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State-Dependent Modification of Voltage-Gated Sodium Channels by Pyrethroids

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Abstract

Pyrethroids disrupt nerve function by altering the rapid kinetic transitions between conducting and nonconducting states of voltage-gated sodium channels that underlie the generation of nerve action potentials. Recent studies of pyrethroid action on cloned insect and mammalian sodium channel isoforms expressed in *Xenopus laevis* oocytes show that in some cases pyrethroid modification is either absolutely dependent on or significantly enhanced by repeated channel activation. These use-dependent effects have been interpreted as evidence of preferential binding of at least some pyrethroids to the open, rather than resting, state of the sodium channel. This paper reviews the evidence for state-dependent modification of insect and mammalian sodium channels expressed in oocytes by pyrethroids and considers the implications of state-dependent effects for understanding the molecular mechanism of pyrethroid action and the development and testing of models of the pyrethroid receptor.

Keywords

pyrethroid; cismethrin; cypermethrin; deltamethrin; permethrin; tefluthrin; sodium channel; insect; rat; human

1. Introduction

The transient permeability of neuronal cell membranes to sodium ions is responsible for the nerve action potentials that are the fundamental units of information transfer within neurons. These transient permeability changes are mediated by voltage-gated sodium channels, large proteins in the cell membrane that contain an intrinsic sodium-selective ion pore that opens (activates) briefly in response to a depolarizing change in the transmembrane potential and closes (inactivates) by a mechanism that is independent of membrane repolarization [1]. The simplest model for sodium channel function postulates three distinct channel states: closed or resting (available for activation); open; and inactivated (closed but not available for activation). The transitions between these states can be observed in the classic voltage clamp experiment, which is illustrated in Fig. 1. At hyperpolarized membrane potentials, channels are closed and available for activation. Depolarization of the membrane causes a rapid opening of sodium channels, seen as an inward current. When the membrane is held at a

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depolarized potential, the duration of the sodium current is limited to a few ms by the onset of inactivation, which renders the channel nonconducting. Returning the membrane to a hyperpolarized potential converts inactivated channels to closed channels, making them available once again for activation.

These state transitions require a minimum of two independent gating processes, one for activation and one for inactivation, which were described conceptually by Hodgkin and Huxley more than 50 years ago [2]. In the closed state, the activation gate of the channel is closed and the inactivation gate is open. Depolarization of the membrane opens the activation gate, creating an open channel, which is quickly followed by the closing of the inactivation gate to give the nonconducting inactivated state. The conversion of inactivated to closed channels upon membrane repolarization involves two processes: closing of the activation gate ("deactivation") followed by opening of the inactivation gate. Advances in the structural analysis of voltage-gated sodium channels and other members of the voltage-gated cation channel superfamily have identified specific structural elements that mediate the activation and inactivation gating events proposed by Hodgkin and Huxley [3-5].

The insecticidal activity of pyrethroids depends on their ability to modify the gating kinetics of voltage-gated sodium channels, thereby disrupting normal cellular communication [6]. Voltage clamp techniques have been employed extensively to document the effects of pyrethroids on sodium currents recorded from invertebrate and vertebrate neurons [7,8]. The hallmarks of pyrethroid action measured under these conditions are a slowing of inactivation during a depolarizing pulse and delayed deactivation, which is evident as a slowly-decaying sodium tail current conducted by channels that remain open following a depolarization - repolarization cycle (Fig. 2). Whereas delayed inactivation and deactivation are universal elements of pyrethroid-modified sodium currents, the actions of some pyrethroids in some experimental systems may also produce channels with altered activation kinetics or voltage dependence of either channel activation or steady-state inactivation.

The majority of studies of pyrethroid actions on sodium channel gating under voltage clamp conditions have been performed by equilibrating channels with pyrethroids at hyperpolarized membrane potentials and assessing the effects of pyrethroids upon depolarization. This approach is biased toward the detection of closed-state modification, and most of these studies have not considered the contribution of other channel states to pyrethroid action. However, there is evidence from studies with voltage-clamped squid and crayfish axons that modification by some pyrethroids is enhanced by repetitive depolarization, implying that channel opening increases the affinity of the pyrethroid binding site for these compounds [9-11]. More recently, the discovery that modification by cypermethrin and deltamethrin of cloned insect sodium channels expressed in *Xenopus laevis* oocytes is absolutely dependent on repeated depolarization [12,13] has led to the conclusion that these compounds bind preferentially to open sodium channels. These findings have prompted renewed interest in the broader significance of pyrethroid binding to the open state of sodium channels and have led to the development of a new structural model for the molecular interactions between pyrethroids and the open state of the house fly sodium channel pore region [14].

The properties that distinguish closed-state and open-state modification of sodium channels by pyrethroids are summarized in Fig. 3. Closed-state modification requires that closed channels exhibit an affinity for pyrethroids that is equal to or greater than that of open channels. When pyrethroids bind to channels in the closed state, these modified channels may exhibit altered kinetics and voltage dependence of activation, but repetitive activation will not increase the extent of channel modification. In contrast, pure open-state modification requires that the affinity of open channels be substantially greater than that of

closed channels. Binding to the open state requires prior channel activation, so that the kinetics and voltage dependence of activation are not affected but the extent of channel modification exhibits use dependence. It is important to note that effects of pyrethroids on the kinetics and voltage dependence of inactivation and the kinetics of deactivation originate from the pyrethroid-modified open state and are not affected by the route to that state. Also, closed and open channel modification are not mutually exclusive; if the affinity of open channels for a given pyrethroid is greater than the affinity of closed channels, closed-channel modification will be enhanced by repeated activation.

Studies over the past decade of the actions of pyrethroids on cloned insect and mammalian sodium channels expressed in the *Xenopus* oocyte system have provided new insight into state-dependent sodium channel modification by pyrethroids. The following sections summarize these data and their importance to an understanding of pyrethroid mode of action.

2. Insect sodium channels

2.1 Insect sodium channel genes

The pore-forming α subunits of voltage-gated sodium channels in insects are the products of a single gene, the *para* gene in *Drosophila melanogaster* [15] and its orthologs in other species [16]. The differential expression and functional diversity of sodium channels in insects is achieved through the extensive alternative splicing. In both the *D. melanogaster para* and house fly (*Musca domestica*) *Vssc1* genes, alternative splicing of seven optional exons and two mutually exclusive exon pairs could theoretically generate 512 unique *para* transcripts, although only a small number of these (typically <10) are found in pools of transcribed mRNAs [16].

Initial efforts to express functional sodium channels from synthetic *para* RNA in *Xenopus* oocytes gave barely detectable sodium currents, but coexpression with synthetic RNA corresponding to the *D. melanogaster tipE* coding sequence greatly enhanced sodium current expression [17,18]. The TipE protein appears to function as a sodium channel auxiliary subunit, modifying the gating properties of Para sodium channels as well as enhancing expression [18]. Recently, four homologs of *tipE* have been identified in the *D. melanogaster* genome and found to encode proteins that also modulate the expression of Para sodium channels in oocytes [19]. Coexpression with TipE also enhances and modulates the expression of house fly *Vssc1* and *Blattella germanica* sodium channels in oocytes [20-22], thereby implying the existence of TipE-like sodium channel auxiliary subunits in these species. The only TipE ortholog identified to date is the *Vssc β* protein of the house fly, which is a more effective modulator of *Vssc1* sodium channels in oocytes than TipE [20].

2.2 Resting modification by cismethrin

Cismethrin provides the clearest example of a compound that modifies insect sodium channels in the resting or closed state. Initial experiments with house fly *Vssc1*/TipE sodium channels in oocytes showed extensive modification of sodium currents in the first depolarizing pulse following equilibration at a hyperpolarized holding potential (Fig. 4A) [12]. Typical of the actions of pyrethroids on sodium channels in other systems, cismethrin caused a pronounced non-inactivating “late current” that persisted at the end of a depolarizing pulse and a slowly-decaying tail current following repolarization. Measurement of control and cismethrin-modified currents following depolarization to a range of membrane potentials showed that the voltage dependence of activation of the late current was shifted by ~7 mV relative to the peak current (Fig. 4B). This shift in the voltage dependence of the cismethrin-modified current permitted the selective activation of cismethrin-modified channels by depolarizing oocytes to a membrane potential (e.g., -40

mV; arrow in Fig. 4B), at which cismethrin-modified channels are preferentially activated. Selective activation of cismethrin-modified channels caused the development of a slowly-activating, noninactivating current not seen in the absence of cismethrin (Fig. 4C). The appearance of modified currents during the first depolarizing pulse after equilibration, the shift in the voltage dependence of activation of the cismethrin-modified channels, and the induction of a slowly-activating current in the presence of cismethrin at test potentials that failed to activate unmodified channels provide clear evidence that cismethrin modifies Vssc1 sodium channels in the closed state. Repetitive depolarization did not enhance the degree of modification observed during the first depolarizing pulse [12]. Therefore, open channels do not exhibit enhanced affinity for this compound, but this result does not rule out the possibility that cismethrin may act with equal effectiveness on both closed and open channels.

2.3 Use-dependent modification by cypermethrin and deltamethrin

In contrast to cismethrin, cypermethrin (1*R*,*cis*, α *S* isomer) failed to modify Vssc1/TipE sodium channels expressed in oocytes during the first depolarizing pulse after equilibration (Fig. 5) [12]. However, the application of increasing numbers depolarizing prepulses prior to a standard test depolarization caused corresponding increases in the amplitude of the cypermethrin-modified currents measured during the test pulse. The absolute dependence of cypermethrin modification on channel activation implies that this compound, unlike cismethrin, binds preferentially to the open state of the channel.

A subsequent study [13] employed deltamethrin, a close structural relative of cypermethrin, and *D. melanogaster* Para/TipE sodium channels expressed in oocytes to characterize in greater detail pyrethroid modification of insect channels in the open state. In this study, multiple brief depolarizations promoted deltamethrin modification, whereas a single long pulse producing an equivalent period of depolarization had little effect. This result was interpreted as evidence for the binding of deltamethrin to open but not inactivated channels. Further evidence for binding to open channels was obtained in experiments performed in the presence of *Anemonia sulcata* toxin II (ATX-II), a polypeptide toxin that abolishes fast inactivation of Para sodium channels. In the presence of ATX-II, deltamethrin induced a slowly-developing current during a long depolarizing pulse, which was interpreted as the progressive modification by deltamethrin of persistently open channels. The results obtained with deltamethrin provide the clearest evidence that the modification of insect channels by this compound and its close structural relatives involves preferential binding to the open state.

2.4 Mixed-state modification by permethrin

Available information on the action of permethrin on insect sodium channels expressed in oocytes suggests that this compound interacts with both closed and open channels. The first report of the action of permethrin on *D. melanogaster* Para/TipE channels in oocytes showed clear modification from the closed state, with modified currents evident in the first depolarizing pulse after equilibration and selective activation of permethrin-modified channels with altered kinetics upon depolarization to potentials that did not activate unmodified channels [18]. In this regard, the behavior of permethrin-modified Para channels was identical to that observed for cismethrin and Vssc1 sodium channels (Fig. 4). Subsequent studies in other laboratories confirmed that permethrin effectively modified both Para/TipE [23] and Vssc1/Vssc β [24] channels in the resting state. However, the application of trains of depolarizing prepulses greatly enhanced the modification of Para/TipE channels by permethrin [25]. Taken together, these results suggest that permethrin binds to and modifies insect sodium channels in both the closed and open states and that permethrin binds with greater affinity to the open state.

3. Mammalian sodium channels

3.1 Mammalian sodium channel gene families

In mammals the pore-forming α subunits of voltage-gated sodium channels are encoded by a family of nine genes [26]. These channels, designated $\text{Na}_v1.1$ - $\text{Na}_v1.9$, are differentially distributed in excitable cells and exhibit unique functional and pharmacological properties (Fig. 6.). In the most cases, sodium channel α subunits are coexpressed with either one or two auxiliary β subunits that modulate channel gating and kinetics and regulate channel expression [27]. In mammals, there are four sodium channel β subunits (designated $\beta1$ - $\beta4$) that are structurally unrelated to the TipE family of auxiliary subunits found in insects. Typically, a single neuron expresses multiple sodium channel α and β subunits and therefore may contain several distinct heteromultimeric complexes, making the correlation of pharmacological sensitivity with channel structure impossible. The development of heterologous expression systems, such as the *Xenopus* oocyte, has provided new insight into both the gating and pharmacological properties of individual isoforms.

3.2 $\text{Na}_v1.8$ sodium channels

The most extensive body of information on the action of pyrethroids on mammalian sodium channel isoforms has been obtained using the rat $\text{Na}_v1.8$ isoform. $\text{Na}_v1.8$ channels are expressed in the peripheral nervous system, primarily in sensory neurons [26], and are likely to carry the TTX-resistant, pyrethroid-sensitive component of sodium current found in rat dorsal root ganglion neurons [28]. Whereas actions on peripheral nerve channels such as $\text{Na}_v1.8$ are not relevant to the systemic neurotoxic effects of pyrethroids, they may underlie the paresthesia that can occur following dermal exposure to pyrethroids [29].

Initial studies of $\text{Na}_v1.8$ channels in oocytes documented modification in the closed state by cismethrin, cypermethrin, and deltamethrin [30-33]. Figure 7 shows typical results obtained with cismethrin [30]. As found previously with insect (*Vssc1/TipE*) sodium channels, cismethrin produced a population of slowly inactivating and deactivating channels that gave pronounced late currents and tail currents (Figs. 7A and 7B). Cismethrin did not alter the voltage dependence of activation the peak transient sodium current, but the voltage dependence of the late current was shifted in the hyperpolarizing direction (Fig. 7C). Depolarization to potentials that selectively activate only the cismethrin-modified population of channels permitted the direct observation of the slow kinetics of activation and inactivation of these channels [33]. All of these effects are hallmarks of sodium channel modification by pyrethroids in the closed state.

A subsequent study examined 11 structurally diverse pyrethroids as modifiers of $\text{Na}_v1.8$ channels in oocytes [34]. All 11 compounds produced clearly detectable closed-state modification. Manual subtraction of scaled control sodium currents from currents obtained following pyrethroid exposure permitted reconstruction and kinetic characterization of currents carried only by the pyrethroid-modified channel population. Figure 8 shows example reconstructed traces for cismethrin-modified (Fig. 8A) and cypermethrin-modified (Fig. 8B) currents. In all cases, the pyrethroid-modified currents activated and inactivated much more slowly than currents carried by unmodified channels. There was a strong positive correlation between the kinetics of channel activation during depolarization and the kinetics of deactivation following repolarization (Fig. 9).

This study also evaluated the effects of repetitive depolarization on the extent of pyrethroid modification. Deltamethrin and three structurally related compounds produced statistically-significant use-dependent enhancement of modification. The specificity of this effect for compounds containing the α -cyano-3-phenoxybenzyl alcohol moiety is illustrated in Fig. 10. Cypermethrin (1*R,cis*, α S isomer) but not permethrin (1*R,cis* isomer) gave enhanced

modification with repeated activation. Use-dependent enhancement was restricted to the four compounds with the slowest kinetics of activation and deactivation (see Fig. 9). It is possible, therefore, that these effects are due to the accumulation of persistently open channels upon repeated depolarization rather than to enhanced binding of these compounds to the open state of the $\text{Na}_v1.8$ channel.

3.3 $\text{Na}_v1.2$ sodium channels

The $\text{Na}_v1.2$ sodium channel isoform is the most extensively characterized of the isoforms expressed in the brain. $\text{Na}_v1.2$ sodium channels are expressed widely in the CNS and are localized in both myelinated and unmyelinated axons [26]. Sodium channels in brain are complexes of a pore-forming α subunit and two auxiliary β subunits, most commonly the $\beta1$ and $\beta2$ subunits [27]. Therefore, heterologous expression experiments with brain isoforms typically involve co-expression with β subunits to produce channels that mimic the properties of channels in the CNS.

Several studies of the rat $\text{Na}_v1.2$ sodium channel isoform expressed in oocytes have documented the very low sensitivity of these channels to modification by pyrethroids. $\text{Na}_v1.2$ channels expressed either alone or in combination with the $\beta1$ subunit ($\text{Na}_v1.2+\beta1$ channels) were insensitive to resting modification by cismethrin, permethrin and tetramethrin and only weakly sensitive to resting modification by cypermethrin and other Type II (α -cyano-3-phenoxybenzyl) compounds [35-38]. In assays with $\text{Na}_v1.2+\beta1$ channels and 10 μM deltamethrin, repeated brief depolarizations caused progressive channel modification, suggesting that deltamethrin binds preferentially to the open state of the $\text{Na}_v1.2+\beta1$ channel [38]. Tefluthrin produced weak resting modification of $\text{Na}_v1.2+\beta1+\beta2$ channels, but repeated depolarizations enhanced the extent of modification approximately four-fold [39]. Taken together, these studies suggest that pyrethroid modification of the $\text{Na}_v1.2$ isoform occurs primarily in the open state. However, the overall insensitivity of this isoform to pyrethroid modification suggests that it is not likely to be an important target for the central neurotoxic effects of these insecticides.

3.4 $\text{Na}_v1.3$ sodium channels

$\text{Na}_v1.3$ sodium channels are the predominant sodium channels in the embryonic and neonatal CNS of rats [40,41], where their expression parallels that of the $\beta3$ auxiliary subunit. $\text{Na}_v1.3$ α subunits and $\beta3$ subunits are also highly expressed in the developing CNS of humans [42,43] but are much more abundant in the human adult brain than in the rat adult brain [42,44]. These data suggest that $\text{Na}_v1.3+\beta3$ channels may be important targets for pyrethroid action in the developing nervous system in both rats and humans, whereas $\text{Na}_v1.3+\beta1+\beta2$ channels may represent targets that are expressed more abundantly in the adult human brain than in the adult rat brain.

The first report of the action of pyrethroids on $\text{Na}_v1.3$ sodium channels in expressed in oocytes examined resting modification by deltamethrin and six other pyrethroids and assessed the impact of coexpression of the rat $\text{Na}_v1.3$ α subunit with either the $\beta1$ or $\beta3$ subunit [35]. Resting modification by deltamethrin was enhanced by coexpression with either the $\beta1$ or $\beta3$ subunit, with $\text{Na}_v1.3+\beta3$ channels showing the greatest sensitivity to this compound. In all cases $\text{Na}_v1.3$ channels, expressed either alone or with the $\beta1$ or $\beta3$ subunit, were more sensitive to resting modification by deltamethrin than rat $\text{Na}_v1.2$ channels expressed alone or in combination with either β subunit. $\text{Na}_v1.3+\beta3$ channels were also more sensitive to resting modification by β -cyfluthrin, cypermethrin, esfenvalerate and fenpropathrin than $\text{Na}_v1.2+\beta1$ channels, but neither isoform was sensitive to resting modification by permethrin or tetramethrin.

Recently, studies on the Na_v1.3 isoform were expanded to consider both the resting and use-dependent modification by tefluthrin of rat and human Na_v1.3 sodium channels expressed in oocytes [39]. Rat Na_v1.3 sodium channels, expressed either alone or in combination with the rat β 1 and β 2 subunits (Na_v1.3+ β 1+ β 2 channels) were equally sensitive to resting modification by 100 μ M tefluthrin (Fig. 11). However, application of trains of depolarizing prepulses enhanced modification of Na_v1.3+ β 1+ β 2 channels approximately two-fold but had no effect on the extent of modification of Na_v1.3 channels in the absence of β subunits. Rat Na_v1.3+ β 1+ β 2 channels were substantially more sensitive to both resting and use-dependent modification by tefluthrin than rat Na_v1.2+ β 1+ β 2 channels, confirming the difference in the sensitivity of these isoforms found in the study of Meacham *et al.* [35]. Surprisingly human Na_v1.3+ β 1+ β 2 channels were much less sensitive to either resting or use-dependent modification than rat Na_v1.3+ β 1+ β 2 channels (Fig. 11).

These studies with Na_v1.3 sodium channels, though limited, reveal several novel aspects of the action of pyrethroids on mammalian sodium channels. First, in rats the Na_v1.3 isoform is much more sensitive to pyrethroids than the Na_v1.2 isoform; using the extent of modification by tefluthrin as an index, the Na_v1.3 isoform exhibits the greatest pyrethroid sensitivity of any rat sodium channel isoform examined to date. Second, coexpression with auxiliary β subunits affects both the resting and use-dependent modification of this channel. This result implies that the properties of the pyrethroid binding site on the sodium channel α subunit are somehow altered in a heteromultimeric α/β subunit complex. Finally, the unexpected and substantial difference in the sensitivity of rat and human Na_v1.3+ β 1+ β 2 channels to tefluthrin raises questions about the reliability of studies with rat target sites as models for the corresponding targets in humans.

4. Conclusions and Implications

The conformational changes that underlie the gating of sodium channels make them “moving targets” for drugs and a wide structural variety of natural and synthetic neurotoxicants [45]. The studies reviewed here of pyrethroid action using cloned insect and mammalian channels expressed in *Xenopus* oocytes shed new light on the significance of the conformational changes associated with sodium channel activation as determinants of pyrethroid sensitivity. Although the number of different pyrethroid structures examined to date in these assays is small, it is clear that the relative importance of resting and use-dependent modification varies from pyrethroid to pyrethroid. This structural specificity of state-dependent modification implies that channel activation produces a pyrethroid receptor with properties that are distinct from the pyrethroid receptor of channels in the resting or closed state.

The conventional interpretation of use-dependent channel modification is that it signifies preferential binding of an agent to the open state of the channel. This understanding is widely accepted as the explanation of use-dependent channel modification by pyrethroids and is implicit in this review. However, there is little direct evidence of open-channel modification by pyrethroids. So far, the best evidence has been obtained using *D. melanogaster* Para/TipE sodium channels that have been chemically modified with ATX-II to remove fast inactivation. However, allosteric coupling of the binding sites for ATX-II and pyrethroids [46] suggests that the properties of the pyrethroid receptor of channels modified by ATX-II may differ from those of native channels. It would be valuable to expand the exploration of open channel modification by pyrethroids using techniques, such as site-directed mutagenesis, that remove fast channel inactivation without pharmacological modification.

If pyrethroids can modify open sodium channels, it is puzzling that they apparently cannot modify inactivated channels. Studies with Para/TipE channels and deltamethrin clearly show that a single long depolarization, which favors the production of inactivated channels, does not promote modification [13]. Current models of activation and inactivation gating suggest that open and fast-inactivated channels differ in structure only by the occlusion of the channel by the fast inactivation gate, a highly conserved intracellular domain that is thought to act as a “hinged lid” at the intracellular mouth of the channel [47,48]. The failure of pyrethroids to modify inactivated channels suggests that inactivation prevents access of the pyrethroid to its binding site or that pyrethroid binding and channel inactivation are mutually exclusive processes, but neither of these seems likely. Permanently charged molecules, such as some local anesthetics, are known to require an open channel to reach their binding site in the inner pore region but uncharged lipophilic agents can gain access to their binding site by diffusion through the membrane and do not require the aqueous pathway provided by the open channel [49]. The physical properties of pyrethroids argue against the need for an aqueous pathway for access to their binding site. Also, pyrethroid-modified channels clearly undergo inactivation, albeit with retarded kinetics (see Fig. 8A), and the removal of fast inactivation of pyrethroid-modified channels by repolarization causes a characteristic “hooked” tail current (partially visible for cismethrin-modified Vssc1/TipE channels in Fig. 4A), in which sodium conductance is transiently increased before the decay of the tail current due to slow deactivation. The delayed onset and removal of fast inactivation following pyrethroid modification that is evident in these experiments suggests that channel inactivation does not preclude pyrethroid binding.

There are two possible explanations for the use-dependent modification of sodium channels by pyrethroids that do not require binding to the open state of the channel. The first, proposed initially to account for the use-dependent enhancement of modification of TTX-resistant sodium channels in rat DRG neurons by deltamethrin [50], attributes use-dependent effects to the accumulation of persistently open channels that are modified by compounds such as deltamethrin, which produce long-lived open modified states. This mechanism may also account for the use-dependent enhancement of the modification of rat Na_v1.8 channels in oocytes by deltamethrin and related compounds, all of which exhibit very slow rates of deactivation [34] (see Fig. 9). Alternatively, use-dependent modification may result from preferential binding to an intermediate channel state that is transiently present during the activation gating process. This explanation, which requires channel activation but not an open channel to promote binding, could account for the failure of pyrethroids that exhibit use dependence to modify inactivated channels. A recent study suggests that an analogous mechanism, involving binding to transitional nonconducting states, contributes to sodium channel block by lidocaine and benzocaine [51]. Further work is required to determine whether the clearly evident use-dependent modification of sodium channels by pyrethroids involves binding to the open state of the channel.

The inference that some pyrethroids bind preferentially to open channels stimulated the development of a model of the pyrethroid receptor for the house fly Vssc1 sodium channel in the open state [14]. This model, which postulates molecular interactions between key elements of pyrethroid structure and specific residues forming a hydrophobic pocket in the inner pore region of the channel, is a rich source of new hypotheses about the structure of the pyrethroid receptor that are amenable to testing by site-directed mutagenesis of these residues [52]. In light of the evidence for closed-state modification by some pyrethroids, it would be of interest to generate a comparable model of the Vssc1 sodium channel in the resting state. The available data from studies with Vssc1/tipE and Para/TipE channels expressed in oocytes suggest that the pyrethroid receptor of resting channels should accommodate compounds such as cismethrin and permethrin, which are capable of modifying closed channels, but exclude compounds such as deltamethrin and cypermethrin,

which require activation for channel modification. The modeling of state-specific modification has the potential to generate new and important insight into the pyrethroid receptor as a “moving target.”

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Fig. 1. Sodium channel state transitions associated with transient sodium currents measured under voltage clamp conditions.

Fig. 2. Control and pyrethroid-modified sodium currents measured under voltage clamp conditions, illustrating the pyrethroid-induced late current during a depolarizing pulse and tail current following repolarization.

Fig. 3. Conceptual model illustrating the differences in the modification of closed (C) and open (O) sodium channels by pyrethroids (Py). Pyrethroid-modified states are marked with asterisks.

Fig. 4. Modification of Vssc1/TipE sodium channels expressed in oocytes by cismethrin in the resting (closed) state. (A) Sodium current traces measured in the same oocyte before and after bath application of cismethrin. Cismethrin was applied at a holding potential of -100 mV and the modified sodium current was recorded during the first depolarizing pulse after equilibration. (B) Conductance-voltage plots for the activation of peak transient sodium currents prior to cismethrin treatment and late currents (measured at the end of a depolarizing pulse) following cismethrin treatment, illustrating the shift in the voltage dependence of activation of cismethrin-modified channels. (C) Selective activation of cismethrin-modified channels during a long depolarization to -40 mV, a potential that does not activate unmodified channels (see arrow in Panel B). Typical control and pyrethroid-modified currents are observed during and after a second depolarization to -10 mV. Figures are re-drawn from the data of Smith *et al.* [12].

Fig. 5. Depolarization-dependent modification of Vssc1/TipE sodium channels expressed in oocytes by cypermethrin. Traces were recorded during test pulses that followed the indicated number of short depolarizing prepulses. The figure is re-drawn from the data of Smith *et al.* [12].

Fig. 6.

Dendrogram depicting the evolutionary relationships among the nine rat Na_v1 isoforms calculated from alignment of the published amino acid sequences. Older synonyms for each isoform are shown together with principal sites of expression (CNS, central nervous system; PNS, peripheral nervous system) and relative sensitivity to block by tetrodotoxin (TTX).

Fig. 7.

Modification of rat Na_v1.8 sodium channels expressed in oocytes by cismethrin in the resting (closed) state. Sodium current traces were measured in the same oocyte before (A) and after (B) bath application of cismethrin. Oocytes were clamped at a holding potential of -100 mV; traces represent sodium currents evoked by 40-ms depolarizing pulses to potentials ranging from -50 mV to 50 mV. (B) Current-voltage plots for the activation of peak transient sodium currents before (filled squares, panel A) and after cismethrin (filled circles, panel B) treatment and late currents measured at the end of a depolarizing pulse following cismethrin treatment (open circles, panel B), illustrating the shift in the voltage dependence of activation of cismethrin-modified channels. Figures are re-drawn from the data of Choi and Soderlund [30].

Fig. 8.

Visualization of sodium currents carried by populations of rat $\text{Na}_v1.8$ sodium channels expressed in oocytes modified by either cismethrin (A) or (B) cypermethrin. The top panels show total current traces obtained from the same oocyte before and after pyrethroid application. The bottom panels show calculated net currents for pyrethroid-modified channels that were obtained by the subtraction of scaled control currents from currents from composite currents in the presence of pyrethroids. The segments of the net currents used to calculate time constants for activation (τ_{act}), inactivation (τ_{inact}), and tail current decay (τ_{tail}) are indicated. Figures are redrawn from the data of Choi and Soderlund [34].

Fig. 9. Correlation between the time constants of activation and tail current decay, obtained from traces such as those shown in Fig. 8, for rat Na_v1.8 sodium channels modified by different pyrethroids. The compounds enclosed in the rectangle exhibited statistically significant use-dependent enhancement of channel modification. The figure is re-drawn from the data of Choi and Soderlund [34].

Fig. 10.

Plot of the effect of prepulse number on the extent of modification of rat $\text{Na}_v1.8$ sodium channels by cypermethrin and permethrin. Dashed rectangles enclose the single moiety that differs in the structures of cypermethrin and permethrin. The figure is re-drawn from the data of Choi and Soderlund [34].

Fig. 11.

The effect of prepulse number on the extent of modification of rat Na_v1.3 sodium channels expressed either in the absence or presence of the rat β 1 and β 2 subunits and of human Na_v1.3 sodium channels expressed in presence of the human β 1 and β 2 subunits by tefluthrin. The figure is re-drawn from the data of Tan and Soderlund [39] and unpublished data.