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Differential state-dependent modification of inactivation-deficient $Na_v1.6$ sodium channels by the pyrethroid insecticides *S*-bioallethrin, tefluthrin and deltamethrin

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ABSTRACT

Pyrethroid insecticides disrupt nerve function by modifying the gating kinetics of transitions between the conducting and nonconducting states of voltage-gated sodium channels. Pyrethroids modify rat $Na_v1.6 + \beta1 + \beta2$ channels expressed in *Xenopus* oocytes in both the resting state and in one or more states that require channel activation by repeated depolarization. The state dependence of modification depends on the pyrethroid examined: deltamethrin modification requires repeated channel activation, tefluthrin modification is significantly enhanced by repeated channel activation, and *S*-bioallethrin modification is unaffected by repeated activation. Use-dependent modification by deltamethrin and tefluthrin implies that these compounds bind preferentially to open channels. We constructed the rat $Na_v1.6Q3$ cDNA, which contained the IFM/QQQ mutation in the inactivation gate domain that prevents fast inactivation and results in a persistently open channel. We expressed $Na_v1.6Q3 + \beta1 + \beta2$ sodium channels in *Xenopus* oocytes and assessed the modification of open channels by pyrethroids by determining the effect of depolarizing pulse length on the normalized conductance of the pyrethroid-induced sodium tail current. Deltamethrin caused little modification of $Na_v1.6Q3$ following short (10 ms) depolarizations, but prolonged depolarizations (up to 150 ms) caused a progressive increase in channel modification measured as an increase in the conductance of the pyrethroid-induced sodium tail current. Modification by tefluthrin was clearly detectable following short depolarizations and was increased by long depolarizations. By contrast modification by *S*-bioallethrin following short depolarizations was not altered by prolonged depolarization. These studies provide direct evidence for the preferential binding of deltamethrin and tefluthrin (but not *S*-bioallethrin) to $Na_v1.6Q3$ channels in the open state and imply that the pyrethroid receptor of resting and open channels occupies different conformations that exhibit distinct structure–activity relationships.

1. Introduction

Effects on voltage-gated sodium channels underlie both the insecticidal actions of pyrethroids and their neurotoxicity to nontarget organisms (Soderlund, 2012; Soderlund et al., 2002). Pyrethroids modify the gating of voltage-gated sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the rising phase of the nerve action potential (reviewed in: Bloomquist, 1993; Narahashi, 1996; Soderlund, 1995). Voltage- and patch-clamp analyses of isolated sodium currents show that pyrethroids retard the kinetics of sodium channel activation, inactivation, and deactivation to produce persistently open channels. The hallmark of pyrethroid modification in all experimental systems is the induction of a sodium tail current following membrane depolarization–repolarization cycles under voltage clamp whose persistence is correlated with pyrethroid structure.

The majority of studies of pyrethroid action on sodium currents in neurons under voltage-clamp conditions have been performed by equilibrating preparations with insecticide at hyperpolarized membrane potentials and assessing the effects of pyrethroids upon depolarization (Bloomquist, 1993; Narahashi, 1996; Soderlund, 1995). This approach is biased toward the detection of the modification of channels in the closed state by pyrethroids. Consequently, only a few studies have explored possible use-dependent modification by assessing the impact of trains of depolarizing prepulses on the amplitude of the pyrethroid-induced sodium tail current. Use-dependent enhancement of modification

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is detected in assays of fenvalerate on sodium currents in crayfish giant axons (Salgado and Narahashi, 1993), assays of deltamethrin on the tetrodotoxin (TTX)-sensitive and TTX-resistant components of the sodium current in rat dorsal root ganglion neurons (Tabarean and Narahashi, 2001), and assays of tetramethrin and permethrin on sodium currents in honeybee antennal olfactory neurons (Kadala et al., 2011). By contrast, repetitive depolarization did not alter the time-dependent increase in the sodium tail current elicited by perfusion of squid giant axon preparations with phenothrin (de Weille et al., 1988).

Most of the evidence for use-dependent sodium channel modification comes from studies of pyrethroid action on cloned sodium channels expressed transiently in oocytes of the frog *Xenopus laevis*. Modification by some pyrethroids of both insect and mammalian channels expressed in *X. laevis* oocytes either depends on or is enhanced by trains of high-frequency depolarizing prepulses (reviewed in: Soderlund, 2010). Recent studies of the rat $Na_v1.6$ sodium channel isoform in the *Xenopus* oocyte system show that the relative importance of resting and use-dependent channel modification depends on the pyrethroid examined (Tan and Soderlund, 2010) and that use-dependent effects require coexpression of the $Na_v1.6$ α subunit isoform with the auxiliary β1 and β2 subunits (Tan and Soderlund, 2011a).

The use-dependent effects of pyrethroids on insect sodium channels expressed in *Xenopus* oocytes have been interpreted as evidence for the existence of a high-affinity receptor conformation on channels in the open state (Vais et al., 2000). Despite the inferred importance of pyrethroid binding to open channels there is little direct evidence for open-channel modification by pyrethroids. Assays of *Drosophila melanogaster* Para sodium channels expressed in *Xenopus* oocytes employed *Anemonia sulcata* toxin II (ATX-II) to eliminate fast inactivation and document pyrethroid modification of persistently open channels (Vais et al., 2000). However, the allosteric coupling of the ATX-II and pyrethroid binding sites on mammalian sodium channels (Lombet et al., 1988) suggests that the pyrethroid receptor of ATX-II-modified Para channels may exhibit pharmacological properties that differ from those of native channels.

To study pyrethroid interactions with open channels directly in the absence of pharmacological manipulation we introduced mutations in the inactivation gate region of the rat $Na_v1.6$ sodium channel that are known to eliminate fast inactivation (West et al., 1992; Zhou and Goldin, 2004) and employed these inactivation-deficient channels to assess pyrethroid modification in the *Xenopus* oocyte expression system. We employed three pyrethroids that exhibit compound-specific differences in the relative importance of resting and use-dependent modification of native $Na_v1.6$ sodium channels in the oocyte expression system. Deltamethrin modification requires repeated channel activation, tefluthrin modification is significantly enhanced by repeated channel activation, and *S*-bioallethrin modification is unaffected by repeated activation (Tan and Soderlund, 2010). Our results provide direct evidence that the differences in resting and use-dependent modification of native $Na_v1.6$ channels among these three pyrethroids result from their differential modification of resting and open channels. Our results imply that the pyrethroid receptor of resting and open channels occupies different conformations that exhibit distinct structure–activity relationships.

2. Materials and methods

2.1. Sodium channel subunit cDNAs

Cloned rat voltage-sensitive sodium channel subunit cDNAs were obtained from the following sources: the $Na_v1.6\alpha$ subunit (Dietrich et al., 1998) from L. Sangameswaran (Roche Bioscience, Palo Alto, CA) and the β1 and β2 subunits (Isom et al., 1992, 1995) from W.A. Catterall (University of Washington, Seattle, WA). The cDNA for the $Na_v1.6Q3$ sodium channel α subunit, containing the IFM to QQQ mutation at amino acid sequence positions 1477–1479, was prepared by oligonucleotide-mediated site-directed mutagenesis on the parental $Na_v1.6$ cDNA using a commercial kit (QuikChange XL, Stratagene, La Jolla, CA) and the structure of the $Na_v1.6Q3$ cDNA was confirmed by DNA sequencing. 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.

2.2. Expression in oocytes

Freshly dissected *X. laevis* ovaries obtained from a commercial source (Nasco, Ft. Atkinson, WI) were employed as a source of stage V–VI oocytes, which were isolated as described elsewhere (Smith and Soderlund, 2001). Each data set was derived from oocytes isolated from three different ovaries. Oocytes were injected with a 1:1:1 (mass ratio) mixture 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 desired binary or ternary α + β complexes (Tan and Soderlund, 2009, 2011b). 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 6% horse serum (Sigma–Aldrich, St. Louis, MO), 0.5% streptomycin/penicillin, and 1% sodium pyruvate (Goldin, 1992) at 16 °C for 3–5 days until electrophysiological analysis of sodium currents.

2.3. 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 0.3–1.0 MΩ 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 test pulses to potentials to 40 mV in 5-mV increments. Maximal peak transient currents were obtained upon depolarization to −10 mV. For determinations of time-dependent modification of $Na_v1.6Q3$ channels, oocytes were clamped at a membrane potential of −100 mV and currents were measured during and after test pulses increasing in duration from 3 ms to 150 ms in 3, 5 or 10 ms increments. All experiments employed 30-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).

2.4. Assays with pyrethroids

S-bioallethrin (92.9% purity) and deltamethrin (99.5%) were obtained from Bayer CropScience (Research Triangle Park, NC) and tefluthrin (98.8%) was obtained from Syngenta (Bracknell, Berks., UK). Pyrethroids were prepared as stock solutions in dimethyl sulfoxide (DMSO) and diluted with ND-96 immediately before use to final concentrations of 10 μM (deltamethrin) or 100 μM (*S*-bioallethrin and tefluthrin), the highest concentrations achievable