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Actions of tefluthrin on rat $Na_v1.7$ voltage-gated sodium channels expressed in *Xenopus* oocytes

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ABSTRACT

In rats expression of the $Na_v1.7$ voltage-gated sodium channel isoform is restricted to the peripheral nervous system and is abundant in the sensory neurons of the dorsal root ganglion. We expressed the rat $Na_v1.7$ sodium channel α subunit together with the rat auxiliary β1 and β2 subunits in *Xenopus laevis* oocytes and assessed the effects of the pyrethroid insecticide tefluthrin on the expressed currents using the two-electrode voltage clamp method. Tefluthrin at 100 μM modified of $Na_v1.7$ channels to prolong inactivation of the peak current during a depolarizing pulse, resulting in a marked "late current" at the end of a 40 ms depolarization, and induced a sodium tail current following repolarization. Tefluthrin modification was enhanced up to twofold by the application of a train of up to 100 5 ms depolarizing prepulses. These effects of tefluthrin on $Na_v1.7$ channels were qualitatively similar to its effects on rat $Na_v1.2$, $Na_v1.3$ and $Na_v1.6$ channels assayed previously under identical conditions. However, $Na_v1.7$ sodium channels were distinguished by their low sensitivity to modification by tefluthrin, especially compared to $Na_v1.3$ and $Na_v1.6$ channels. It is likely that $Na_v1.7$ channels contribute significantly to the tetrodotoxin-sensitive, pyrethroid-resistant current found in cultured dorsal root ganglion neurons. We aligned the complete amino acid sequences of four pyrethroid-sensitive isoforms (house fly Vssc1; rat $Na_v1.3$, $Na_v1.6$ and $Na_v1.8$) and two pyrethroid-resistant isoforms (rat $Na_v1.2$ and $Na_v1.7$) and found only a single site, located in transmembrane segment 6 of homology domain I, at which the amino acid sequence was conserved among all four sensitive isoform sequences but differed in the two resistant isoform sequences. This position, corresponding to Val410 of the house fly Vssc1 sequence, also aligns with sites of multiple amino acid substitutions identified in the sodium channel sequences of pyrethroid-resistant insect populations. These results implicate this single amino acid polymorphism in transmembrane segment 6 of sodium channel homology domain I as a determinant of the differential pyrethroid sensitivity of rat sodium channel isoforms.

1. Introduction

Actions on voltage-gated sodium channels are intrinsic to both the insecticidal effects of pyrethroids and their neurotoxic effects in mammals [1]. Pyrethroids disrupt nerve function by altering the kinetics of the transitions between conducting (open) and nonconducting (closed; inactivated) states of voltage-gated sodium channels that underlie the generation of nerve action potentials [2]. Pyrethroid modification causes persistent channel opening, which in turn causes repetitive nerve firing following single stimuli or use-dependent conduction block depending on the duration of the open time of the pyrethroid-modified channel.

Native sodium channels in mammalian tissues are hetero multimeric complexes comprised of one large (~260 kDa) α subunit and either one or two smaller (33–36 kDa) auxiliary β subunits [3,4]. Sodium channel α subunits form the ion pore and confer the fundamental functional and pharmacological properties of the channel [3]. Sodium channel β subunits modulate channel function, regulate channel expression at the level of the plasma membrane, and contribute to cell adhesion and cell-cell communication [4].

Mammalian genomes contain nine genes encoding sodium channel α subunit isoforms, designated $Na_v1.1$–$Na_v1.9$ [5,6], and four genes encoding sodium channel β subunits, designated β1–β4 [4]. Overlapping patterns of sodium channel α subunit expression in the central and peripheral neurons [7,8] 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*. This system has

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been employed to assess the pyrethroid sensitivity of four rat sodium channel isoforms. $Na_v1.2$ sodium channels exhibit very low sensitivity to modification by deltamethrin and other pyrethroids [9,10]. By contrast rat $Na_v1.3$, $Na_v1.6$ and $Na_v1.8$ channels are more sensitive to pyrethroid modification [11–16].

The dorsal root ganglion (DRG), a cluster of cell bodies of afferent sensory nerves, has been widely used to study the electrical properties of mammalian peripheral neurons [17]. Electrophysiological studies with cultured DRG neurons have identified two populations of sodium channels that are distinguished by their gating properties and their sensitivity to block by tetrodotoxin (TTX) [18]. The TTX-resistant channel population is sensitive to pyrethroid modification, whereas the TTX-sensitive population is relatively insensitive to pyrethroids [19,20]. The TTX-resistant sodium current has been correlated with expression of the $Na_v1.8$ isoform, but the coordinate expression of multiple TTX-sensitive isoforms within individual DRG cells [17] complicates the identification of the molecular basis of the TTX-sensitive current that is resistant to pyrethroids.

The $Na_v1.7$ sodium channel isoform (formerly designated PN1) was first identified by cDNA cloning from peripheral nervous tissue [21,22] and is abundantly expressed in the dorsal root ganglion [17]. Here we describe the action of the potent pyrethroid insecticide tefluthrin on the rat the $Na_v1.7$ sodium channel α subunit isoform coexpressed with the rat β1 and β2 subunits in *Xenopus* oocytes. Our results identify $Na_v1.7$ as a pyrethroid-resistant isoform that is likely to contribute to the TTX-sensitive, pyrethroid-resistant sodium current of DRG neurons.

2. Materials and methods

2.1. Expression in oocytes

The rat $Na_v1.7$ voltage-gated sodium channel α subunit cDNA was provided by G. Mandel (State University of New York, Stony Brook, NY) and the β1 and β2 subunit cDNAs were provided by 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 [12]. 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 a 1:1:1 (mass ratio) mixtures of α subunit, β1 subunit and β2 subunit 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 the desired ternary α + β complex [23]. 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 at room temperature with NaOH) supplemented with 5% horse serum (Sigma–Aldrich), 1% streptomycin/penicillin, and 1% sodium pyruvate [24] at 19 °C for 3–5 days until electrophysiologic 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, Foster City, 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 MΩ when immersed in ND-96 medium. Currents were filtered at 2 kHz with a low-pass four-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 to 20 mV in 5 mV increments. Maximal peak transient currents were obtained upon depolarization to −10 mV. To determine the voltage dependence of steady-state inactivation, oocytes were clamped at a membrane potential of −140 mV followed by a 100 ms conditioning prepulse to potentials from −100 to 5 mV in 5 mV increments and then a 40 ms test pulse to −10 mV. For determinations of use dependence, oocytes were given trains of 1–100 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. 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 [25].

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 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 tefluthrin in ND-96 for 6 min and then with ND-96 for 3 min prior to recording. Washout of tefluthrin during perfusion with ND-96 for up to 30 min was negligible under these conditions. All experiments with pyrethroids employed a disposable capillary perfusion system [20] and custom-fabricated single-use recording chambers [12] to prevent cross-contamination between oocytes.

2.4. Data analysis

Data were acquired and analyzed using pClamp 10.2 (Molecular Devices, Burlingame, CA) 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 7.0 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 currents measured following 40 ms step depolarizations from −100 to −10 mV 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 [20]. Statistical analyses were performed using the Prism software package (GraphPad Software, La Jolla, CA).

3. Results

3.1. Expression, gating and kinetics of $Na_v1.7$ sodium channels

We expressed the $Na_v1.7$ α subunit in combination with the β1 and β2 auxiliary subunits to give channel complexes of comparable subunit composition to those employed in our previous studies of