly effected by depolarization potential. At all test potentials between −15 mV and 10 mV the majority of $rNa_v1.3$ channels inactivated with slow kinetics, whereas the majority of $hNa_v1.3$ channels inactivated with fast kinetics.

The voltage-dependent gating of $rNa_v1.3$ and $hNa_v1.3$ sodium channels is illustrated in Fig. 2 and the statistical analyses of these data are summarized in Table 1. There was no difference between the rat and human channels in the midpoint potential for activation, but rat channels

exhibited a significantly steeper voltage response (Fig. 2A). The midpoint potentials for steady-state inactivation of the $rNa_v1.3$ and $hNa_v1.3$ channels differed by ~10 mV (Fig. 2B).

Effects of tefluthrin

We employed 100 μM tefluthrin, the highest concentration achievable in ND-96 perfusion medium, to maximize the extent of sodium channel modification by this pyrethroid. We assessed the modification of channels in the resting state by tefluthrin by holding the oocyte membrane at a hyperpolarized potential during perfusion and measuring the pyrethroid-modified current during the first depolarizing pulse. Figure 3 show representative currents recorded from single oocytes expressing either $rNa_v1.3$ (Fig. 3A) or $hNa_v1.3$ (Fig. 3B) channels before and after exposure to 100 μM tefluthrin. In assays with both channels tefluthrin modification was evident in the prolonged and incomplete inactivation of the peak current during a depolarizing pulse and in the induction of a prominent sodium tail current following repolarization.

Figures 3C and 3D illustrate the effect of tefluthrin on the τ_{fast} and τ_{slow} components of inactivation measured at test potentials from −15 mV to 10 mV. Tefluthrin prolonged τ_{fast} for both channels at −15 mV but had little effect at other test potentials (Fig. 3C). In contrast, tefluthrin retarded the slower component of inactivation of $rNa_v1.3$ and $hNa_v1.3$ channels by at least two-fold over a range of test potentials (Fig. 3D). With $rNa_v1.3$ channels depolarized to −15 mV, the value for τ_{slow} was too large to be measurable during a standard 40-ms test depolarization. In addition to these effects on the kinetics of the slow component of inactivation, tefluthrin also increased the proportion of current for each channel that exhibited slow inactivation kinetics (Fig. 4).

The modified sodium currents shown in Fig. 3 are composites of separate currents carried by populations of unmodified and tefluthrin-modified channels. To identify the kinetic properties of the tefluthrin-modified channel population, we reconstructed tefluthrin-modified currents by subtracting a scaled control current measured prior to tefluthrin exposure from the current measured in the same oocyte in the presence of tefluthrin. Figure 5 shows example reconstructed currents for tefluthrin-modified $rNa_v1.3$ (Fig. 5A) and $hNa_v1.3$ (Fig. 5B) channels recorded during and after a depolarizing pulse. In each case, the delay in the onset of each current after depolarization is an artifact of the imperfect subtraction of control currents and oocyte membrane capacitance. Reconstructed currents showed that tefluthrin retarded the activation and inactivation of both channels and that tefluthrin-modified $rNa_v1.3$ channels activated and inactivated much more slowly than tefluthrin-modified $hNa_v1.3$ channels. First-order fits of the rising phases of these currents yielded time constants for activation (τ_{act}) for both channels (Table 2). Similar fits of the falling phase of currents from $hNa_v1.3$ channels also yielded time constants for inactivation (τ_{inact}) for this channel (Table 2), but the inactivation of $rNa_v1.3$ channels during a standard 40-ms depolarization was too slow to be determined.

We also employed the reconstructed tefluthrin-modified currents (as in Fig. 5) to determine the kinetics of sodium tail current decay. The decay of tefluthrin-induced tail currents was fitted best by a two-component, single exponential decay model that yielded first-order constants for the fast initial phase (τ_1) and slower secondary phase (τ_2) of decay (Table 2). Differences in tail current decay between $rNa_v1.3$ and $hNa_v1.3$ channels were limited to the fast component of decay.

We employed the normalized conductance of the tefluthrin-induced sodium tail current to calculate the fraction of sodium channels that were modified by tefluthrin (Tatebayashi and Narahashi, 1994). Tefluthrin at 100 μM modified 41.5 ± 3.0% of $rNa_v1.3$ channels (n = 6) but only 9.9 ± 1.0% of $hNa_v1.3$ channels (n = 8). By this criterion, $rNa_v1.3$ channels were

approximately four-fold more sensitive to resting modification by 100 μM tefluthrin than $hNa_v1.3$ channels under these conditions.

Modification of both insect (Smith et al., 1998; Vais et al., 2000) and mammalian (Choi and Soderlund, 2006; Tabarean and Narahashi, 2001; Vais et al., 2000) sodium channels by some pyrethroids is enhanced by repeated depolarization. We therefore determined the effect of repeated high-frequency depolarization on the extent of modification of $rNa_v1.3$ and $hNa_v1.3$ sodium channels by tefluthrin. In the absence of insecticide, repeated stimulation caused a slight initial decline in the amplitudes of peak transient sodium currents obtained with both channels during the first five depolarizing pulses, but current amplitudes stabilized at ~90% of the initial amplitude and then returned to control levels following longer pulse trains (Fig. 6A). Fig. 6B shows the effect of 0–100 depolarizing prepulses on the extent of modification of $rNa_v1.3$ and $hNa_v1.3$ sodium channels. With both channels, the extent of modification increased as a function of prepulse number over the first 20 prepulses and then stabilized. For both channels, maximal use-dependent modification was nearly two-fold greater than resting modification. The use-dependent modification of $rNa_v1.3$ channels by 100 μM tefluthrin was four-fold greater than that of $hNa_v1.3$ channels.

To place the difference in tefluthrin sensitivity between $rNa_v1.3$ and $hNa_v1.3$ sodium channels in context, we compared the tefluthrin sensitivities of these two channels to the tefluthrin sensitivities of two other rat sodium channel isoforms. We performed experiments identical to those in Fig. 6B using 100 μM tefluthrin and the $rNa_v1.2$ sodium channel α subunit coexpressed with the rβ1 and rβ2 subunits (data not shown). Figure 7 compares the extent of both resting and use-dependent (after 100 prepulses) modification by tefluthrin of $rNa_v1.2$, $rNa_v1.3$ and $hNa_v1.3$ channels. For comparative purposes, Fig. 7 also includes comparable data from a previous study of the rat $Na_v1.8$ ($rNa_v1.8$) isoform expressed in the absence of β subunits (Choi and Soderlund, 2006). Modification of the $rNa_v1.2$ isoform by tefluthrin was enhanced by a factor of almost four-fold by repeated depolarization. In contrast, repeated depolarization caused a small increase in the mean modification of $rNa_v1.8$ channels but the effect was not statistically significant. Using either resting or use-dependent modification as the criterion, $rNa_v1.3$ channels were most sensitive to tefluthrin among the three rat isoforms. In contrast $hNa_v1.3$ sodium channels were less sensitive to tefluthrin than $rNa_v1.2$ channels, which were the least sensitive among the three rat isoforms.

Discussion

The amino acid sequences of orthologous voltage-gated sodium channel isoforms from rats and humans are highly conserved, and it is often assumed that the functional and pharmacological properties of orthologous channel isoforms are similarly conserved. However, there is little published information bearing on this issue. Previous studies have described the properties of the $rNa_v1.3$ or $hNa_v1.3$ sodium channels following heterologous expression in *Xenopus* oocytes (Joho et al., 1990; Meacham et al., 2008; Patton et al., 1994; Shah et al., 2001; Thimmapaya et al., 2005) or in mammalian cells (Chen et al., 2000; Cummins et al., 2001), but these studies have focused exclusively on either the rat or the human ortholog and have employed different splice variants as well as different α/β subunit combinations. In this study, we conducted a direct comparison of the same splice variant of the rat and human $Na_v1.3$ sodium channel α subunits. We coexpressed each α subunit in *Xenopus* oocytes with its conspecific β1 and β2 subunits to mimic the presumed native subunit structure of the most abundant $Na_v1.3$-containing sodium channel complexes in the adult brain. Our study, the first direct comparison of orthologous rat and human sodium channel isoforms, identified marked differences between these channels in both their inactivation properties and their sensitivities to tefluthrin, a potent neurotoxic pyrethroid insecticide.

Biochemical purification studies, coupled with *in situ* hybridization and immunocytochemical localization of sodium channel subunit isoforms in specific brain regions and cell types, imply that sodium channels in the adult brain are heteromultimeric complexes of one α subunit and two β subunits (Meadows and Isom, 2005). In the *Xenopus* oocyte expression system, coexpression of the $Na_v1.1$, $Na_v1.2$ or $Na_v1.6$ sodium channel α subunit isoforms with the β1 and β2 subunits confers the fast inactivation kinetics typical of sodium currents recorded from neurons (Smith et al., 1998). The rapid inactivation of $hNa_v1.3$+β1+β2 channels in our assays is therefore consistent with the behavior of other isoforms expressed with the β1 and β2 subunits in oocytes. In contrast to these results, coexpression of the $rNa_v1.3$ α subunit isoform with the β1 and β2 subunits produced channels that inactivated slowly. The unusual inactivation kinetics of $rNa_v1.3$ channels was also accompanied by a depolarizing shift in the steady-state inactivation curve of these channels relative to $hNa_v1.3$ channels. The predominance of the slow-inactivating component in our assays of $rNa_v1.3$ channels coexpressed with the β1 and β2 subunits is consistent with the results of other studies of the $rNa_v1.3$ isoform in oocytes, which involved coexpression with either the β1 or β3 subunit (Meacham et al., 2008; Patton et al., 1994; Shah et al., 2001). Moreover, preliminary experiments with the $rNa_v1.3$ isoform expressed in the absence of β subunits show that the inactivation properties of $rNa_v1.3$ channels coexpressed with the rβ1 and rβ2 differ from those measured for $rNa_v1.3$ channels expressed alone (J. Tan and D. M. Soderlund, unpublished observations). Therefore the unusually slow inactivation of $rNa_v1.3$ channels described here and in other studies does not result from the failure of the injected mixture of cRNAs to express the desired heteromultimeric complex in oocytes. This conclusion is supported by the observation that the $rNa_v1.3$ channels coexpressed with the rβ1 subunit exhibit inactivation kinetics similar to those described here for $rNa_v1.3$ channels across a 50-fold range of rβ1 subunit concentrations (Patton et al., 1994).

We employed tefluthrin as pharmacological probe to characterize and compare the effects of pyrethroids on $rNa_v1.3$ and $hNa_v1.3$ channels. Tefluthrin is a pyrethroid of the Type I structural class (lacking the α-cyano-3-phenoxybenzyl alcohol moiety) that exhibits the highest acute toxicity to rodents of all pyrethroids currently registered for use in the United States (Soderlund et al., 2002). It is not known whether tefluthrin causes the T (tremor) syndrome of intoxication, typical of most other Type I pyrethroid structures, but its properties as a modifier of sodium channel function cluster with those of other Type I compounds that produce the T syndrome rather than with the properties of Type II compounds (α-cyano-3-phenoxybenzyl esters) that produce the CS (choreoathetosis with salivation) syndrome (Choi and Soderlund, 2006). Tefluthrin was also approximately as effective as deltamethrin and more effective than 5 other Type I compounds in increasing motor activity in rats, an index of behavioral toxicity at sublethal doses (Wolansky et al., 2006).

Consistent with its high toxicity to rodents, tefluthrin exhibits high relative potency as a modifier of sodium channels in the *Xenopus* oocyte expression system. Tefluthrin was among the most potent of 11 pyrethroids examined in assays of the resting and use-dependent modification of rat $Na_v1.8$ sodium channels expressed in oocytes (Choi and Soderlund, 2006). Also, tefluthrin was more potent than deltamethrin in assays of the resting and use-dependent modification of rat $Na_v1.6$ sodium channels coexpressed with the rat β1 and β2 subunits in oocytes (Tan et al., 2008).

Exposure of $rNa_v1.3$ and $hNa_v1.3$ channels in the resting state to a high concentration of tefluthrin slowed the kinetics of channel activation, inactivation and deactivation. Tefluthrin prolonged inactivation increased the proportion of channels in the slow-inactivating population. Reconstructed currents for the tefluthrin-modified channel population showed that these channels opened very slowly compared to unmodified channels. Sodium tail currents resulting from the slow deactivation of tefluthrin-modified channels exhibited similar kinetics for both $rNa_v1.3$ and $hNa_v1.3$ channels. The actions of tefluthrin on rat and human $Na_v1.3$

channels in these assays are typical of the effects of pyrethroids on mammalian sodium channel isoforms in the oocyte expression system (Choi and Soderlund, 2006; Meacham et al., 2008; Smith and Soderlund, 1998; Smith and Soderlund, 2001; Soderlund and Lee, 2001; Vais et al., 2000).

The modification of both $rNa_v1.3$ and $hNa_v1.3$ channels by tefluthrin was enhanced approximately two-fold by the application of trains of brief depolarizing pulses prior to a standard test depolarization. This use-dependent enhancement of modification suggests that tefluthrin may bind to both the open and resting states of $rNa_v1.3$ and $hNa_v1.3$ channels. The significance of use-dependent pyrethroid modification depends on the particular pyrethroid-channel combination examined. We found greater use-dependent enhancement of tefluthrin modification in assays with $rNa_v1.2$ channels than with $rNa_v1.3$ or $hNa_v1.3$ channels. In contrast, a previous study found no significant use-dependent enhancement of the modification of $rNa_v1.8$ channels by tefluthrin, even though other pyrethroids caused substantial use-dependent enhancement of modification of this isoform (Choi and Soderlund, 2006).

Despite the qualitative similarities between $rNa_v1.3$ and $hNa_v1.3$ channels in terms of the kinetics and use dependence of pyrethroid modification, these two channels differed markedly in their sensitivity to tefluthrin. Our finding that $rNa_v1.3$ channels are much more sensitive than $rNa_v1.2$ channels to resting and use-dependent modification by tefluthrin confirms and extends the results of a recent study comparing these two isoforms, which employed different pyrethroids and did not consider use-dependent effects (Meacham et al., 2008). Using sensitivity to tefluthrin as a basis for comparison, the $rNa_v1.3$ channel is also more sensitive to pyrethroid modification than the $rNa_v1.8$ channel (Choi and Soderlund, 2006), which is considered to be a pyrethroid-sensitive isoform. In contrast, $hNa_v1.3$ channels were less sensitive to tefluthrin than all three of the rat sodium channel isoforms when assayed under identical conditions.

The four-fold difference in the extent of channel modification of $rNa_v1.3$ and $hNa_v1.3$ channels in the presence of 100 μM tefluthrin probably understates the difference in sensitivity of these channels. Concentration-effect curves for sodium channel modification by pyrethroids in the oocyte expression system are shallow and incomplete (Choi and Soderlund, 2006; Soderlund and Lee, 2001) due to both the limited solubility of pyrethroids in aqueous media and the loss of bioavailable pyrethroid during perfusion by partitioning into lipid-rich compartments in the oocyte (Harrill et al., 2005). The four-fold difference in the tefluthrin sensitivity of $rNa_v1.3$ and $hNa_v1.3$ at a single concentration may therefore correspond to a >10-fold difference in the concentration required to produce the same degree of channel modification (Choi and Soderlund, 2006; Soderlund and Lee, 2001). The low sensitivity of $hNa_v1.3$ channels to 100 μM tefluthrin precluded a more detailed comparison of the relative sensitivities of $rNa_v1.3$ and $hNa_v1.3$ channels using a range of lower tefluthrin concentrations.

Studies of pyrethroid action modification of sodium currents under voltage clamp conditions, such as those in the present study, require high concentrations of insecticide to produce detectable populations of pyrethroid-modified channels. However, the concentrations required to disrupt normal action potential generation in neurons are orders of magnitude lower because only a small fraction of the available population of channels must be modified in order to disrupt electrical signaling. This relationship was demonstrated directly by Song and Narahashi (1996) in a study of the action of tetramethrin on sodium channels in rat Purkinje neurons. In this study, micromolar concentrations of tetramethrin were required to produce sufficient channel modification (5–25%) to be detected readily under voltage clamp conditions, whereas disruption of action potential generation in the same cells was achieved at 100 nM tetramethrin, a concentration calculated to modify fewer than 1% of sodium channels based on voltage clamp data. Similarly, concentrations of deltamethrin in the micromolar range are required to produce

detectable modification various rat sodium channel isoforms expressed in oocytes under voltage clamp conditions (Choi and Soderlund, 2006; Meacham et al., 2008; Vais et al., 2000) but deltamethrin concentrations in the nanomolar range are sufficient to disrupt normal electrical activity in primary cultures of mouse frontal cortex and spinal cord neurons (Shafer et al., 2008). The high pyrethroid concentrations employed in voltage-clamp studies are valuable to characterize the effects of pyrethroids on sodium currents in detail and to assess the relative sensitivity of channels to pyrethroid modification, but they greatly exceed the concentrations of pyrethroids required to disrupt electrical signaling in intact neurons.

The differences in the inactivation properties and pyrethroid sensitivity of the orthologous rat and human $Na_v1.3$ isoforms identified in this study are likely to be determined by differences in the structure of these α subunits rather than by the modulatory effects of the auxiliary β subunits. However, these divergent properties are not readily explained by differences in the amino acid sequences of these channels. The predicted amino acid sequences of the $rNa_v1.3$ and $hNa_v1.3$ clones used in this study differ at only 54 out of 1951 sequence positions. None of these polymorphisms occur at sites that have been identified as determinants of fast inactivation (Goldin, 2003; Ulbricht, 2005). Also, none of the polymorphisms in rat and human $Na_v1.3$ channels align with the 22 sequence positions in insect sodium channel α subunits that are the sites of mutations associated with pyrethroid resistance (Dong, 2007). A systematic analysis of the functional and pharmacological impact of sequence differences between the $rNa_v1.3$ and $hNa_v1.3$ α subunits, using a combination of chimeric channels and site-directed mutagenesis, has the potential to identify new domains of sodium channel α subunits that determine both fast inactivation and pyrethroid sensitivity.

Our results have important implications for understanding the value and limitations of toxicological studies in rats as the basis for assessing risk to humans. The $Na_v1.3$ sodium channel isoform is abundantly expressed in the embryonic and neonatal CNS of rats but is much less abundant than the $Na_v1.1$, $Na_v1.2$ and $Na_v1.6$ isoforms in the CNS of adult rats (Felts et al., 1997; Shah et al., 2001). The $Na_v1.3$ isoform is also highly expressed in the embryonic human CNS (Thimmapaya et al., 2005) but, in contrast to the rat, is also strongly expressed in some regions of the adult CNS (Thimmapaya et al., 2005; Whitaker et al., 2000; Whitaker et al., 2001). The high pyrethroid sensitivity of sodium channels formed from the $rNa_v1.3$ isoform, expressed as a heterodimer with either the β1 or β3 subunit (Meacham et al., 2008) or as a heterotrimer with the β1 and β2 subunits (this study), suggests that this isoform might be a significant target for developmental neurotoxic effects of pyrethroids in rats (Meacham et al., 2008). The lower pyrethroid sensitivity of $hNa_v1.3$ channels raises the possibility that the rat model may overestimate the sensitivity of the developing human CNS to pyrethroids. Further studies involving additional pyrethroids, channel complexes reflecting the subunit combinations expected to be expressed in the developing CNS, and heterologous expression systems other than *Xenopus* oocytes will be required to confirm that this species difference in pyrethroid sensitivity is a fundamental property of the $Na_v1.3$ sodium channel α subunit.

At a more general level, our data challenge the assumption that the pharmacological properties of orthologous rat and human sodium channel isoforms are conserved. It is therefore important to determine whether species differences in sensitivity also exist for other orthologous pairs of sodium channel isoforms, not only for pyrethroids but also for other insecticides and therapeutic agents that act on voltage-gated sodium channels.

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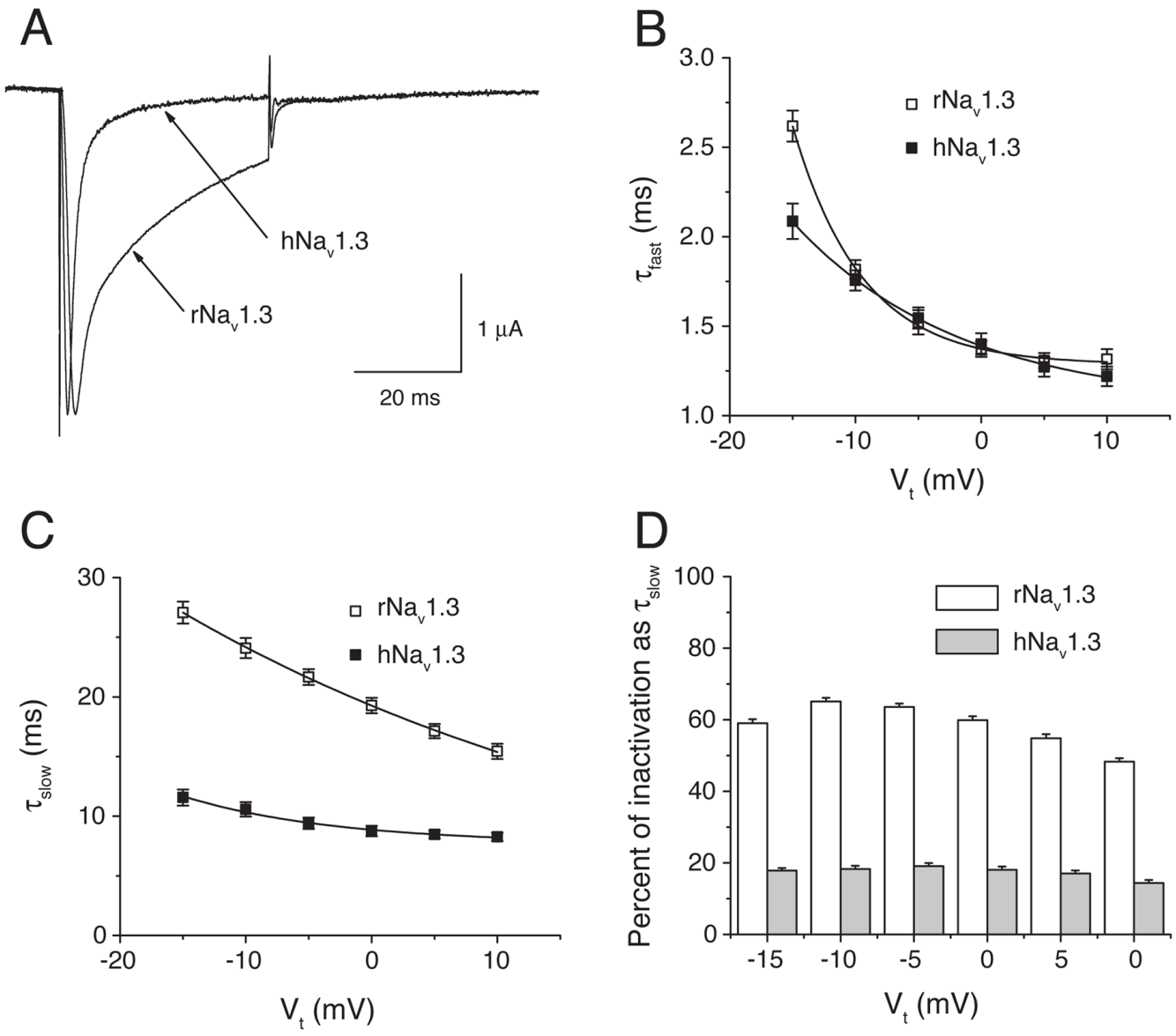

Figure 1.

Gating kinetics of rat and human $Na_v1.3$ sodium channels expressed in *Xenopus* oocytes. (A) Representative peak transient currents recorded from oocytes expressing $rNa_v1.3$ or $hNa_v1.3$ sodium channels; currents were recorded during and after a 40-ms depolarizing pulse from a holding potential of −100 mV to either −10 mV ($rNa_v1.3$ channels) or −5 mV ($hNa_v1.3$ channels). (B) Voltage dependence of the first-order time constants for the fast component of inactivation (τ_{fast}) of $rNa_v1.3$ and $hNa_v1.3$ sodium channels obtained from currents recorded during 40-ms depolarizations from −100 mV to the indicated test potential (V_t). (C) Voltage dependence of the first-order time constants for the slow component of inactivation (τ_{slow}) of $rNa_v1.3$ and $hNa_v1.3$ sodium channels obtained from currents recorded during 40-ms depolarizations from −100 mV to the indicated test potential (V_t). (D) Percentage of $rNa_v1.3$ and $hNa_v1.3$ sodium channels exhibiting the slow component of inactivation at various test potentials (V_t); values were calculated from the proportionality constants of the two-component, single exponential fits of the falling phases of peak transient sodium currents.

Values in panels B-D are means ± SE of 7 ($rNa_v1.3$ channels) or 9 ($hNa_v1.3$ channels) separate experiments with different oocytes.

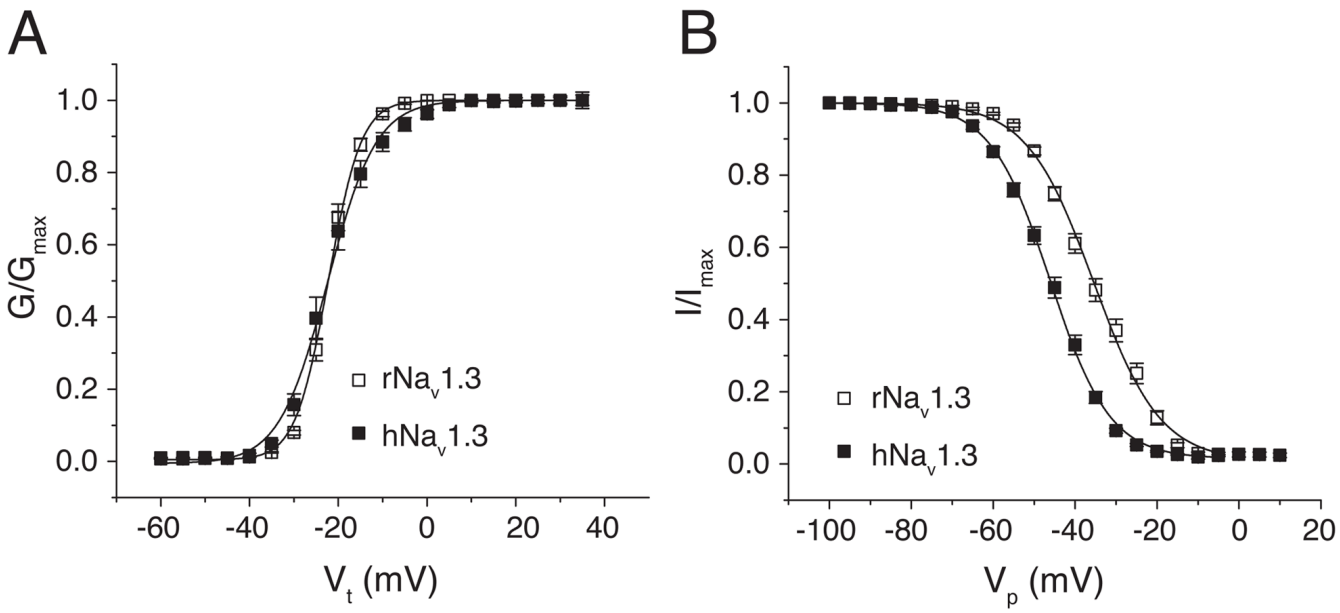

Figure 2.

Voltage-dependent activation and steady-state inactivation of rat and human $Na_v1.3$ sodium channels expressed in *Xenopus* oocytes. (A) Conductance-voltage plots for channel activation; peak sodium currents obtained on depolarizations from −100 mV to test potentials ranging from −60 mV to 35 mV were transformed to conductances (G) using the equation $G = I/(V_t - V_{rev})$, where I is the peak current, V_{rev} is the reversal potential, and V_t is the voltage of the test potential; conductances were then normalized to the maximum conductance (G_{max}) for that oocyte; values are means ± SE of 10 ($rNa_v1.3$ channels) or 9 ($hNa_v1.3$ channels) separate experiments with different oocytes; curves were fitted to the mean values using the Boltzmann equation. (B) Voltage dependence of steady-state inactivation; conditioning pulses (100 ms) from −100 mV to potentials ranging from −100 mV to 10 mV (V_p) were followed immediately by 40-ms test pulses to either −10 mV ($rNa_v1.3$ channels) or −5 mV ($hNa_v1.3$ channels); peak currents were normalized to the maximum current obtained during the inactivation protocol for that oocyte; values are means ± SE of 10 ($rNa_v1.3$ channels) or 9 ($hNa_v1.3$ channels) separate experiments with different oocytes; curves were fitted to the mean values using the Boltzmann equation.

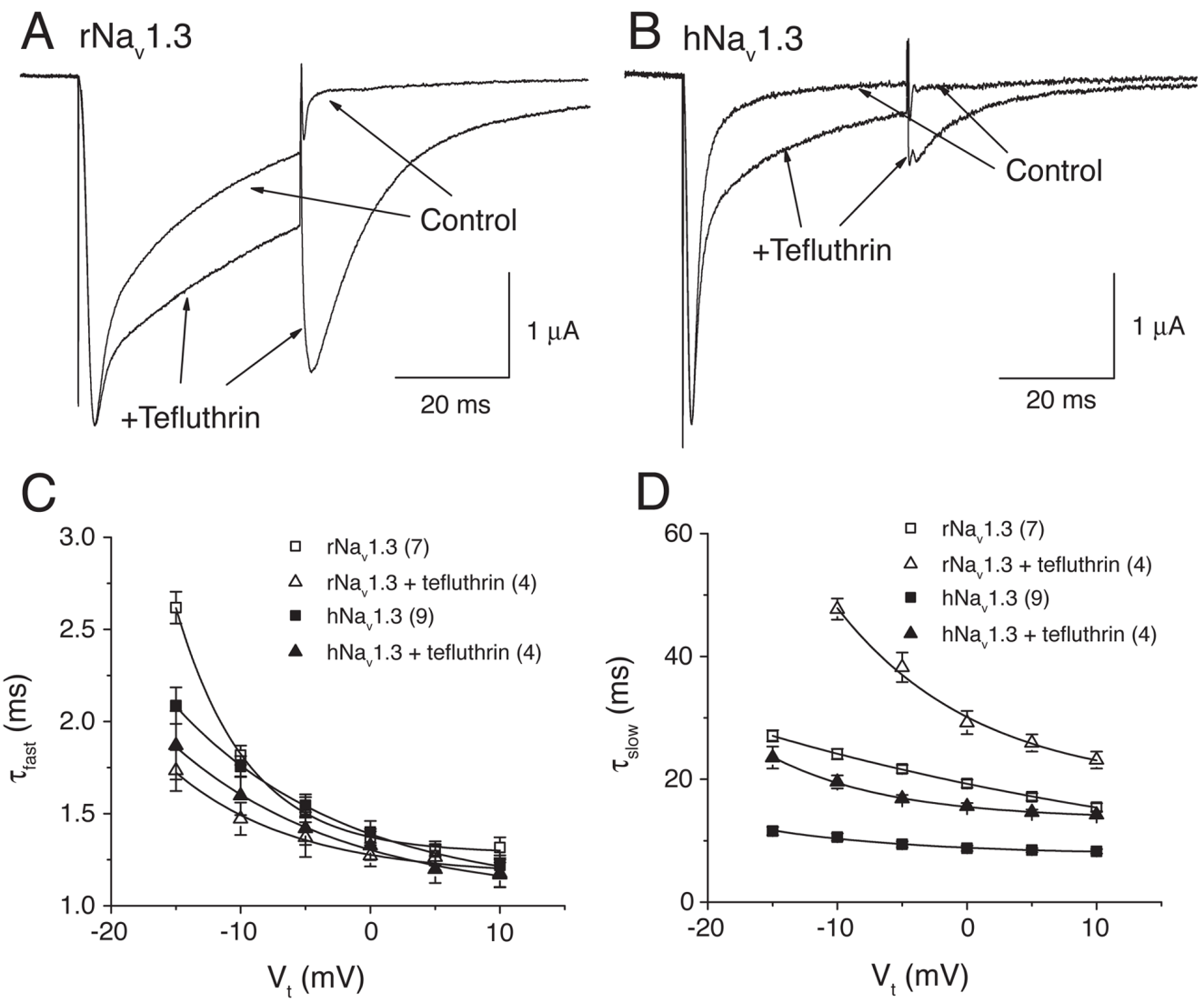

Figure 3.

Resting modification of rat and human $Na_v1.3$ sodium channels expressed in *Xenopus* oocytes by tefluthrin. (A) Representative sodium currents from the same oocyte expressing $rNa_v1.3$ sodium channels before and after exposure to 100 μM tefluthrin; currents were recorded during and after 40-ms depolarizing pulses to −10 mV from a holding potential of −100 mV. (B) Representative sodium currents from the same oocyte expressing $hNa_v1.3$ sodium channels before and after exposure to 100 μM tefluthrin; currents were recorded during and after 40-ms depolarizing pulses to −5 mV from a holding potential of −100 mV. (C) Effects of tefluthrin on the first-order time constants for the fast component of inactivation (τ_{fast}) of $rNa_v1.3$ and $hNa_v1.3$ sodium channels obtained from currents recorded during 40-ms depolarizations from −100 mV to the indicated test potential (V_t); values are means ± SE of the indicated number of separate experiments with different oocytes. (D) Effects of tefluthrin on the first-order time constants for the slow component of inactivation (τ_{slow}) of $rNa_v1.3$ and $hNa_v1.3$ sodium channels obtained from currents recorded during 40-ms depolarizations from −100 mV to the indicated test potential (V_t); values are means ± SE of the indicated number of separate experiments with different oocytes.

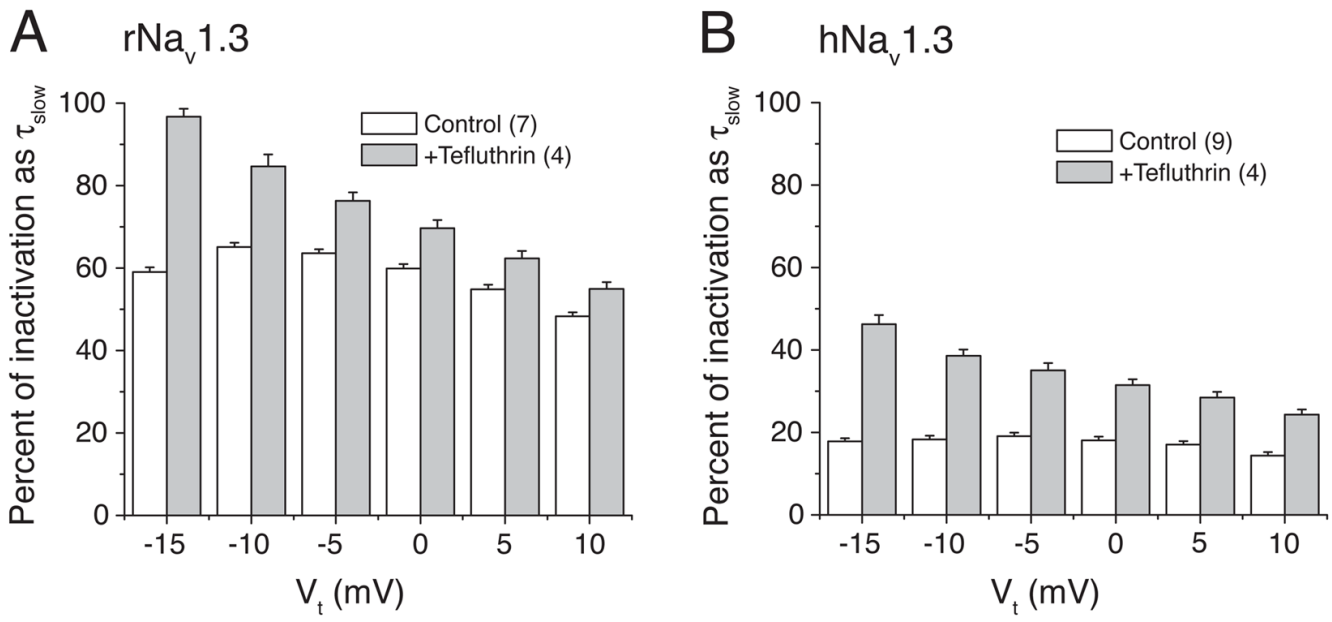

Figure 4.

Effect of tefluthrin on the fraction of rNa$_v$1.3 (A) or hNa$_v$1.3 (B) sodium channels exhibiting the slow component of inactivation at various test potentials (V_t). Values were calculated from the proportionality constants of the two-component, single exponential fits of the falling phases of peak transient sodium currents and are expressed as means ± SE of the indicated number of separate experiments with different oocytes.

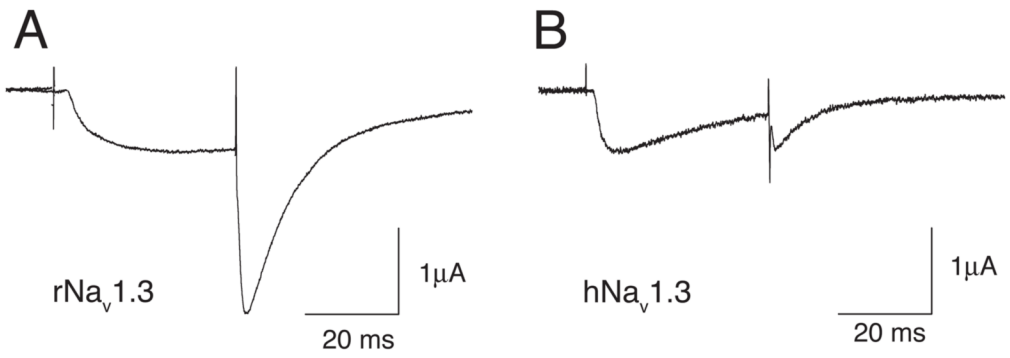

Figure 5.

Representative net sodium currents carried by tefluthrin-modified rNa$_v$1.3 (A) or hNa$_v$1.3 (B) sodium channels. Net currents were reconstructed by the subtraction of scaled control currents from tefluthrin-modified currents measured in the same oocyte (as in Figs. 3A and 3B).

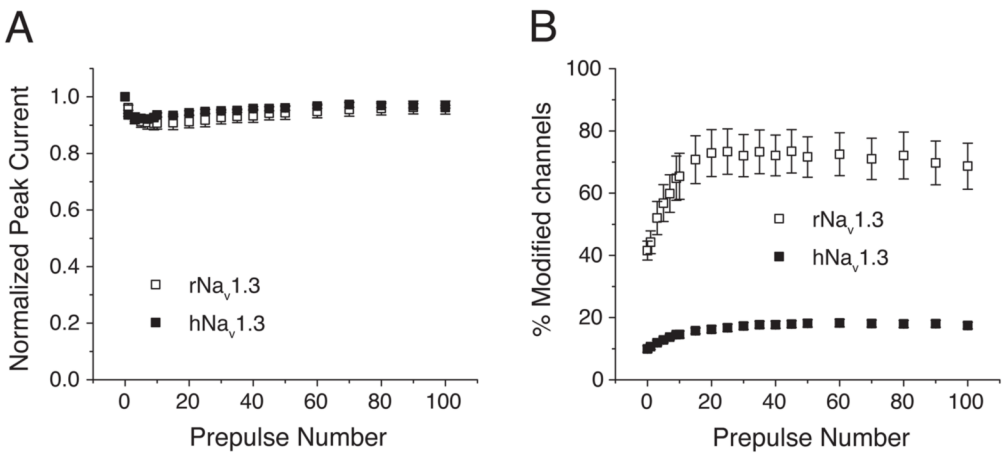

Figure 6.

Effect of repeated depolarization on sodium current stability and modification by tefluthrin for rat and human $Na_v1.3$ sodium channels. (A) Effect of repeated depolarizing pulses on the normalized amplitude of peak transient sodium currents; values were normalized to the amplitude of the peak current from the same oocyte prior to repetitive depolarization and are expressed as means ± SE of 7 ($rNa_v1.3$ channels) or 8 ($hNa_v1.3$ channels) separate experiments with different oocytes. (B) Effect of repeated depolarizing pulses on the extent of tefluthrin modification of $rNa_v1.3$ or $hNa_v1.3$ channels; for fractional modification were calculated from the normalized conductances of sodium tail currents and are expressed as means ± SE of 6 ($rNa_v1.3$ channels) or 8 ($hNa_v1.3$ channels) separate experiments with different oocytes.

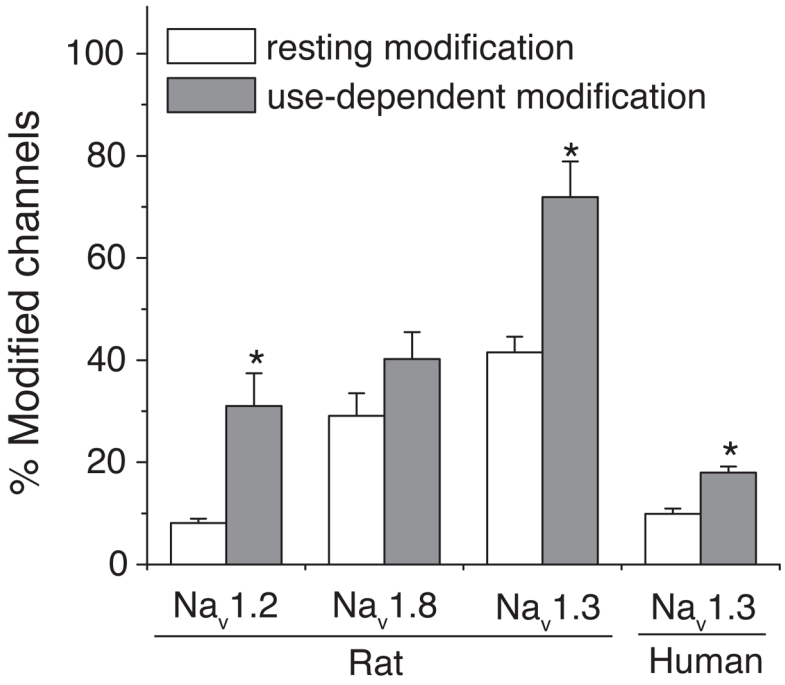

Figure 7.

Comparison of the resting and use-dependent (after 100 prepulses) modification of rat and human sodium channel isoforms expressed in *Xenopus* oocytes. Data for rat $Na_v1.2$, rat $Na_v1.3$ and human $Na_v1.3$ channels were obtained upon coexpression with the conspecific β1 and β2 subunits and are means ± SE of 6 (rat $Na_v1.3$) or 8 (rat $Na_v1.2$, human $Na_v1.3$) separate experiments with different oocytes. Data for rat $Na_v1.8$ channels (assayed in the absence of β subunits) are from a previous study in this laboratory (Choi and Soderlund, 2006) and are included for comparative purposes; these data are means ± SE of 3 separate experiments with different oocytes. Values for use-dependent modification marked with asterisks are significantly different from values for the resting modification of the same channel (paired t-tests, $P < 0.05$).

Table 1

Activation and inactivation gating parameters of rat and human $Na_v1.3$ sodium channels expressed in *Xenopus* oocytes in the absence or presence of tefluthrin[a].

		Activation			Inactivation		
Species	**Condition**	**$V_{0.5}$**	**K**	***n***	**$V_{0.5}$**	**K**	***n***
Rat	Control	−22.6 ± 0.3	3.20 ± 0.11	9	−35.5 ± 1.2	7.74 ± 0.19	10
	+Tefluthrin	−20.4 ± 0.4[b]	4.27 ± 0.34[b]	5	−35.3 ± 1.5	7.94 ± 0.19	7
Human	Control	−22.2 ± 1.2	4.85 ± 1.13[c]	9	−46.0[c] ± 0.8	7.17 ± 0.10[d]	9
	+Tefluthrin	−20.2 ± 2.4	6.14 ± 0.48	5	−45.3 ± 2.4	7.32 ± 0.11	5

[a] Values calculated from fits of data obtained in the absence (Fig. 2) or presence of 100 μM tefluthin to the Boltzmann equation; $V_{0.5}$, midpoint potential (mV) for voltage-dependent activation or inactivation; K, slope factor.

[b] Significantly different from control (unpaired t-test, $P < 0.01$).

[c] Significantly different from rat (unpaired t-test, $P < 0.01$).

[d] Significantly different from rat (unpaired t-test, $P < 0.05$).

Table 2

Kinetics of activation, fast inactivation, and tail current decay of tefluthrin-modified rat and human $Na_v1.3$ sodium channels expressed in *Xenopus* oocytes.

	Peak Current[a]			Tail Current[b]		
Species	τ_{act}	τ_{inact}	***n***	τ_1	τ_2	***n***
Rat	5.3 ± 0.4	nd[c]	7	0.89 ± 0.07	10.6 ± 0.6	5
Human	1.1 ± 0.2[d]	29.8 ± 3.2	6	4.1 ± 1.6[d]	9.8 ± 1.6	5

[a] Time constants (ms) for the activation (τ_{act}) and inactivation (τ_{inact}) of pyrethroid-modified channels based on reconstructed currents such as those shown in Fig. 5.

[b] Time constants (ms) for the fast (τ_1) and slow (τ_2) components of tail current decay based on fits to currents such as those shown in Fig. 5.

[c] No measurable inactivation of tefluthrin-modified current during a 40-ms depolarizing pulse.

[d] Significantly different from rat (unpaired t-test, $P < 0.001$).