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Coexpression with Auxiliary β Subunits Modulates the Action of Tefluthrin on Rat Na_v1.6 and Na_v1.3 Sodium Channels

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Abstract

We expressed the rat Na_v1.3 and Na_v1.6 sodium channel α subunit isoforms in *Xenopus* oocytes either alone or with the rat β 1 and β 2 auxiliary subunits in various combinations and assessed the sensitivity of the expressed channels to resting and use-dependent modification by the pyrethroid insecticide tefluthrin using the two-electrode voltage clamp technique. Coexpression with the β 1 and β 2 subunits, either individually or in combination, did not affecting the resting sensitivity of Na_v1.6 channels to tefluthrin. Modification by tefluthrin of Na_v1.6 channels in the absence of β subunits was not altered by the application of trains of high-frequency depolarizing prepulses. By contrast, coexpression of the Na_v1.6 channel with the β 1 subunit enhanced the extent of channel modification twofold following repeated depolarization. Coexpression of Na_v1.6 with the β 2 subunit also slightly enhanced modification following repeated depolarization, but coexpression of Na_v1.6 with both β subunits caused enhanced modification following repeated depolarization that was indistinguishable from that found with Na_v1.6+ β 1 channels. In contrast to Na_v1.6, the resting modification by tefluthrin of Na_v1.3 channels expressed in the absence of β subunits was reduced by repeated depolarization. However, tefluthrin modification of the Na_v1.3 α subunit expressed with both β subunits was enhanced 1.7-fold by repeated depolarization, thereby confirming that β subunit modulation of use-dependent effects was not confined to the Na_v1.6 isoform. These results show that the actions of pyrethroids on mammalian sodium channels in the *Xenopus* oocyte expression system are determined in part by the interactions of the sodium channel α subunit with the auxiliary β subunits that are part of the heteromultimeric sodium channel complexes found in neurons and other excitable cells.

Keywords

voltage-gated sodium channels; Na_v1.6 isoform; Na_v1.3 isoform; β subunit; voltage clamp; tefluthrin

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1. Introduction

Pyrethroid insecticides bind to voltage-gated sodium channels and alter their gating properties, thereby disrupting electrical signaling in the nervous system and causing acute intoxication in both insects and nontarget organisms [1,2]. Potential sodium channel targets for pyrethroid intoxication in mammals comprise nine different pore-forming α subunit isoforms ($\text{Na}_v1.1$ – $\text{Na}_v1.9$) that exhibit unique patterns of developmental and anatomical expression and varied functional and pharmacological properties [3]. Four α subunits ($\text{Na}_v1.1$, $\text{Na}_v1.2$, $\text{Na}_v1.3$, and $\text{Na}_v1.6$) are abundantly expressed in either the embryonic or adult brain and represent possible targets for pyrethroid neurotoxicity in the central nervous system. The $\text{Na}_v1.3$ and $\text{Na}_v1.6$ isoforms are of particular interest because they are the most abundantly-expressed isoforms in the embryonic ($\text{Na}_v1.3$) and adult ($\text{Na}_v1.6$) brain. Moreover, the $\text{Na}_v1.3$ and $\text{Na}_v1.6$ isoforms have recently been identified as being more sensitive to pyrethroids than the $\text{Na}_v1.2$ isoform [4–6].

Additional diversity among sodium channels results from their coassembly in the nerve membrane with one or two auxiliary β subunits. Channels in the adult brain are heterotrimeric complexes of one α subunit and two β subunits that differ in structure and their mode of association (covalent or noncovalent) with the α subunit [7]. Although there are four β subunits in mammals, the ubiquitous expression of the $\beta1$ and $\beta2$ subunits in the adult brain implies that the majority of brain channels are $\alpha+\beta1+\beta2$ complexes [8–10]. Heterologous expression experiments identify important roles for β subunits in the modulation of channel gating and the regulation of expression in the cell membrane [11].

The covalent labeling of the sodium channel α subunit protein by a pyrethroid photoaffinity ligand [12] and the functional modification by pyrethroids of channels heterologously expressed only from cloned α subunits [12–14] demonstrate that the pyrethroid binding site is located on the α subunit protein. Nevertheless, there is evidence that sodium channel β subunits modulate the action of pyrethroids on sodium channels transiently expressed in *Xenopus laevis* oocytes. Specifically, coexpression of the rat $\text{Na}_v1.2$ or $\text{Na}_v1.3$ sodium channel α subunit with either the rat $\beta1$ or $\beta3$ subunit enhances the sensitivity of these channels to modification by pyrethroids in the resting state [4,13]. However, there is no information available on the effect of the $\beta2$ subunit, either alone or in combination with the $\beta1$ subunit, on sodium channel modification by pyrethroids. Moreover, there is no information on the effect of β subunits on use-dependent sodium channel modification by pyrethroids, an effect that is particularly important in the action of deltamethrin and other Type II pyrethroids on both insect and mammalian sodium channels when expressed in the *Xenopus* oocyte system [15].

Here we describe the effect of coexpression with the rat $\beta1$ and $\beta2$ subunits on the sensitivity of rat $\text{Na}_v1.3$ and $\text{Na}_v1.6$ sodium channels to modification by the pyrethroid insecticide tefluthrin. Our studies of the $\text{Na}_v1.6$ isoform considered the independent and joint effects of the $\beta1$ and $\beta2$ subunits whereas our studies of the $\text{Na}_v1.3$ considered only the combined effects of the two β subunits. Our results identify a critical role for the β subunits, and in particular the $\beta1$ subunit, in conferring sensitivity of $\text{Na}_v1.3$ and $\text{Na}_v1.6$ sodium channels to the use-dependent component of channel modification by tefluthrin.

2. Materials and Methods

2.1 Expression in oocytes

Cloned rat voltage-sensitive sodium channel subunit cDNAs were obtained from the following sources: the $\text{Na}_v1.6$ α subunit from L. Sangameswaran (Roche Bioscience, Palo Alto, CA); the $\text{Na}_v1.3$ α subunit (adult splice variant) from A. L. Goldin (University of

California, Irvine, CA); and, the $\beta 1$ and $\beta 2$ subunits from W.A. Catterall (University of Washington, Seattle, WA). 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% (w/v) agarose – formaldehyde gels.

Stage V–VI oocytes were removed from female *X. laevis* frogs (Nasco, Ft. Atkinson, WI) as described elsewhere [14]. This procedure was performed in accordance with National Institutes of Health guidelines and followed a protocol that was approved by the Cornell University Institutional Animal Care and Use Committee. Each data set was derived from oocytes isolated from at least two different frogs. Oocytes were injected with 1:1 or 1:1:1 (mass ratio) mixtures of α subunit, $\beta 1$ subunit and $\beta 2$ subunit cRNAs (0.5 – 5 ng/oocyte); this mixture provided a ~9-fold molar excess of $\beta 1$ and $\beta 2$ cRNAs to ensure the preferential expression of desired binary or ternary $\alpha + \beta$ complexes [5,16]. Injected oocytes were incubated in ND-96 medium (in mM: 96 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, and 5 HEPES; adjusted to pH 7.6 at room temperature with NaOH) supplemented with 5% horse serum (Sigma-Aldrich, St. Louis, MO), 1% streptomycin/penicillin, and 1% sodium pyruvate [17] at 19°C for 3–5 days until electrophysiological analysis of sodium currents.

2.2 Electrophysiology

Sodium currents were recorded from oocytes perfused with ND-96 at 21–23°C in the two-electrode voltage clamp configuration using an Axon Geneclamp 500B amplifier (Molecular Devices, Sunnyvale, CA). Microelectrodes were pulled from borosilicate glass capillary tubes (1.0 mm O.D.; 0.5 mm I.D.; World Precision Instruments Inc., Sarasota, FL) and filled with 3 M KCl. Filled electrodes had resistances of 0.7–1.5 M Ω when immersed in ND-96 medium. Currents were filtered at 2 kHz with a low-pass 4-pole Bessel filter and digitized at 50 kHz (Digidata 1320A; Molecular Devices). 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 0 mV (Na_v1.6 channels) or –10 mV (Na_v1.3 channels). To determine the voltage dependence of steady-state inactivation, oocytes were clamped at a membrane potential of –140 mV (Na_v1.6 channels) or –120 mV (Na_v1.3 channels) followed by a 100-ms conditioning prepulse to potentials from –130 mV to 20 mV (Na_v1.6 channels) or from –120 mV to 20 mV (Na_v1.3 channels) in 5-mV increments and then a 40-ms test pulse to 0 mV (Na_v1.6 channels) or –10 mV (Na_v1.3 channels). 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 0 mV (Na_v1.6 channels) or –10 mV (Na_v1.3 channels). All experiments employed 10-s intervals between pulses or pulse trains to permit complete recovery from tefluthrin modification. Capacitive transients and leak currents were subtracted using the P/4 method [18].

2.3 Assays with tefluthrin

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 μ M. 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 tefluthrin in ND-96 for 6 min and then with ND-96 for 3 min prior to recording. All experiments with pyrethroids employed a disposable capillary perfusion system [19] and custom-fabricated single-use recording chambers [14] to prevent cross-contamination between oocytes.

2.4 Data analysis

Data were acquired and analyzed using pClamp 10.2 (Molecular Devices) and Origin 8.0 (OriginLab Corp., Northampton, MA). The Boltzmann equation $[y = (A_1 - A_2)/(1 + e^{(x-x_0)/dx}) + A_2]$ was used to fit conductance-voltage and sodium current inactivation data. Time constants for fast sodium channel inactivation were obtained by using the Chebyshev method in Origin 8.0 to fit the falling phase of the peak transient current to a double exponential decay model. Similarly, 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 [19]. Statistical analyses were performed in Prism 5.0 (GraphPad Software, La Jolla, CA). Comparisons of two or more mean values to a common control data set employed one-way analysis of variance (ANOVA) followed by an appropriate *post hoc* test for statistical significance. Comparisons of two means employed either a paired or unpaired Student's t-test depending on the design of the experiment. Details of statistical analyses and levels of significance are given in footnotes to the Tables and in the Figure legends.

3. Results

3.1 Effects of β Subunits on Na_v1.6 Sodium Channel Modification by Tefluthrin

In a previous study [16] we described the independent and joint effects of the rat $\beta 1$ and $\beta 2$ auxiliary subunits on the expression, kinetics and gating of rat Na_v1.6 sodium channels in the *Xenopus* oocyte system. Here we present the results of a parallel series of experiments to define the modulatory effects of the $\beta 1$ and $\beta 2$ subunits on the action of tefluthrin on Na_v1.6 sodium channels. We employed 100 μ M tefluthrin, the highest concentration achievable in ND-96 perfusion medium, to maximize the extent of sodium channel modification by this pyrethroid. Figure 1A shows representative currents recorded from a single oocyte expressing Na_v1.6+ $\beta 1$ + $\beta 2$ channels before exposure to tefluthrin and in the first depolarizing pulse after equilibration with 100 μ M tefluthrin. Modification of the sodium current by tefluthrin was evident in the enhancement of the persistent current during a depolarizing pulse and the induction of a prominent sodium tail current following repolarization. Similar results were obtained in assays with Na_v1.6, Na_v1.6+ $\beta 1$, and Na_v1.6+ $\beta 2$ channels (data not shown). With all four channel variants tefluthrin increased the persistent current measured at the end of a 40-ms depolarization (Fig. 1B).

Previous studies [5,6,20] showed that the decay of pyrethroid-induced sodium tail currents observed in the oocyte expression system is typically best fit by a two-component first-order model but that only one component of the decay is pyrethroid-specific. Figure 1C summarizes the effects of coexpression with β subunits on the compound-specific decay component of tefluthrin-induced tail currents. Coexpression with the $\beta 2$ subunit alone prolonged tail current decay significantly but the decay constants for Na_v1.6+ $\beta 1$ or Na_v1.6+ $\beta 1$ + $\beta 2$ channels did not differ significantly from that for Na_v1.6 channels.

Table 1 summarizes the effects of tefluthrin on the voltage dependence of activation and steady-state inactivation for Na_v1.6 sodium channels in the absence or presence of the $\beta 1$ and $\beta 2$ subunits. Tefluthrin did not alter the $V_{0.5}$ value or slope factor for activation of any of the channel variants. However, tefluthrin caused a small hyperpolarizing shift in the $V_{0.5}$ value for steady-state inactivation of Na_v1.6+ $\beta 1$ + $\beta 2$ channels and also altered the slope of the inactivation curve for both Na_v1.6+ $\beta 2$ and Na_v1.6+ $\beta 1$ + $\beta 2$ channels.

Previous studies [5,6,21] showed that the extent of modification by tefluthrin of rat Na_v1.2, Na_v1.3, Na_v1.6 and Na_v1.7 sodium channels, expressed in oocytes as α + β 1+ β 2 complexes, is significantly enhanced by repeated channel activation. This effect is illustrated for Na_v1.6+ β 1+ β 2 channels in Fig. 2, which shows tefluthrin-induced tail currents recorded from the same oocyte following a single depolarizing test pulse (0 prepulses) or after a train of 100 prepulses applied at a frequency of 66.7 Hz to a potential that caused maximal channel activation.

Figure 3A shows the effect of 0–100 depolarizing prepulses on the extent of modification by tefluthrin of Na_v1.6 channels expressed either alone or with all combinations of the β 1 and β 2 subunits, and Fig. 3B compares the extent of resting (0 prepulses) and maximal use-dependent (100 prepulses) modification for all four channel variants. In these experiments the tail current recorded following each prepulse train was normalized to the peak current obtained from the same oocyte after the same number of prepulses prior to pyrethroid exposure to isolate effects of the β subunits on channel modification from their effects on peak current stability in response to repeated depolarization [16]. Modification of Na_v1.6 channels in the resting state (0 prepulses) was not affected by coexpression with the β 1 and β 2 subunits individually or in combination. Repeated depolarization had no effect on channel modification in the absence of β subunits but enhanced the modification of Na_v1.6+ β 1 and Na_v1.6+ β 1+ β 2 channels by approximately twofold. Coexpression with the β 2 subunit alone also slightly enhanced resting modification, but inclusion of the β 2 subunit did not further increase use-dependent modification of Na_v1.6+ β 1+ β 2 channels when compared to Na_v1.6+ β 1 channels.

3.2 Effects of β Subunits on the Kinetics and Gating of Na_v1.3 Sodium Channels

To determine whether the effects of sodium channel β subunits on the modification of Na_v1.6 sodium channels by tefluthrin were specific only to the Na_v1.6 isoform, we performed similar experiments comparing the effects of tefluthrin on the rat Na_v1.3 isoform expressed either alone or in combination with the β 1 and β 2 subunits. Our initial experiments characterized the effects of the β 1 and β 2 subunits on the kinetic and gating properties of Na_v1.3 sodium channels to provide a basis for assessing the effects of tefluthrin.

Typical sodium currents recorded from oocytes expressing Na_v1.3 and Na_v1.3+ β 1+ β 2 sodium channels are shown in Fig. 4A. In the absence of β subunits Na_v1.3 channels activated and inactivated very slowly during a depolarizing pulse. Expression of Na_v1.3 with the β 1 and β 2 subunits gave currents that activated more rapidly but exhibited mixed kinetics for inactivation and did not inactivate completely during a 40-ms depolarizing pulse. The decay of peak transient currents such as those shown in Fig. 4A was fitted best by a two-component, single exponential decay model that yielded first-order decay constants for the initial fast phase (τ_{fast}) and the slower secondary phase (τ_{slow}) (Fig. 4B). Coexpression of the Na_v1.3 α subunit with the β 1 and β 2 subunits significantly accelerated the fast component of inactivation. In contrast to the effect observed on τ_{fast} , coexpression of the Na_v1.3 α subunit with the β 1 and β 2 subunits significantly prolonged the slow component of inactivation. We used the proportionality constants of the double exponential fits of peak current decay to calculate the fraction of the current carried by Na_v1.3 and Na_v1.3+ β 1+ β 2 channels that represented the slower component of inactivation. Coexpression with β subunits significantly reduced the relative abundance of slow gating component from $91.6 \pm 0.6\%$ for Na_v1.3 channels to $59.9 \pm 1.1\%$ for Na_v1.3+ β 1+ β 2 channels ($P < 0.005$; unpaired t-test).

The voltage-dependent gating of Na_v1.3 and Na_v1.3+ β 1+ β 2 sodium channels is illustrated in Fig. 5 and the statistical analyses of these data are summarized in Table 2. Coexpression

of Na_v1.3 channels with the β 1 and β 2 subunits caused a ~5-mV hyperpolarizing shift in the midpoint potential for activation and a small but significant increase in the steepness of the voltage response (numerical decrease in the slope factor) compared to Na_v1.3 channels (Fig. 5A). Coexpression of Na_v1.3 channels with the β 1 and β 2 subunits caused a ~10-mV hyperpolarizing shift in the midpoint potential for inactivation accompanied by a significant decrease in the steepness of the voltage response compared to rNa_v1.3 channels (Fig. 5B).

3.3 Effects of β Subunits on Na_v1.3 Sodium Channel Modification by Tefluthrin

Figure 6 show representative currents recorded from a single oocyte expressing either Na_v1.3 or Na_v1.3+ β 1+ β 2 channels before exposure to tefluthrin and in the first depolarizing pulse after equilibration with 100 μ M tefluthrin. Tefluthrin increased the persistent component of current measured during a depolarizing pulse and induced a prominent sodium tail current following repolarization. Fits of tail currents using a two-component first-order model yielded tail current time constants of 8.3 ± 0.9 ms (mean $\pm$ SE, $n = 5$) for Na_v1.3 channels and 10.6 ± 0.6 ms ($n = 4$) for Na_v1.3+ β 1+ β 2 channels. The difference between these values was not statistically significant ($P > 0.05$; unpaired t-test).

Table 2 summarizes the effects of tefluthrin on the voltage dependence of activation and steady-state inactivation of Na_v1.3 and Na_v1.3+ β 1+ β 2 channels. Tefluthrin had no effect on the activation curve for Na_v1.3 channels but produced a small depolarizing shift in the $V_{0.5}$ for the activation of Na_v1.3+ β 1+ β 2 channels that was accompanied by a significant change in the slope factor for the activation curve. By contrast, tefluthrin had no effect on the voltage dependence of steady-state inactivation of either Na_v1.3 or Na_v1.3+ β 1+ β 2 channels.

The effects of the β 1 and β 2 subunits on the use-dependent modification of rat Na_v1.3 channels by tefluthrin are summarized in Fig. 7 and Fig. 8. We previously showed that currents carried by Na_v1.6 channels declined rapidly to about 60% of the initial amplitude when challenged with trains of high-frequency depolarizing prepulses in the absence of the β 1 subunit [16]. Therefore, prior to determining the use-dependent effects of tefluthrin on Na_v1.3 and Na_v1.3+ β 1+ β 2 channels we assessed the effect of the β subunits on sodium current stability (Fig. 7). In the absence of β subunits, currents carried by Na_v1.3 channels declined rapidly in response to high-frequency trains of depolarizing prepulses to ~70% of the control current after 20 prepulses and remained stable at that level for up to 100 prepulses. By contrast, currents carried by Na_v1.3+ β 1+ β 2 channels were more stable, declining to ~90% of the control current over the first 10 prepulses and remained stable thereafter.

Figure 8A shows the effect of 0–100 depolarizing prepulses on the extent of modification of Na_v1.3 and Na_v1.3+ β 1+ β 2 channels by tefluthrin. In these assays, the fractional modification for each oocyte was normalized using the conductance of the peak transient current obtained after the same number of prepulses prior to the application of tefluthrin (as in Fig. 7). This enabled us to isolate the effects of the β subunits on channel modification from their effects on peak current stability. In the absence of β subunits, repeated depolarization decreased the extent of Na_v1.3 channel modification compared to resting channels (0 prepulses). By contrast, in the presence of the β 1 and β 2 subunits repeated depolarization caused a sharp increase in the extent of Na_v1.3 channel modification over the first 20 prepulses to a level that remained stable for up to 100 prepulses. Figure 8B compares the effect of the β 1 and β 2 subunits on resting (0 prepulses) and maximal use-dependent (100 prepulses) modification by tefluthrin. Repetitive depolarization in the absence of the β subunits decreased channel modification to ~77% of that observed for resting channels. However, repetitive depolarization of oocytes expressing Na_v1.3+ β 1+ β 2 channels caused a ~1.7-fold increase in the extent of channel modification by tefluthrin.

4. Discussion

4.1 Effects of β subunits on sodium channel kinetics, gating and peak current stability

In the *Xenopus* oocyte system sodium channel β subunits typically modify both the kinetics and gating of expressed sodium channels, but the nature and magnitude of these effects vary with both the α subunit isoform and the specific combinations of β subunits that are expressed [11,22,23]. We previously demonstrated that the independent and joint effects of the $\beta 1$ and $\beta 2$ subunits on $\text{Na}_v 1.6$ channel kinetics and gating in oocytes were distinct but subtle, involving acceleration of the rate of decay of the peak transient current and inducing small (< 5 mV) shifts in the voltage dependence of activation [16]. Moreover, we documented effects of the $\beta 1$ and $\beta 2$ subunits expressed together that were not predicted by the sum of the effects of these subunits expressed individually.

The combined effect of the $\beta 1$ and $\beta 2$ subunits on $\text{Na}_v 1.3$ kinetics and gating in this study differed substantially from their effects on the $\text{Na}_v 1.6$ channel described previously [16]. The $\beta 1$ and $\beta 2$ subunits did not eliminate the slow component of peak transient current decay observed for the rat $\text{Na}_v 1.3$ channel although they were expressed at a large molar excess to the α subunit. The heterogeneous inactivation kinetics and predominance of the slow-inactivating component in our assays of $\text{Na}_v 1.3+\beta 1+\beta 2$ channels are consistent with the results of other studies of the rat $\text{Na}_v 1.3$ isoform in oocytes, which involved coexpression with either the $\beta 1$ or $\beta 3$ subunit [4,9,24]. Moreover, the unusual inactivation properties of $\text{Na}_v 1.3+\beta 1+\beta 2$ channels do not result from the failure of the injected mixture of cRNAs to express a homogeneous population of the desired heteromultimeric complex in oocytes, because rat $\text{Na}_v 1.3+\beta 1$ channels exhibit inactivation kinetics similar to those described here for $\text{Na}_v 1.3+\beta 1+\beta 2$ channels across a 50-fold range of $\beta 1$ subunit concentrations [24]. These unusual inactivation properties appear to be unique to the rat $\text{Na}_v 1.3$ ortholog because human $\text{Na}_v 1.3+\beta 1+\beta 2$ channels expressed in oocytes exhibit the fast inactivation kinetics typical of most sodium channel $\alpha+\beta$ complexes in this system [5]. In addition to these effects on peak current kinetics, the $\beta 1$ and $\beta 2$ subunits not only caused a hyperpolarizing shift the voltage dependence of activation but also caused a larger hyperpolarizing shift in the voltage dependence of steady-state inactivation, effects not seen in parallel experiments with the $\text{Na}_v 1.6$ isoform [16].

Coexpression of $\text{Na}_v 1.3$ channels with the $\beta 1$ and $\beta 2$ subunits also affected peak current stability in response to high-frequency depolarizations in a manner similar to that found previously for $\text{Na}_v 1.6$ channels. Both the $\text{Na}_v 1.6$ isoform [16] and the $\text{Na}_v 1.3$ isoform (this study) expressed in the absence of β subunits gave currents that declined rapidly in amplitude following repeated depolarization. This effect, which is also observed with $\text{Na}_v 1.2$ and $\text{Na}_v 1.4$ channels expressed in oocytes [25,26], has been attributed to rapid entry of these channels into slow-inactivated states. Coexpression of $\text{Na}_v 1.6$ with the $\beta 1$ subunit or the $\beta 1$ and $\beta 2$ subunits in combination, but not the $\beta 2$ subunit alone, preserved the stability of peak transient currents following trains of high-frequency depolarizations [16]. The present study documented a similar effect of the $\beta 1$ and $\beta 2$ subunits in combination on the stability of peak currents carried by $\text{Na}_v 1.3$ channels.

4.2 Effects of β subunits on sodium channel modification by tefluthrin

The principal finding of the present study was that coexpression with β subunits, and in particular the $\beta 1$ subunit, was necessary to confer use-dependent enhancement of tefluthrin modification for both $\text{Na}_v 1.6$ and $\text{Na}_v 1.3$ sodium channels expressed in oocytes. Expression of $\text{Na}_v 1.6$ with only the $\beta 2$ subunit conferred a small but statistically significant degree of enhancement, but this effect was not additive with the much larger effect of the $\beta 1$ subunit in experiments with $\text{Na}_v 1.6+\beta 1+\beta 2$ channels. In a preliminary study we observed similar

effects of the $\beta 1$ and $\beta 2$ subunits on use-dependent modification of $\text{Na}_v 1.6$ channels by deltamethrin [27]. The $\beta 1$ and $\beta 2$ subunits also conferred sensitivity to use-dependent modification when coexpressed with the $\text{Na}_v 1.3$ α subunit isoform, thereby confirming that this effect is not limited to the $\text{Na}_v 1.6$ channel. The present study held the tefluthrin concentration constant to facilitate the comparison of different sodium channel subunit combinations. However, we previously showed that the enhancement of the modification of $\text{Na}_v 1.6+\beta 1+\beta 2$ channels by 100 μM tefluthrin corresponds to more than a 10-fold increase in the apparent affinity of these channels for tefluthrin based on partial concentration curves for resting and use-dependent modification [6].

The use-dependent enhancement of tefluthrin modification in these assays implies that channel opening increases the affinity of the pyrethroid binding site for this compound [15]. The requirement for coassembly with the $\beta 1$ subunit to observe use-dependent effects therefore implies that the $\beta 1$ subunit modifies the conformation of the inner pore region of the open channel, the proposed location of the pyrethroid receptor [28]. There are only a few other examples of the effects of β subunits on the pharmacological properties of binding sites located on the sodium channel α subunit. Coexpression of the rat $\beta 1$ subunit increased the affinity of resting or inactivated rat $\text{Na}_v 1.4$ channels to block by lidocaine but decreased the affinity resting human $\text{Na}_v 1.5$ channels to this drug without affecting block of inactivated channels [29,30]. Also, coexpression of the rat $\text{Na}_v 1.3$ channel with the $\beta 1$ subunit reduced use-dependent block by lidocaine whereas coexpression with the $\beta 3$ subunit had no effect [31]. Finally, coexpression with the $\beta 1$ subunit decreased the sensitivity of rat $\text{Na}_v 1.2$ channels to block in the slow-inactivated state by the sodium channel inhibitor insecticide RH 3421 [32]. In all three of these cases, coexpression with the $\beta 1$ subunit alters the sensitivity of sodium channels to block by compounds that bind at or near the local anesthetic receptor in the inner pore region of the channel, a location close to the site of the proposed pyrethroid receptor. However, the molecular mechanisms by which sodium channel β subunits alter pharmacological properties of binding sites in the sodium channel α subunit inner pore region are not known.

The $\beta 1$ nor the $\beta 2$ subunits expressed individually or together did not affect the tefluthrin sensitivity of $\text{Na}_v 1.6$ channels in the resting state. By contrast, coexpression of $\text{Na}_v 1.3$ channels with the $\beta 1$ and $\beta 2$ subunits significantly reduced the resting sensitivity of these channels to tefluthrin. These results differ from those of previous studies, which document enhancement of the sensitivity of rat $\text{Na}_v 1.2$ and $\text{Na}_v 1.3$ channels to pyrethroid modification in the resting state by coexpression in binary complexes with either the $\beta 1$ or $\beta 3$ subunit [4,13]. The absence of data in the present study for the same combinations of sodium channel subunits and pyrethroids that were employed previously prevented us from determining whether this discrepancy reflected differences between various α subunit isoforms when expressed as a binary complex with the $\beta 1$ subunit, differences between binary ($\alpha+\beta 1$) and ternary ($\alpha+\beta 1+\beta 2$) complexes, or differences between tefluthrin and the pyrethroids employed in other studies.

Results of the present study, together with those of previous studies [4,13], provide evidence that the actions of pyrethroids on mammalian sodium channels in the *Xenopus* oocyte expression system are determined in part by the interactions of the sodium channel α subunit, which contains both the channel pore and the pyrethroid receptor, with the auxiliary β subunits that are part of the heteromultimeric sodium channel complexes found in neurons and other excitable cells. Sodium channel β subunits often produce different effects on the gating and kinetics of a single sodium channel α subunit isoform when expressed in different cellular backgrounds [33,34]. Therefore, the effects of β subunits on resting and use-dependent sodium channel modification by pyrethroids documented in our results and the results of previous studies [4,13] are specific to the *Xenopus* oocyte expression system

and are not necessarily predictive of the pharmacological properties of sodium channel $\alpha+\beta$ channel complexes either in other heterologous expression systems (e.g., mammalian cell lines) or in neurons. Nevertheless, future studies of the action of pyrethroids on sodium channels in any heterologous expression system must account for the potential effects of auxiliary β subunits in shaping the pharmacological properties of the expressed channel.

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Research Highlights

- Tefluthrin caused only resting modification of channels expressed without β subunits
- The $\beta 1$ and $\beta 2$ subunits did not affect resting modification of $\text{Na}_v 1.6$ channels
- Coexpression of $\text{Na}_v 1.3$ with the $\beta 1$ and $\beta 2$ subunits reduced resting modification
- β subunits were required for use-dependent modification of $\text{Na}_v 1.6$ and $\text{Na}_v 1.3$ channels

Fig. 1.

Effects of tefluthrin (100 μM) on sodium currents carried by rat Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 sodium channels. (A) Representative control and tefluthrin-modified sodium currents recorded from an oocyte expressing rat Na_v1.6+β1+β2 sodium channels using the indicated depolarization protocol. (B) Effect of tefluthrin on persistent currents carried by Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 sodium channels. Currents were measured at the end of a 40-ms depolarizing pulse as shown in Panel A. Values are means ± SE of 5–10 separate experiments with different oocytes. Values within the control and tefluthrin treatment groups marked with different letters were significantly different (one-way ANOVA with Tukey's multiple comparison test; $P < 0.05$). The value for each channel measured in the presence of tefluthrin was significantly different from the control value for the same channel (paired t-tests, $P < 0.05$). (C) Time constants for the fast component of decay of the tefluthrin-induced tail current for Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 sodium channels. Values (means ± SE of 5–17 separate experiments with different oocytes) marked with different letters were significantly different (one-way ANOVA with Tukey's multiple comparison test; $P < 0.05$).

Fig. 2. Representative traces showing tefluthrin-induced sodium tail currents recorded from a single oocyte expressing Na_v1.6+β1+β2 sodium channels following either 0 or 100 depolarizing prepulses and a 40-ms test pulse using the indicated pulse protocol.

Fig. 3.

(A) Effect of repeated depolarizing prepulses on the extent of modification of rat Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 by tefluthrin. Values are means of 5–8 separate experiments with different oocytes; bars show SE values larger than the data point symbols. (B) Comparison of the extent of resting (after 0 prepulses) and maximal use-dependent (after 100 prepulses) modification of rat Na_v1.6, Na_v1.6+β1, Na_v1.6+β2, and Na_v1.6+β1+β2 by tefluthrin. Values for use-dependent modification marked with asterisks were significantly different from values for the resting modification of the same channel (paired t-tests, $P < 0.05$).

Fig. 4. Sodium currents carried by rat $\text{Na}_v1.3$ and $\text{Na}_v1.3+\beta1+\beta2$ sodium channels. (A) Representative current traces from oocytes expressing either $\text{Na}_v1.3$ or $\text{Na}_v1.3+\beta1+\beta2$ sodium channels recorded using the indicated pulse protocol. (B) Time constants for fast and slow components of the decay of peak transient currents such as those shown in Panel A. Values are means $\pm$ SE of 10 ($\text{Na}_v1.3$ channels) or 7 ($\text{Na}_v1.3+\beta1+\beta2$ channels) separate experiments with different oocytes; values for $\text{Na}_v1.3+\beta1+\beta2$ channels marked with asterisks were significantly different from the corresponding values for $\text{Na}_v1.3$ channels (unpaired t -test, $P < 0.01$).

Fig. 5.

Voltage dependence of activation and steady-state inactivation of rat $Na_v1.3$ and $Na_v1.3+\beta1+\beta2$ sodium channels. (A) Conductance-voltage plots for channel activation. Peak transient sodium currents were measured during 40-ms depolarizations from a holding potential of -100 mV to test potentials (V_t) from -60 mV to 40 mV; peak sodium currents were transformed to conductances (G) using the equation $G = I/(V_{rev}-V_t)$, 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 of 9 separate experiments with different oocytes; bars show SE values larger than the data point symbols. Curves were fitted to the mean values using the Boltzmann equation. (B) Current-voltage plots for steady-state inactivation. Conditioning pulses (100 ms) from -120 mV to voltages ranging from -120 mV to 20 mV (V_p) were followed immediately by 40-ms test pulses to -10 mV; peak currents measured during the test pulse were normalized to the maximum peak current for that oocyte. Values are means of 9 ($Na_v1.3$ channels) or 10 ($Na_v1.3+\beta1+\beta2$ channels) separate experiments with different oocytes; bars show SE values larger than the data point symbols. Curves were fitted to the mean values using the Boltzmann equation.

Fig. 6. Representative sodium currents recorded from oocytes expressing rat $\text{Na}_v1.3$ (A) or $\text{Na}_v1.3+\beta1+\beta2$ (B) sodium channels before and after exposure to tefluthrin ($100 \mu\text{M}$).

Fig. 7. Effect of repeated depolarizing prepulses on sodium current stability in assays with rat $\text{Na}_v1.3$ and $\text{Na}_v1.3+\beta1+\beta2$ sodium channels. Amplitudes of peak transient sodium currents were normalized to the amplitude of the peak current from the same oocyte prior to repetitive depolarizations; values are means of 8 separate experiments with different oocytes; bars show SE values larger than the data point symbols.

Fig. 8.

(A) Effect of repeated depolarizing prepulses on the extent of modification of rat Na_v1.3 and Na_v1.3+β1+β2 channels by tefluthrin. Values are means ± SE of 5 (Na_v1.3) or 6 (Na_v1.3+β1+β2) separate experiments with different oocytes. (B) Comparison of the extent of resting (after 0 prepulses) and maximal use-dependent (after 100 prepulses) modification of rat Na_v1.3 and Na_v1.3+β1+β2 channels by tefluthrin. Values for use-dependent modification marked with asterisks were significantly different from values for the resting modification of the same channel (paired t-tests, $P < 0.05$).

Effects of coexpression with the $\beta 1$ and $\beta 2$ subunits on the voltage dependence of activation and steady-state inactivation of $\text{Na}_v 1.6$ sodium channels in the absence or presence of tefluthrin.^a

Table 1

Channel	Condition	Activation			Inactivation		
		$V_{0.5}$	K	n	$V_{0.5}$	K	n
$\text{Na}_v 1.6$	Control	$-13.4 \pm 0.8^{*b}$	$8.77 \pm 0.16^{*}$	15	-51.5 ± 0.4	$8.84 \pm 0.28^{*}$	11
	+Tefluthrin	-12.0 ± 1.7	8.57 ± 0.21	4	-51.9 ± 1.0	8.54 ± 0.54	5
$\text{Na}_v 1.6+\beta 1$	Control	$-14.2 \pm 0.3^{*}$	$9.04 \pm 0.14^{*†}$	48	-50.8 ± 0.3	$7.09 \pm 0.15^{†}$	26
	+Tefluthrin	-13.4 ± 0.7	8.66 ± 0.30	16	-51.0 ± 0.7	7.15 ± 0.08	14
$\text{Na}_v 1.6+\beta 2$	Control	$-11.3 \pm 0.4^{†}$	$8.57 \pm 0.21^{*†}$	20	-50.9 ± 0.4	$8.05 \pm 0.08^{†}$	26
	+Tefluthrin	-10.9 ± 0.8	9.34 ± 0.24	6	-51.6 ± 1.0	7.27 ± 0.28^c	6
$\text{Na}_v 1.6+\beta 1+\beta 2$	Control	$-16.8 \pm 0.3^{†}$	$7.93 \pm 0.15^{†}$	32	-51.1 ± 0.3	$7.19 \pm 0.08^{†}$	34
	+Tefluthrin	-17.5 ± 0.5	7.64 ± 0.21	12	-52.6 ± 0.7^c	6.37 ± 0.19^c	11

^aValues calculated from fits of the data from the indicated number of individual experiments to the Boltzmann equation; $V_{0.5}$, midpoint potential (mV) for voltage-dependent activation or inactivation; K, slope factor; data in the absence of tefluthrin are from Tan and Soderlund [16].

^bValues in each column obtained in the absence of tefluthrin that are marked with different symbols were significantly different by one-way ANOVA with Tukey's multiple comparison test ($P < 0.01$).

^cSignificantly different from control value for the same channel (unpaired t -test, $P < 0.05$).

Effects of coexpression with the $\beta 1$ and $\beta 2$ subunits on the voltage dependence of activation and steady-state inactivation of $\text{Na}_v 1.3$ sodium channels in the absence or presence of tefluthrin.^a

Table 2

Channel	Condition	Activation		Inactivation	
		$V_{0.5}$	K	$V_{0.5}$	K
$\text{Na}_v 1.3$	Control	-17.3 ± 0.9	4.06 ± 0.15	-26.0 ± 0.8	6.59 ± 0.26
	+Tefluthrin	-18.4 ± 0.9	4.09 ± 0.25	-26.7 ± 0.6	6.70 ± 0.22
$\text{Na}_v 1.3+\beta 1+\beta 2$	Control	-22.6 ± 0.3^b	3.20 ± 0.11^b	-35.5 ± 1.2^b	7.74 ± 0.19^b
	+Tefluthrin	-20.4 ± 0.4^c	4.27 ± 0.34^c	-35.3 ± 1.5	7.94 ± 0.19

^a Values calculated from fits of the data from the indicated number of individual experiments to the Boltzmann equation; $V_{0.5}$, midpoint potential (mV) for voltage-dependent activation or inactivation; K, slope factor.

^b Significantly different from $\text{Na}_v 1.3$ (unpaired t -test, $P < 0.01$).

^c Significantly different from control value for $\text{Na}_v 1.3+\beta 1+\beta 2$ (unpaired t -test, $P < 0.01$)