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Compound-specific effects of mutations at Val787 in DII-S6 of $Na_v1.4$ sodium channels on the action of sodium channel inhibitor insecticides

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ABSTRACT

Sodium channel inhibitor (SCI) insecticides are hypothesized to inhibit voltage-gated sodium channels by binding selectively to the slow-inactivated state. Replacement of valine at position 787 in the S6 segment of homology domain II of the rat $Na_v1.4$ sodium channel by lysine (V787K) enchances slow inactivation of this channel whereas replacement by alanine or cysteine (V787A and V787C) inhibits slow inactivation. To test the hypothesis that SCI insecticides bind selectively to the slow-inactivated state, we constructed mutated $Na_v1.4$/V787A, $Na_v1.4$/V787C, and $Na_v1.4$/V787K cDNAs, expressed wildtype and mutated channels with the auxiliary β1 subunit in *Xenopus* oocytes, and used the two-electrode voltage clamp technique to examine the effects of these mutations on channel inhibition by four SCI insecticides (indoxacarb, its bioactivated metabolite DCJW, metaflumizone, and RH3421). Mutations at Val787 affected SCI insecticide sensitivity in a manner that was independent of mutation-induced changes in slow inactivation gating. Sensitivity to inhibition by 10 μM indoxacarb was significantly increased in all three mutated channels, whereas sensitivity to inhibition by 10 μM metaflumizone was significantly reduced in $Na_v1.4$/V787A channels and completely abolished in $Na_v1.4$/V787K channels. The effects of Val787 mutations on metaflumizone were correlated with the hydrophobicity of the substituted amino acid rather than the extent of slow inactivation. None of the mutations at Val787 significantly affected the sensitivity to inhibition by DCJW or RH3421. These results demonstrate that the impact of mutations at Val787 on sodium channel inhibition by SCI insecticides depend on the specific insecticide examined and is independent of mutation-induced changes in slow inactivation gating. We propose that Val787 may be a unique determinant of metaflumizone binding.
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1. Introduction

Sodium channel inhibitor (SCI) insecticides are an emerging class of neurotoxic insect control agents that cause ataxia, convulsions, paralysis, and mortality in arthropods (Salgado, 1990). SCI insecticides were first discovered in the early 1970s, though compounds in this insecticide class have only become commercially available within the last decade. The oxadiazine indoxacarb (Fig. 1A), the first SCI insecticide to be commercially registered, is a proinsecticide that is efficiently converted in insects but not in mammals to its more potent *N*-decarbomethoxylated metabolite, DCJW (Fig. 1A) (Wing et al., 2005). The novel semicarbazone metaflumizone (Fig. 1B), the second SCI insecticide to be commercialized, is the first member of this insecticide class introduced into the animal health market (Salgado and Hayashi, 2007). Dihydropyrazoles (*e.g.* RH-3421 and RH-4841; Fig. 1C) exemplify earlier members of the SCI family that exhibited high insecticidal activity but were not developed commercially because of their high subchronic neurotoxicity to mammals (Payne et al., 1998; Silver and Soderlund, 2005a). The continued efforts of the agrochemical industry to develop new, commercially viable SCI compounds reinforces both the value of this type of biological activity and the need to better understand the actions of these compounds that underlie their toxicological properties.

The insecticidal activity of SCI insecticides results from their disruption of electrical signaling in the nervous system by inhibiting voltage-gated sodium channels (Salgado, 1992). Voltage-gated sodium channels are also considered to be the primary targets for the central neurotoxic effects of SCI insecticides in mammals, but there is limited information on the mammalian toxicity of these compounds available in the public literature. Acute oral administration of RH-3421 causes ataxia, tremors,

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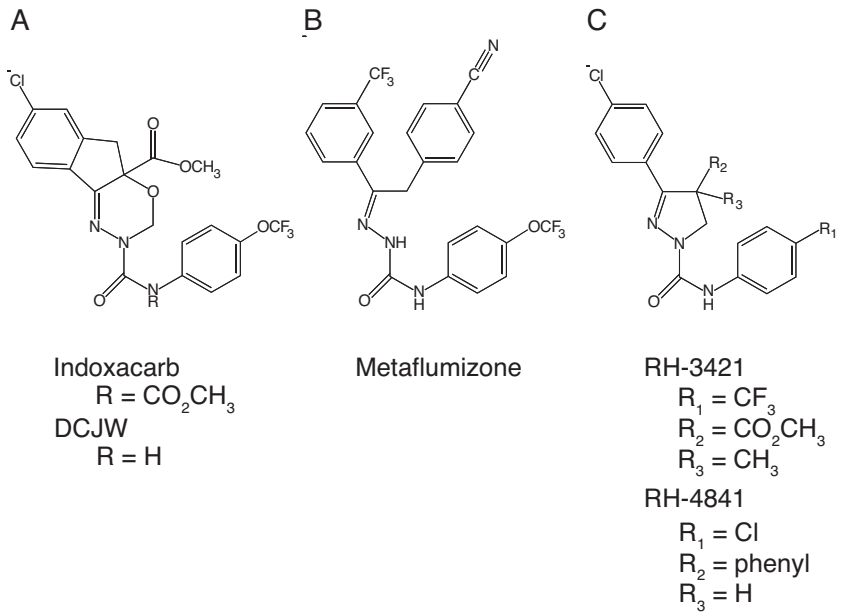

Fig. 1. Structures of SCI insecticides. (A) Indoxacarb and DCJW. (B) Metaflumizone. (C) RH-3421 and RH-4841.

salivation, spasms, and a hunched posture in rats (Salgado, 1992; Silver and Soderlund, 2005a), and similar symptoms were reported in rats administered a high oral dose of indoxacarb (Zhao et al., 1999). A direct linkage between effects of SCIs on sodium channel function and the neurotoxic actions of these compounds in mammals remain to be established.

Voltage-gated sodium channels are integral membrane proteins composed of a large (~260 kDa) α subunit that confers all of the major structural, functional, and pharmacological properties of native channels and either one or two small auxiliary β subunits that modulate channel gating and regulate expression in the cell membrane (Catterall, 2000; Isom, 2001). The α subunit comprises 24 transmembrane α-helical segments that are organized into four homologous domains (DI-DIV) around a central ion-conducting pore. Each domain contains six transmembrane segments, designated S1–S6.

Voltage-gated sodium channels undergo changes in conformation and functional state in response to transient or persistent changes in the voltage difference across the plasma membrane. Transient depolarization from a negative resting potential initiates a cycle of channel activation and fast inactivation that underlies the initiation and termination of a nerve action potential. By contrast, persistent or prolonged depolarizations cause channels to enter non-conducting slow-inactivated states (Goldin, 2003). The transitioning of sodium channels between conformational states not only alters sodium ion permeability but also determines the sensitivity of channels to many neurotoxic and therapeutic agents (McDonough and Bean, 2006).

SCI insecticides are state-dependent inhibitors of sodium channels. They have no effect on channels in the resting state but disrupt channel function through a selective interaction with channels in the non-conducting slow-inactivated state (Salgado, 1992; Lapied et al., 2001; Zhao et al., 2003; Silver and Soderlund, 2005b; von Stein and Soderlund, 2012). However, previous studies provide indirect evidence that some SCIs may also interact with other sodium channel states. Experiments with crayfish axons showed that inhibition of sodium channels by an experimental SCI insecticide (RH-1211) was not disrupted by progressive proteolytic removal of slow and fast inactivation by intracellular perfusion of trypsin and *N*-bromoacetamide, respectively (Salgado, 1992). Furthermore, metaflumizone differed from other SCI insecticides in its ability to antagonize the inhibition of $Na_v1.4$ sodium channels by the local anesthetic lidocaine under experimental conditions that promoted lidocaine binding to fast-inactivated channels but did not cause insecticide inhibition (von Stein and Soderlund, 2012). These results not only suggest that metaflumizone may bind to fast-inactivated channels without inhibiting them but also imply a fundamental difference in the state-dependent action of metaflumizone compared to other SCI insecticides.

SCI drugs (local anesthetics, class I anticonvulsants and antiarrhythmics), which bind inside the inner pore at the local anesthetic (LA) receptor, preferentially target open or fast-inactivated channels (Ragsdale et al., 1994, 1996). Studies employing site-directed mutagenesis have identified putative drug binding determinants in the S6 transmembrane segments in all domains except domain II (Ragsdale et al., 1994; Yarov-Yarovoy et al., 2001, 2002). Residues in domain IV-S6, particularly Phe1579 and Tyr1586 (rat $Na_v1.4$ numbering) appear to be essential for state-dependent inhibition by SCI drugs. Inhibition of slow-inactivated $Na_v1.4$ channels by SCI insecticides is also modulated by mutations at Phe1579 and Tyr1586 (Silver and Soderlund, 2007; von Stein and Soderlund, 2012). These findings are consistent with the hypothesis that SCI insecticides bind selectively to slow-inactivated sodium channels at or near the LA receptor.

The present study was initiated to test the hypothesis that the extent of sodium channel inhibition by SCI insecticides depends directly on the availability of slow-inactivated channel states. We employed experimental mutations at the highly conserved Val787 residue in sodium channel DII-S6 that either increase or decrease the propensity of channels to enter slow inactivated states (O'Reilly et al., 2001). Replacement of Val787 by lysine (V787K) profoundly enhances slow inactivation, whereas replacement by either alanine or cysteine (V787A and V787C) inhibits slow inactivation compared to wildtype channels. We anticipated that mutations at Val787 would selectively modify slow inactivation without affecting binding of SCI insecticides to the channel because this residue has not been identified previously as part of the LA receptor (Wang et al., 2001; Kondratiev and Tomaselli, 2003). We expressed wildtype $Na_v1.4$ channels and $Na_v1.4$ channels possessing the V787A, V787C, or V787K mutation in combination with the rat β1 subunit in *Xenopus laevis* oocytes and assessed the sensitivity of channels to inhibition by SCI insecticides. Surprisingly, the impact of mutations

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