Chapter 3

Pyrethroids and Their Effects on Ion Channels

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Additional information is available at the end of the chapter

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1. Introduction

Ion channels are integral membrane proteins that are critical for neuronal function. They form pores in the plasma membrane that allow certain ions to travel with their concentration gradient across the membrane. Those that open in response to a change in membrane potential are called voltage-gated ion channels. Channels that open in response to binding by a chemical signal or molecule are ligand-gated ion channels. In neurons, ion channels are essential for chemical communication between cells, or synaptic transmission. Ion channels also function to maintain membrane potential and initiate and propagate electrical impulses.

Due to their importance in neurons, ion channels are often the molecular targets of neurotoxins. Pyrethrin compounds first identified in the pyrethrum extract of *Chrysanthemum cinerariaefolium* are neurotoxins that disrupt the normal function of voltage-gated sodium channels (VGSCs). Pyrethrum extract is a very effective natural insecticide [1], however, it rapidly degrades upon exposure to light, making it unsuitable for commercial agriculture use. Beginning in the mid 20th century, synthetic analogs of pyrethrins, pyrethroid insecticides, were developed by modifying the structures of pyrethrins to increase photostability and insecticidal activity. Pyrethroid insecticides target VGSCs, but also affect the function of voltage-gated calcium and voltage-gated chloride channels. This chapter provides an overview of pyrethroid structure and toxicological properties in insects and mammals concentrating on the effects of pyrethroid on various ion channels.

2. Pyrethroid structure and toxicity

Initially isolated in 1924, two pyrethrin compounds were determined to be responsible for the insecticidal action of pyrethrum. They were designated pyrethrin I and II; pyrethrin I had a monocarboxylic acid moiety (chrysanthemic acid) while pyrethrin II had a dicarboxylic acid (pyrethric acid) [2, 3]. The other main chemical moiety of interest in the

pyrethrins was an alcohol, a substituted cyclopentenolone. Of the two compounds, pyrethrin I was more potent in insecticidal assays with *Aphis rumincis L.* (Aphids) [4, 5], which concurred with earlier work on cockroaches. Together the early insecticidal assays of the two compounds indicated that the insecticidal action was likely driven by pyrethrin I. However, subsequent studies conflicted regarding the potency of pyrethrin I and II: some studies indicated that pyrethrin I was more potent whereas others indicated pyrethrin II was more potent [3, 5, 6]. These discrepancies were ascribed to the difficulties encountered by early researchers in isolating pure pyrethrin I and II, photodegredation of stored fractions, and differences in test species selection. Eventually it was determined that pyrethrin I was more effective for killing insects while pyrethrin II was more effective for knockdown [1]. This combination of knockdown and lethal effects was largely responsible for the effectiveness of pyrethrum extracts against biting insects and prompted investigations designed to improve insecticidal potency by modifying the chemical moieties of the pyrethrin compounds.

In the first generation of the synthetic pyrethroids, which included *all*ethrin (*All*yl analog), *tetrame*thrin (*tetra*hydrophthalimido*me*thyl analog), and *res*methrin (discovered at *R*othamsted *E*xperimental *S*tation), the acid moiety was based on the chrysanthemic acid present in pyrethrin I. This acid has a cyclopropane ring which is not present in more recent synthetic pyrethroids, such as fenvalerate (*phen*ylisov*alerate* pyrethroid). In terms of insecticidal potency, the major breakthrough was the introduction of a cyano (CN) residue at the α carbon of the 3-phenoxybenzyl-alcohol moiety. Addition of this CN group enhanced insecticidal activity roughly 3-6 fold compared to non-cyano pyrethroids.

This cyano group in synthetic pyrethroids allowed for the classification of pyrethroids into two distinct subclasses. Type II compounds have the cyano group while type I agents do not. For example, permethrin is a common type I pyrethroid while cypermethrin (named for the addition of the cyano group) is a type II (**Figure 1**). Pyrethroids are highly flexible with many theoretically attainable geometries; most have several potentially stereogenic carbons [7, 8]. Due to this complex geometry, pyrethroids can be present as mixtures of isomers; for example, allethrin can be used as a mixture of four isomers (4*RS*) or as a single isomer (S-bioallethrin). In contrast the synthetic pathway used to generate deltamethrin and esfenvalerate results in the enrichment of a single isomer [7]. In general *R* esters of type I pyrethroids and S esters of type II pyrethroids exhibit the highest insecticidal potency [1, 9-11]. The presence of the stereo-inactive isomers can attenuate the effects of the active isomers [12].

Although the potency increased, first and second generation pyrethroids were still highly photolabile [13]. The photodegredation of pyrethroids generally results in decomposition of the parent compound to less toxic products. The first photostable pyrethroids developed was permethrin in 1973 [14], and permethrin is still one of the most heavily used pyrethroids in agricultural settings. Permethrin and other halogen-containing pyrethroids such as decamethrin and fenvalerate can persist on plant cuticles and have prolonged insecticidal action compared to the more photolabile chemicals [1].

Compared to later generation chemicals, first generation pyrethroids have limited outdoor uses due to their photolability. However, they are still used prominently in household insecticide sprays for flying and crawling insects [15]. Indoor pyrethroids are also used in topical creams, sprays, and shampoos for controlling biting insects and head lice in both humans and their domestic pets. Recent studies have shown that sensitive populations such as children and pregnant women are exposed to pyrethroids via these indoor applications [16, 17]. The outdoor pyrethroids are used in agricultural settings and disease vector control. The two most commonly used pyrethkdrroids in agriculture are cypermethrin and permethrin, which together account for an annual usage of 3 million pounds [18, 19]. Several pyrethroids are registered for use in the United States for control of the malaria-bearing mosquito species *Anopheles* [20], and pyrethroid-treated bed nets are used in tropical regions worldwide to prevent malaria. Pyrethroid use in agriculture and vector control has increased in recent years due to the reduced use of chlorinated, carbamate, and organophosphate pesticides [15], resulting in increased human exposure [21].

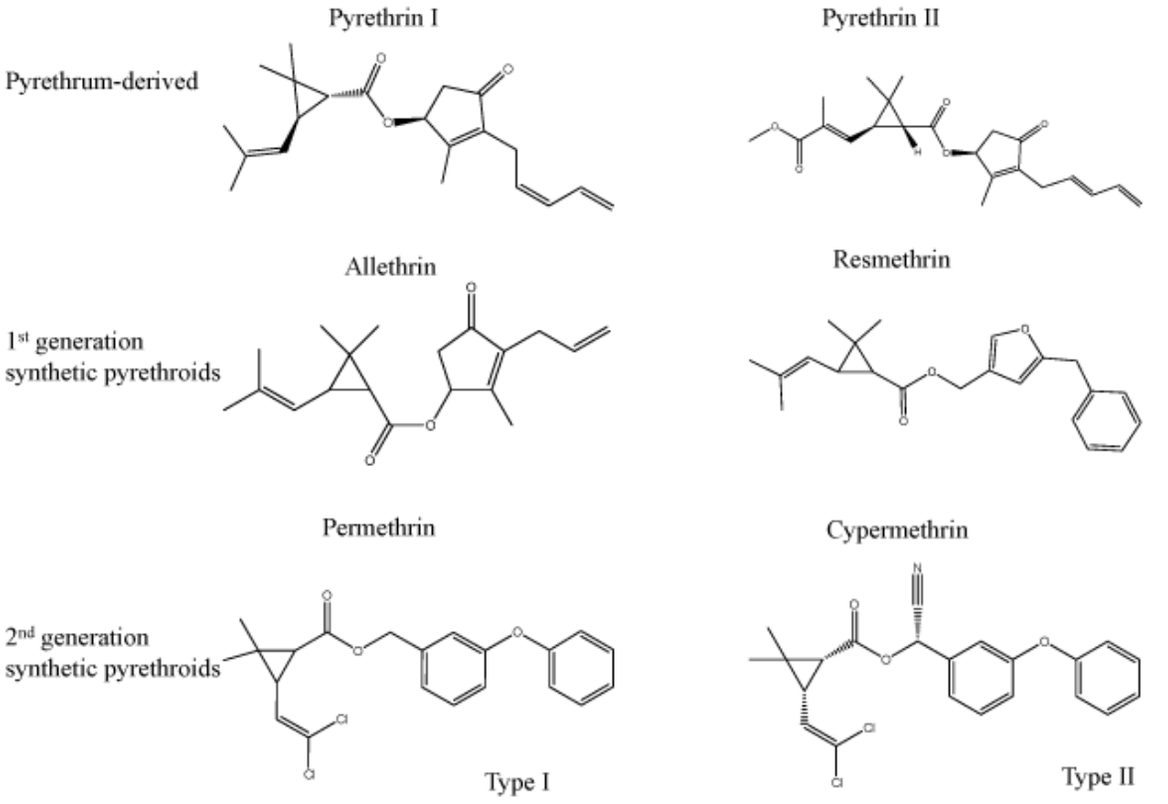

Figure 1. Pyrethrins, prototypes for the synthetic pyrethroids, were derived from the pyrethrum extract of *Chysanthemum* species. Introduction of an α-cyano group determines whether pyrethroids are classified as type I (cyano lacking) or type II (cyano-containing).

Pyrethroid exposure in insects is predominately through the insect cuticle. Rapid absorption, particularly with the halogen-containing pyrethroids, causes disruption of insect neurotransmission causing knockdown and possibly death within seconds to minutes. In contrast to insects, human dermal absorption of pyrethroids is low relative to absorption via lung or gut [15, 22]. Humans absorb approximately 1% of pyrethroid exposure through skin

but up to 36% through ingestion [22]. However, sprays combining pyrethroid formulations with N,N-diethyl-m-toluamide (DEET) can increase dermal absorption of pyrethroids. After absorption, pyrethroids are distributed throughout the body and can cross the blood-brain barrier to access the nervous system. In occupational settings such as agriculture, inhalation of aerosolized pyrethroid formulations can result in toxicity, necessitating the use of personal protective equipment to prevent intoxication. Due to relatively low mammalian toxicity and the adequacy of personal protective gear in occupational settings of high exposure, there have been relatively few cases of human poisoning. The most commonly encountered human symptom of pyrethroids toxicity is paresthesia, which can be treated by decontamination of the exposed skin followed by bathing with oil [22]. Systemic poisonings in humans can be hard to control; central and peripheral nervous system effects in humans must be treated by a multisystem approach due to the numerous targets of pyrethroids in mammals [22].

Studies on pyrethrin I and II and first generation synthetic pyrethroids showed low mammalian toxicity. A large proportion of the administered dose was excreted in an unmetabolized form. Furthermore, the bioavailable dose is metabolized easily in mammals through cleavage by esterases and cytochrome P450 mixed function oxidases in liver or plasma [23-25]. The (+)-trans form of some pyrethroids, such as bioresmethrin, is much more toxic to mammals than the (+)-cis isomer. Mammalian systems metabolize the (+)-trans isomers much faster than the (+)-cis form, effectively removing the most toxic species [26]. Consequently, pyrethroids do not appear to accumulate in mammalian tissues. Clearance from plasma is relatively fast as pyrethroids are distributed throughout the body [27], with plasma half lives ($t_{½}$) of the pyrethroid class ranging from 9-15 hours (hrs) [28]. In animal models pyrethroids exhibit peak concentration in the brain within 2-3 hrs after oral administration and decrease rapidly [27, 29]. Brain pyrethroid levels are relatively low (0.1-0.3% of body burden), even at the time of peak symptom expression [28]. Clearance rates of pyrethroids from the brain can vary; for example in a study comparing clearance rates of several pyrethroids from the brain, deltamethrin was cleared quickest, followed by fenvalerate, cypermethrin, and permethrin [30]. Although the majority of the administered pyrethroid dose is metabolized quickly in plasma and liver, the unmetabolized fraction can partition into fat where pyrethroid metabolism and clearance is much slower [27, 30].

Although efficient metabolism in mammals reduces the likelihood of acute toxicity, high exposures can result in intoxication. Type I and type II pyrethroids produce different toxicological symptoms in mammals, likely due to differences in the effects of the classes on mammalian neurons (**Table 1**) [31, 32]. Mammalian intoxication by type I pyrethroids causes symptoms characterized by tremor (T-class), exaggerated startle response, and hyperexcitability [7, 11, 33]. Intoxication by type II pyrethroids results in burrowing and pawing behavior, followed by salivation and coarse tremor, which evolves into choreoathetosis (involuntary movement and writhing) [34]. This second type of poisoning is referred to as CS-class. Some pyrethroids, such as fenpropathrin and cyphenothrin can result in symptoms from both T and CS-class [9. 31], and have been designated by some to belong to a third class (TS: tremor and salivation) [35]. One hypothesis for the difference in toxicity symptoms is that the cyano group in the type II pyrethroids results in a prolonged effect on neurons, causing a different spectrum of toxicological symptoms [7, 11].

T-Type	CS-Type
Severe fine tremor	Coarse tremor
Marked reflex hyperexcitability	Moderate reflex hyperexcitability
Sympathetic activation	Sympathetic activation
Parethesia (dermal exposure)	Parethesia (dermal exposure)
Clonic seizures†	Tonic seizures
Hyperthermia‡	Choreoathetosis
Uncoordinated twitches‡	Increased extensor tone
Aggressive sparring‡	Profuse watery salivation
	Sinuous writhing†
	Rolling gait‡
	Chewing; nosing; pawing; burrowing‡

Table 1. Pyrethroid toxicity syndromes in mammals. Species-specific effects are indicated †= mouse, ‡= rat. Modified from [21, 34].

The T-, CS-, and TS-syndromes are dose-dependent, acute responses to overt pyrethroid toxicity. They exhibit a fast onset, occurring from within a few minutes to over 1 hr but take about 2-8 hrs to peak [28]. Recovery is generally complete within 24-48 hours after cessation of exposure. The appearance of neurobehavioral symptoms follows this time course; symptoms begin around 1 hr, peak around 4-8 hrs and are resolved 12-48 hrs after oral exposure [34].

The safety of the pyrethrins and early generation pyrethroids is largely due to their instability. By producing more stable pyrethroids, the potential for mammalian toxicity increased. The halogen-containing pyrethroids lack many of the characteristics that originally deemed the earlier pyrethroids and pyrethrins as safe. For example, the halogen-containing pyrethroids are much more photostable. This allows them to persist on the surface of plants much longer than the earlier pyrethroids. They are also highly lipophilic, which promotes higher bioavailability in mammals [1]. In fact, fluorinated pyrethroids induce toxicity symptoms with a much faster onset than non-halogenated compounds [35] and halogenation coupled with the cyano group increases toxic potency in mammals by an order of magnitude [34].

Despite low incidence of acute poisoning in humans, there is substantial interest in the effect of chronic exposure to pyrethroids. The early studies on pyrethroid toxicity focused on their effects on neuronal targets in acute paradigms [35]. However, the identification of other endpoints of concern suggests that there are prolonged effects of pyrethroid exposure, perhaps mediated by active metabolites in addition to the parent compound [7]. Several laboratories have independently identified that chronic pyrethroid exposure causes alterations to the dopaminergic system in rodents [36-40]. This is particularly interesting in the context of motor activity changes during the acute toxicity syndromes [34]. Induction of apoptosis by deltamethrin in dopaminergic neurons was demonstrated *in vitro* [41] and others have shown that sub-chronic exposure to permethrin in rats resulted in neuronal loss in the hippocampus and motor cortex [42]. Repeated exposure to pyrethroids resulted in

altered dopamine transporter function, changes in the number of dopaminergic binding sites in rat brain [36], and possibly increased dopamine turnover resulting in decreased dopaminergic function [38, 49]. Decreased dopamine levels were also observed during chronic exposure to the type II pyrethroid cypermethrin, an effect accompanied by dopaminergic neurodegeneration [40]. The underlying mechanism for dopaminergic sensitivity to pyrethroids is unknown, but the effects observed across laboratories illustrate a consistent picture of pyrethroid effects in the dopamine neurotransmitter system.

The effects of cypermethrin on dopaminergic neurons listed above only occurred in animals exposed to the pyrethroid during both developmental and adult exposure and did not occur in animals exposed only during adulthood [40]. This may indicate that developmental exposure predisposes adult animals to pyrethroid sensitivity. Several studies have suggested that neonatal mammals are more sensitive to pyrethroids than adult animals. Brain levels of pyrethroids in neonatal rats are higher than for adult animals [43]. Further, neonatal rats have less capacity to metabolize pyrethroids than adults, leading to 4-17 fold increased sensitivity to pyrethroids [44, 45]. An additional factor in the susceptibility of developing animals to pyrethroids is the differential expression of pyrethroid targets during development. For example, some developmentally expressed isoforms of the sodium (Na^+) channel are much more sensitive to pyrethroids than isoforms expressed in the adult brain [33, 46]. The differences in sensitivity between neonates and adults are greatest with the type II pyrethroids; type I pyrethroids in neonates produce LD_{50}[1] values roughly 2-3 fold lower than in adults, but type II pyrethroids had 16-24 fold lower LD_{50}s in pups. Besides acute lethality, rats exposed to pyrethroids during postnatal days 10-16 exhibited increased motor activity and lack of habituation. These effects were accompanied by changes in neurotransmitter receptor levels [47], which persisted for up to 5 months after cessation of exposure. Together, results from these studies suggest that neonatal mammals are more sensitive than adult animals, due to a combination of differential expression of pyrethroid targets, reduced metabolic capacity, and increased pyrethroid levels in neuronal tissues.

3. Pyrethroid effects on nerve impulses

Action potentials are the electrical impulses that travel along the axons of neurons and result from the movement of Na^+ and potassium (K^+) ions across the membrane [48] (**Figure 2**). Binding of excitatory neurotransmitters to their receptors opens cation-permeable ion channels causing the membrane to depolarize or become more positive. This depolarization activates (opens) VGSCs allowing Na^+ to enter the neuron further depolarizing the membrane. This increase in membrane permeability to Na^+ is responsible for the rising phase of the action potential, eventually causing the membrane polarity to reverse (overshoot phase). The falling phase of the action potential is caused by the inactivation of the VGSCs and the opening of voltage-gated potassium channels allowing K^+ to leave the cell. The efflux of K^+ ions results in hyperpolarization (undershoot phase) of the membrane.

[1] LD_{50}: The median lethal dose, or the dose required to kill 50 percent of the exposed animals.

Ultimately the voltage-gated K^+ channels close and the membrane potential returns to its resting state.

In microelectrode recordings[2] of action potentials in cockroaches, treatment with pyrethrum extract resulted in repetitive discharges followed by a block in nerve conduction [49, 50]. Allethrin, the first synthetic pyrethroid, also caused repetitive discharges and a subsequent conduction block. This effect was concentration and temperature-dependent [51-53]. Higher concentrations of pyrethrum and allethrin induced conduction block more rapidly and exposure at lower temperatures resulted in greater insecticidal activity. Allethrin treatment also slowed the rising and falling phases of the action potential and resulted in a more positive resting membrane potential. Increasing or decreasing the extracellular K^+ concentration did not alter the effects of allethrin, indicating that pyrethroids alter Na^+ conductance through action on VGSCs. Electrophysiological experiments in frog, squid and crayfish confirmed that type I pyrethroids cause repetitive discharges upon a single stimulus similar to allethrin and pyrethrins, whereas type II pyrethroids cause stimulus-dependent membrane depolarization and conduction block [54-57, 32].

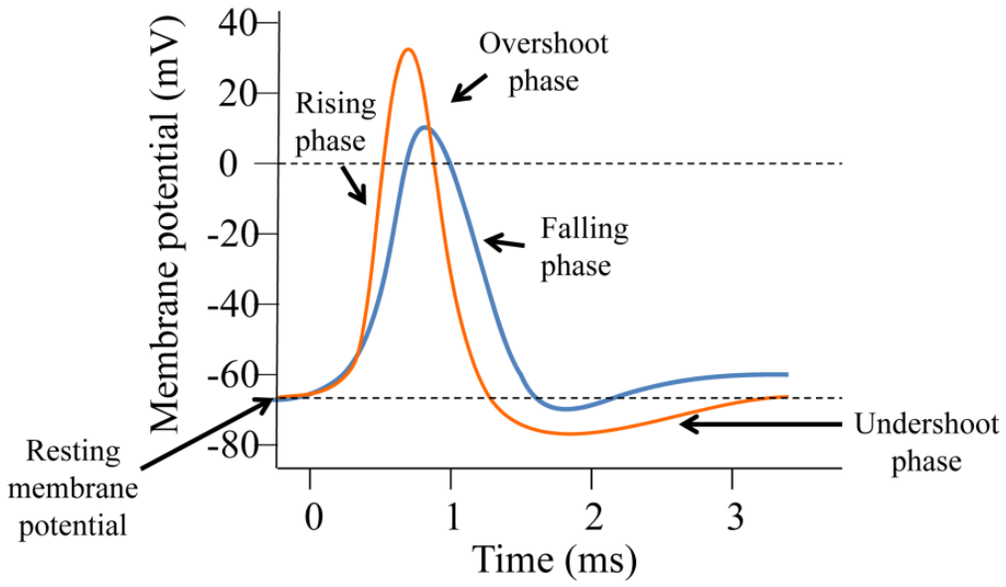

Figure 2. Phases of an action potential. Orange- normal action potential. Blue- action potential in the presence of allethrin. Modified from Motifolio Biomedical PowerPoint Toolkit Suite.

4. Pyrethroid effects on insect voltage-gated sodium channels

VGSCs consist of a pore forming subunit (α subunit) and an auxiliary subunit [58]. The α subunit contains four domains (I-IV) each with 6 transmembrane segments (**Figure 3A**). The amino and carboxy termini of the protein are intracellular. The fifth and sixth transmembrane segments (S5 and S6) and the loop between them form the channel pore and

[2] Microelectrodes are typically made of a glass pipette pulled to a very fine tip and filled with an electrical conductor, such as a high salt solution. When the electrode is connected to a voltmeter and inserted into a cell it can detect changes in transmembrane voltage.

confer selectivity for Na^+. Positively charged amino acids in S4 serve as the voltage sensor initiating a conformation change that opens the channel upon membrane depolarization. The cytoplasmic linker between domains III and IV serves as the inactivation gate. VGSCs can exist in 4 states controlled by the opening and closing of two distinct "gates," known as the activation and inactivation gate, respectively (**Figure 3B**) [48]. At the resting membrane potential the channel is closed and the inactivation gate is open. Upon membrane depolarization, the channel opens allowing Na^+ to enter the cell. During inactivation, the inactivation gate closes occluding the pore. Finally the channel closes during a state called deactivation. Removal of deactivation is needed to restore the "closed" state of the channel and prepare it for another activation (opening).

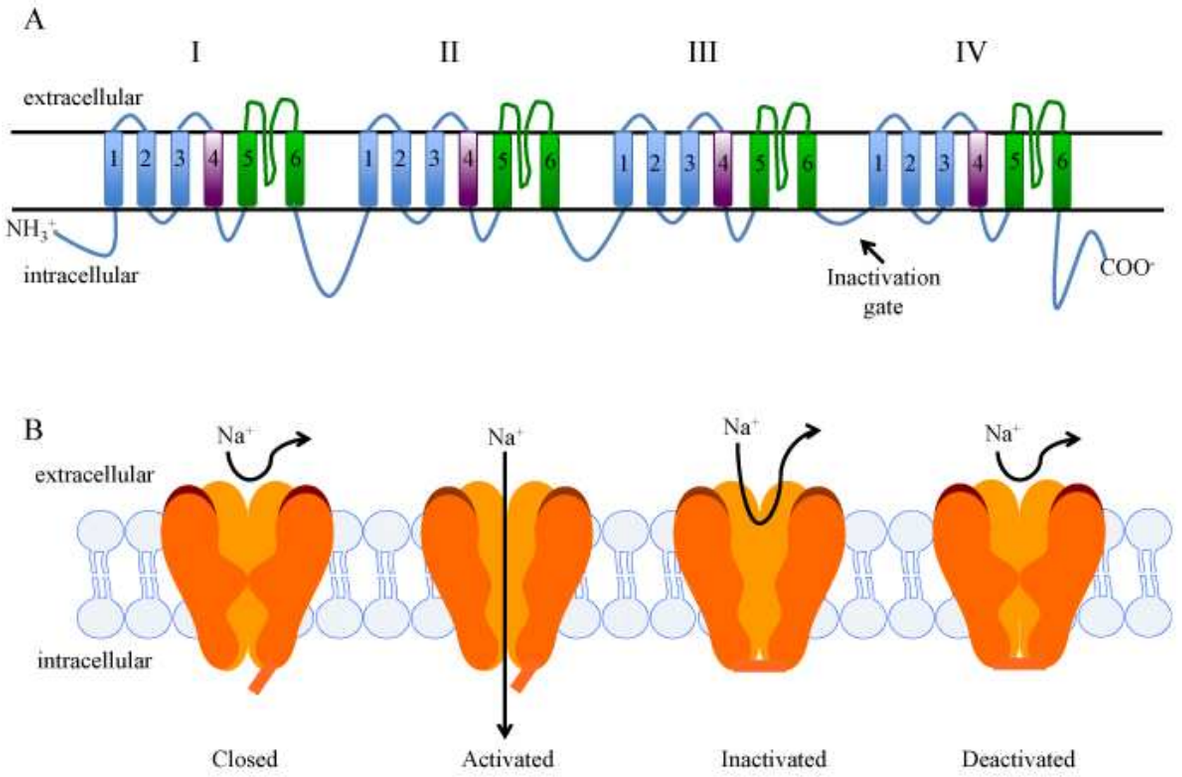

Figure 3. The α subunit of the voltage-gated sodium channel. A. Schematic of VGSC α subunit indicating the four domains (I-IV) and their six transmembrane segments (1-6). The transmembrane segments and loop that form the channel pore are shown in green. The 4th transmembrane segment, shown in purple, acts as the voltage sensor. B. Four states of the VGSC. At resting membrane potentials the channel is closed. During the rising phase of an action potential the channel activates or opens. Channel inactivation contributes to the falling phase. During the undershoot phase the channel deactivates prior to returning to the closed phase once resting membrane resting potential has been restored. Modified from Motifolio Biomedical PowerPoint Toolkit Suite.

In insects, a single gene encodes the VGSC α subunit. In *Drosophila melanogaster* this gene is called *para* [48]. Messenger RNA transcripts from this gene are alternatively spliced resulting in mature transcripts containing different combinations of exons. The transcripts also undergo RNA editing in which some nucleotides are converted from one base to another often changing the amino acid that is encoded. The combination of alternative

splicing and RNA editing results in VGSCs with distinct gating properties. The auxiliary subunit, TipE in *D. melanogaster*, increases cell surface expression of *Para*, enhances the peak Na^+ current and alters the kinetics of channel inactivation.

Advancements in electrophysiological techniques, particularly the development of the voltage and patch-clamp techniques[3], enabled researchers to study the effect of pyrethroids on Na^+ current. Pyrethroids slowed VGSC activation leading to a decrease in peak Na^+ current [53, 55, 59-61]. Pyrethroids slowed VGSC inactivation and deactivation leading to a prolonged VGSC open time. Type II pyrethroids prolonged channel open time more than type I pyrethroids. The longer channel open time results in more Na^+ entering the cell leading to hyperexcitability with type I pyrethroids, membrane depolarization and conduction block with type II pyrethroids. Even though not every VGSC is altered by pyrethroids, modification of a small percentage of VGSCs can increase Na^+ current substantially [91, 95].

In addition to the electrophysiology data, there is genetic evidence that pyrethroids target the VGSC. Pyrethroid resistant, or knockdown-resistant (*kdr*), houseflies were first reported in the 1950s [62, 63]. Genetic mapping of the *kdr* and super-*kdr* trait in houseflies revealed that pyrethroid resistance was tightly linked to the housefly VGSC gene, *Vssc1* [64]. Similar genetic mapping experiments in tobacco budworms, German cockroaches and mosquitoes also linked *kdr* and super-*kdr* traits in those species with their respective VGSC genes [65-67]. Comparative sequence analysis of *kdr* and super-*kdr* housefly *Vssc1* with the wild-type gene identified two point mutations in domain II [68]. The first mutation, a leucine to phenylalanine change in transmembrane segment 6, was found in two *kdr* and six super-*kdr* housefly strains. The second mutation, a methionine to threonine change in the intracellular loop between transmembrane segments 4 and 5, was only found in super-*kdr* strains. Sequencing of the VGSC genes from other species with *kdr* resistance identified several other mutations (**Table 2**) [58, 69-73].

The most common *kdr* mutation is a leucine (L) to phenylalanine (F), histidine (H), or serine (S) change in domain II segment 6 (DIIS6). As this mutation does not alter expression or localization of the VGSC, it was suspected to alter the affinity of the channel for pyrethroids [74]. Expression of this L to F mutant channel in *Xenopus laevis* oocytes resulted in VGSCs that were 10 fold less sensitive to cismethirin as assessed using voltage-clamp experiments [75]. The L to F mutation is found in combination with a methionine (M) to threonine (T) change in highly resistant houseflies. When expressed in *X. laevis* oocytes, the double mutant channels were 200x more resistant to deltamethrin and almost insensitive to permethrin [76]. Several other *kdr* mutations have been assayed in *X. laevis* oocytes and in general mutant channels required much higher concentrations of pyrethroid to produce Na^+ currents that were similar to those produced by pyrethroid treated wild-type channels [77-80].

[3] The voltage-clamp technique typically uses two microelectrodes, allowing the experimenter to control the membrane potential and record transmembrane currents that result from ion channels opening and closing. Patch-clamp is a highly sensitive version of the voltage-clamp technique in which currents flowing through a single ion channel can be measured. A single electrode serves both to measure voltage and pass current.

The clustering of *kdr* and six super-*kdr* mutations in DIIS4-S5 linker, DIIS5 and DIIS6 suggest that these regions are part of the pyrethroid-binding site. This is supported by computer modeling in which fenvalerate is predicted to interact with amino acids in DIIS4-S5 linker, the DIIS5 and the cytoplasmic end of DIIS6 [81]. The computer model is supported by competitive binding assays using active and inactive permethrin [82]. The L to F mutation in DIIS6 was found to reduce binding of the inactive pyrethroid by 16 fold. Alternatively, the *kdr* and six super-*kdr* mutations could alter VGSC gating properties in a way that counteracts pyrethroid action. In support of this second hypothesis, the L to F mutation in DIIS6 and the M to T mutation in DIIS4-S5 linker enhanced inactivation by shifting the voltage-dependence of inactivation to more negative membrane potentials and by increasing the rate of inactivation [76]. Further study of naturally occurring *kdr* mutations and splicing isoforms of VGSCs will provide a greater understanding of the molecular interactions between pyrethroids and VGSCs.

Mutation	Location in VGSC	Species
Leucine to Phenylalanine	DIIS6	*Musca domestica, Blattella germanica, Plutella xylostella, Myzus persicae, Anopheles gambiae, Culex pipiens, Culex quinquefasciatus, Haematobia irritans, Leptinotarsa decemlineata, Frankliniella occidentalis, Cydia pomonella, Ctenocephalides felis*
Leucine to Serine	DIIS6	*Culex pipiens, Anopheles gambiae*
Leucine to Histidine	DIIS6	*Heliothis virescens*
Methionine to Threonine	DIIS4-S5 linker	*Musca domestica, Haematobia irritans, Heliothis virescens*
Aspartate to Glycine	Amino terminus	*Blattella germanica*
Glutamate to Lysine	DI and DII linker	*Blattella germanica*
Cysteine to Arginine	DI and DII linker	*Blattella germanica*
Proline to Leucine	Carboxy terminus	*Blattella germanica*
Valine to Methionine	DIS6	*Heliothis virescens*
Methionine to Isoleucine	DIIS1-S2 linker	*Pediculus capitis*
Leucine to Phenylalanine	DIIS5	*Pediculus capitis*
Threonine to Isoleucine	DIIS5	*Plutella xylostella, Pediculus capitis*
Threonine to Cytosine	DIIS5	*Frankliniella occidentalis*
Threonine to Valine	DIIS5	*Ctenocephalides felis*
Phenylalanine to Isoleucine	DIIIS6	*Boophilus microplus*
Leucine to Proline	DIII-DIV linker	*Varroa destructor*

Table 2. List of some of the species with *kdr* mutations. Mutations in red occur at the same leucine residue. Mutations in blue occur at the same threonine residue.

5. Pyrethroid effects on mammalian voltage-gated sodium channels

The α subunit of VGSCs in mammals is very similar both in structure and amino acid sequence to the insect VGSC [83]. In contrast to insects, mammals have nine VGSC genes (*Na$_v$1.1-1.9*) that differ in channel properties and tissue expression patterns (**Table 3**). Transcripts produced from these genes also undergo alternative splicing to produce more functionally diverse channels. The auxiliary subunit in mammals, or β subunit, is required for proper localization of the α subunit to the plasma membrane. Interaction of the β subunit with the α subunits also modifies the voltage-dependence and kinetics of gating. Mammals have four β subunit genes (*β1-β4*); all four β subunits are expressed in the central nervous system and heart, but skeletal muscle only expresses β1.

Similar to insects, exposure to pyrethroids increases excitability in mammalian neurons by slowing the action potential falling phase [84-87]. Type I and type II pyrethroids delay mammalian VGSC inactivation resulting in a prolonged Na^+ current [88, 89]. The length of channel open time is dependent on the pyrethroid type, with type II pyrethroids holding VGSCs open much longer than type I. For example, tetramethrin increased the open time of mouse neuroblastoma[4] VGSCs ten-fold, whereas deltamethrin increased the open time 200-fold [89, 89]. The ability of pyrethroid to delay mammalian VGSC inactivation was also temperature-dependent, with lower temperatures resulting in slower Na^+ current decay [89-91]. Electrophysiology studies demonstrating mammalian VGSC sensitivity to pyrethroids were also supported by *in vitro* assays. Pyrethroids enhanced $^{22}Na^+$ uptake by toxin activated VGSCs in cultured mouse neuroblastoma cells and rat brain synaptosomes[5] [84, 92]. Binding studies a using radioactive pyrethroid demonstrated specific binding of the pyrethroid to rat brain VGSC α subunits [93].

Channel Name	Former Names	Expression Pattern
Na$_v$ 1.1	brain I	Central and peripheral nervous system
Na$_v$ 1.2	brain II/IIa	Central nervous system
Na$_v$1.3	brain III	Embryonic/neonatal nervous system
Na$_v$1.4	μ1/SkM1	Skeletal muscle
Na$_v$1.5	H1/SkM2	Cardiac muscle
Na$_v$1.6	NaCh6/PN4	Central and peripheral nervous system
Na$_v$1.7	PN1	Central and peripheral nervous system
Na$_v$1.8	SNS/PN3	Peripheral nervous system
Na$_v$1.9	NaN	Peripheral nervous system

Table 3. Mammalian sodium channels and tissue expression pattern. Channels in boldface are resistant to TTX, a voltage-gated sodium channel blocker, but are more sensitive to pyrethroids.

Although, mammalian VGSCs are less sensitive to pyrethroids that insect VGSCs, there is variability in pyrethroid sensitivity among the channel isoforms. Dorsal root ganglion cells

[4] Mouse neuroblastoma cells are an immortal cell-line derived from a spontaneous tumor of neural crest origin.

[5] In this model, neurons are harvested from rat brains and processed via subcellular fractionation to isolate the nerve terminals.

are sensory neurons that express two classes of VGSCs, tetrodotoxin (TTX)-sensitive and TTX resistant channels. TTX is a potent VGSC inhibitor that prevents action potential generation. $Na_v1.8$ and $Na_v1.9$, which are resistant to TTX, are highly sensitive to pyrethroids [94-98]. Allethrin (at 10 μM) was found to have significant effect on TTX-resistant channels but no effect on TTX-sensitive channels. Similar results were obtained with tetramethrin and deltamethrin. Expression and voltage clamp studies in *X. laevis* oocytes demonstrated that $Na_v1.2$ was less susceptible to pyrethroid modification than other sub-types [99-101]. Rat $Na_v1.3$, which is expressed in the developing central nervous system, is more sensitive to type II pyrethroids than $Na_v1.2$ [46,100]. The high pyrethroid sensitivity of $Na_v1.3$, may underlie the increased neurotoxicity of pyrethroid to developing mammals. $Na_v1.6$, which is highly expressed in the adult brain, is fifteen times more sensitive to tefluthrin and deltamethrin than $Na_v1.2$ [101]. VGSCs are also critical for normal cardiac function. Tefluthrin, fenpropathrin and cypermethrin prolonged Na^+ current in rat cardiomyocytes and increased the intervals between heartbeats in perfused hearts [102, 103]. Further investigation into structural differences between mammalian VGSC isoforms and how those differences affect pyrethroid sensitivity will be required to understand the molecular basis of pyrethroid toxicity in mammals. Such studies will be essential to the development of new pyrethroid insecticides with lower mammalian toxicity.

6. Pyrethroid effects on mammalian voltage-gated calcium channels

Voltage gated calcium channels (VGCCs) play essential roles in diverse cellular functions. They mediate Ca^{2+} influx into the cell from the extracellular environment following membrane depolarization. This can alter cell signaling, neurotransmission, and gene expression. VGCCs contain one transmembrane pore-forming and voltage-sensing subunit designated α_1. The association of the transmembrane segments with a water-filled cavity forms the pore, through which Ca^{2+} traverses. A ring of glutamate residues, which line the pore, imparts Ca^{2+} selectivity. Changes in membrane potential cause a conformational shift resulting in the movement of a voltage-sensing domain to open the ionic pore. VGCCs are classified in three groups (Ca_v1-3)[6]. Ca_v1 consists of the L-type high-voltage-activated VGCCs, Ca_v2 consists of P/Q-, N-, and R-type high-voltage activated VGCCs, and Ca_v3 consists of T-type low-voltage activated VGCCs. The terms "high-voltage activated" and "low-voltage activated" derived from the characteristics associated with the voltage-dependence of the two classes. "High-voltage activated" channels require strong levels of depolarization from the resting membrane potential to open, whereas "low-voltage activated" channels need only minimal depolarization from the resting potential to become activated. The α_1 subunit forms the major structural and functional unit of the channel, but VGCC activity can be modulated by accessory subunits α_2, β, δ, and γ. The α_2 and δ

[6] The nomenclature of VGCCs genes is complex since the original naming system, based on the type of Ca^{2+} current observed during electrophysiological recording, was in place before the more systematic HUGO Human Gene Nomenclature was adopted. We will use the nomenclature developed by ion channel researchers throughout this review but refer the reader to (Ertel et al., 2000) for further discussion of VGCC naming conventions.

subunits are the product of a single gene and are covalently linked by a disulfide bond. The four VGCC β subunits (β1-4) are cytoplasmic and play an instrumental role in cellular trafficking, intracellular signaling, and channel activity modulation. The γ subunit (eight isoforms: $Ca_v\gamma 1$- γ8) is only expressed in skeletal muscle and may also play a role in cell trafficking [104]. The ratio of subunits exists in a 1:1:1:1 $\alpha_1\alpha_2\delta\beta\gamma$ ratio, but diversity among subunit isoforms allows for isoform-specific effects.

L-type VGCCs (Ca_v1) are essential to somatodendritic Ca^{2+} influx in mammalian central neurons. They mediate influx in response to back-propagating action potentials, synaptic plasticity, and excitatory activity-dependent modulation of gene transcription. The Ca_v2 family members are all expressed in central neurons, where they are localized to axons, soma, and dendrites. In peripheral neurons, they are expressed differentially. Their localization was determined using specific pharmacological inhibition of isoform-specific currents (**Table 4**). $Ca_v2.1$ (P/Q-type) and $Ca_v2.2$ (N-type) are the predominate VGCCs involved in presynaptic voltage-dependent Ca^{2+} influx which triggers vesicular release of neurotransmitters. However, at some synapses either or both L-type and R-type channels also participate in this essential function. The final family member, Ca_v3, consists of T-type VGCCs that activate at sub-threshold membrane potentials and are critical for regulation of plasma membrane Ca^{2+} permeability near resting membrane potentials and during action potentials. Depending on the brain region, T-type VGCCs are localized to soma, dendrites, or intracellular targets.

The primary target of pyrethroids is widely accepted as the VGSC [105]; however, pyrethroids can interact with other targets. The ciliate protozoa *Paramecium tetraurelia* do not express VGSCs and yet exhibit high sensitivity to type II pyrethroids, via a mechanism involving disrupted Ca^{2+} homeostasis [106]. Though unique in subunit structure, there are fundamental similarities between VGCCs and VGSCs. Both contain a pore-forming α subunit, intracellular β subunits, and similar gating mechanisms, although the VGCC has additional $\alpha_2\delta$ and γ subunits [107]. Several studies have noted effects of pyrethroids on mammalian neuronal VGCCs in the same concentration range as their effects on neuronal VGSCs [11, 98, 108, 109].

Neurons in culture develop spontaneous networks of interconnected neurons that exhibit electrical activity in response to neuronal signaling. This activity can be measured as either excitatory or inhibitory post-synaptic currents (EPSC or IPSC, respectively) using electrophysiological methods. EPSCs can be either action potential-dependent or action potential-independent; in the presence of TTX, EPSCs are present as miniature events and are termed miniature EPSCs (mEPSCs). In contrast, in the absence of TTX, EPSCs have a much larger magnitude. Both type I and type II pyrethroids inhibit spontaneous activity in neurons [110, 111]. Permethrin (type I) and deltamethrin (type II) both decreased the number of EPSCs in hippocampal neuron networks in a concentration-dependent manner; deltamethrin was more potent. In contrast, only the type I permethrin increased the frequency of mEPSCs in hippocampal neurons [112].

The stimulatory effect of permethrin on mEPSCs was dependent on the presence of extracellular Ca^{2+}[112], showing that the effects observed on EPSCs by type I pyrethroids are downstream of Ca^{2+} entry through VGCCs. Effects of type I pyrethroids on VGCCs have been described in several electrophysiological studies. The type I pyrethroid allethrin potently blocked recombinant rat L-, P/Q-, and T-type VGCCs with IC_{50} in the low micromolar range in human embryonic kidney (HEK) 293 cells [108], which is lower than or in the same magnitude of allethrin concentrations that affect insect VGSCs [61, 98]. However, another study found allethrin differentially modulated endogenous VGCC subtypes in rat pheochromocytoma (PC12) cells, with marked differences in sensitivity, stimulating L-type and inhibiting N-type VGCCs. L-type VGCCs exhibited an EC_{50}[7] in the mid-pM range while N-type VGCCs exhibited an IC_{50} in the low μM range [109].

Subtype	Calcium Current	α subunit	Expression Pattern	Pharmacological Inhibitors
$Ca_v1.1$	L-type	α_{1S}	Skeletal muscle	Dihydropyridines (DHPs)
$Ca_v1.2$	L-type	α_{1C}	Heart, smooth muscle, brain, pituitary, adrenal gland	DHPs
$Ca_v1.3$	L-type	α_{1D}	Brain, pancreas, kidney, ovary, cochlea	DHPs
$Ca_v1.4$	L-type	α_{1F}	Retina	DHPs
$Ca_v2.1$	P/Q-type	α_{1A}	Central and peripheral nervous system	ω-agatoxin-IVA/IIIA, ω-grammotoxin SIVA, ω-conotoxin MVIIC
$Ca_v2.2$	N- type	α_{1B}	Central and peripheral nervous system	ω-conotoxin MVIIC/GVIA/CVID, ω-grammotoxin SIVA, farnesol, petidylamines
$Ca_v2.3$	R-type	α_{1E}	Brain, cochlea, pituitary, retina, heart	Tarantula toxin derived peptide SNX-482
$Ca_v3.1$	T-type	α_{1G}	Central and peripheral nervous system	Kurtoxin, ethosuximide, nickel
$Ca_v3.2$	T-type	α_{1H}	Brain, heart, kidney, liver	Kurtoxin, ethosuximide, nickel
$Ca_v3.3$	T-type	α_{1I}	Brain	Kurtoxin, nickel

Table 4. Naming system, tissue localization, and pharmacological agents of VGCC subtypes.

The VGCC subtype-specific effects seen in PC12 cells [109] but not in recombinant assays [108] may be due to differences in VGCC subunit expression. A combination of rat α_{1A}(P/Q), α_{1C}(L) or α_{1G}(T) with the β_{1b} and $\alpha_2\delta$ subunits was expressed in HEK293 cells [108]. While the α subunit composition would remain the same, PC12 cells express β_1, β_2, and β_3 mRNA,

[7] Half maximal effective concentration (EC_{50}) refers to the concentration at which 50% of the maximum response is observed during a stimulatory effect.

and different β subunits associate in the native N-type channel [113]. Additionally, the β subunit can undergo extensive post-translational modification, resulting in numerous splice variants [114]. The β subunit in VGCCs has direct effects on VGCC inactivation, with VGCCs containing the β_{2a} subunit exhibiting slower inactivation than those containing the β_{1b} or β_3 subunit [114-116]. Furthermore, the identity of the β subunit can shift the voltage dependence of VGCC currents, P/Q-type VGCCs containing β_4 exhibit peak current at more hyperpolarized potentials than β_{1b}, β_{2a}, or β_3, [116]. Effects of pyrethroids on VGSCs can be modulated by the identity of the VGSC β subunit [46], so perhaps pyrethroid effects on VGCCs may also be modulated by auxiliary subunit composition.

VGCC sensitivity to pyrethroids may also be modulated by the channel phosphorylation state. N-type VGCCs in particular are heavily influenced by phosphorylation by protein kinase C (PKC). PKC phosphorylates several sites in the N-type VGCC; phosphorylation at threonine-422 (T422) is stimulatory while at serine-425 (S425) it is inhibitory [117, 118]. Effects of PKC phosphorylation are dependent on β subunit expression [119]. In recombinant systems, application of phorbol-12-myristate, 13-acetate (PMA) potentiates current through N-type VGCC [120]. Inhibition of the PKC isoforms βII and ε block this potentiation. Deltamethrin differentially modulated recombinant N-type VGCCs based on channel phosphorylation status at the T422 residue [121]. Deltamethrin exposure of a mutant N-type VGCC that mimics phosphorylation at residue T422 resulted in channel activation. Wild-type channels and mutant channels incapable of phosphorylation are inhibited by deltamethrin. Although effects of type I pyrethroids on this mutant channel have not yet been investigated, this study raises the interesting possibility that VGCCs exhibit phosphorylation-specific sensitivity to pyrethroids

In addition to direct measurement of the effects of pyrethroids on Ca^{2+} current and VGCC properties, there have been many studies on pyrethroids effects on Ca^{2+} influx and neurotransmitter release. These endpoints serve as measures of functional outcomes of pyrethroid modulation of VGCCs. Alternatively, as pyrethroids are lipophilic and may independently partition into cells, results from these assays could indicate pyrethroid action on intracellular Ca^{2+} channels. Several studies were performed using rat synaptosomal preparations. Deltamethrin caused a significant increase in Ca^{2+} influx in rat synaptosomes in a stereospecific manner, with 1R-deltamethrin causing a roughly 1.7 fold increase in Ca^{2+} influx and 1S-deltamethrin having no effect on influx [122]. TTX had no effect on the stimulation of Ca^{2+} influx by deltamethrin, indicating that the influx was independent of VGSCs. However, incubation with ω-conotoxin MVIIC abolished the stimulatory effect of deltamethrin on Ca^{2+} influx, suggesting that the Ca^{2+} influx was via N- or P/Q- type VGCCs. In a follow up study, the deltamethrin-stimulated influx was reduced by 63% in the presence of ω-conotoxin GVIA, suggesting that more than half of the deltamethrin-stimulated influx was via N-type VGCCs.

Cismethrin had a lesser stimulatory effect on Ca^{2+} influx than deltamethrin but only with high (>20 mM) levels of potassium-induced depolarization [121, 123]. The cismethrin-mediated influx was unaffected by ω-conotoxin GVIA but was reduced by 50% with TTX

[123]. In the presence of nimodipine, cismethrin increased Ca^{2+} influx [121]. A similar stimulatory effect on Ca^{2+} influx by cismethrin was observed in the presence of ω-conotoxin GVIA [121]. The results with cismethrin suggested that the pyrethroid is primarily increasing Ca^{2+} via a TTX-sensitive mechanism whereas the mechanism with deltamethrin is likely TTX-insensitive. This may suggest that the Ca^{2+} influx observed with cismethrin is a result of increased Na^+ influx through VGSCs followed by replacement of Na^+ with Ca^{2+} via the Na^+/Ca^{2+} exchanger [121].

Deltamethrin caused a modest, yet significant, increase in glutamate release from rat synaptosomes in a stereospecific manner, with 1R-deltamethrin stimulating glutamate release and 1S-deltamethrin having no effect [122]. TTX had no effect on the stimulation of glutamate release by deltamethrin, indicating that the effect was independent of VGSCs. However, incubation with ω-conotoxin MVIIC abolished the stimulatory effect, suggesting that the release was mediated by activation of N- or P/Q- type VGCCs. In a subsequent study, ω-conotoxin GVIA reduced this deltamethrin-mediated increase in glutamate release by 70%, but nimodipine (L-type VGCC blocker) had no effect, suggesting that the enhanced glutamate release was predominately through stimulation of N-type VGCCs. Two other pyrethroids, cismethrin and bioresmethrin, had no effect on glutamate release in this model system [121, 122].

The studies described above suggested that while both type I and type II pyrethroids stimulate Ca^{2+} influx, type II pyrethroids are more potent modulators of glutamate release. However, as only a limited number of chemicals were tested, further studies are needed to determine if there were inter-class differences in these effects. A study of 11 pyrethroids, showed that they could be separated into 3 groups based on a cluster analysis of their behavior in Ca^{2+} influx and glutamate release assays. Group 1 consisted of cismethrin (type I) and bifenthrin (type I); group 2 consisted of bioallethrin (type I), tefluthrin (type I), and fenpropathrin (type II); and group 3 consisted of permethrin (type I), cyhalothrin (type II), cyfluthrin (type II), deltamethrin (type II), cypermethrin (type II), and esfenvalerate (type II). Thus, Group 1 and 2 were largely composed of type I pyrethroids while Group 3 consisted of mainly type II pyrethroids. Only Groups 1 and 3 elicited a concentration-dependent effect on Ca^{2+} influx into rat synaptosomes, while all three groups elicited a concentration-dependent effect on glutamate release, with the highest potency observed with Group 3 [124]. Thus, in this study the group with the most type II pyrethroids exhibited the highest potency in Ca^{2+} influx and neurotransmitter release assays. These findings were confirmed in another study on 11 pyrethroids. A cluster of 6 pyrethroids (λ-cyhalothrin, cypermethrin, permethrin, deltamethrin, cyfluthrin, and esfenvalerate) had the highest potency in both the Ca^{2+} influx and glutamate release assays. These 6 pyrethroids overlap with the pyrethroids in Group 3 of [124]. Another cluster of pyrethroids (tefluthrin, bio-allethrin, and fenpropathrin) affect glutamate release but not Ca^{2+} influx. These pyrethroids are identical to those in Group 2 of [124] and performed similarly in the Ca^{2+} influx and glutamate release assays in both studies. Finally, bifenthrin and cismethrin had modest effects on Ca^{2+} influx only at the highest concentrations tested and had no effect on glutamate release. These

pyrethroids were in Group 1 [124], and while neither study observed pronounced effects of these pyrethroids on Ca^{2+} influx, [124] did observe effects of these pyrethroids on glutamate release. Thus, there is good agreement between two independent studies that type II pyrethroids generally are more potent against Ca^{2+} influx and glutamate release than are type I agents.

Most pyrethroid formulations used by humans consist of mixtures of various pyrethroids. Thus, an active area of investigation is the way pyrethroid mixtures interact with the pyrethroid targets. Binary mixtures of deltamethrin with the type II pyrethroids λ-cyhalothrin, cypermethrin, and esfenvalerate increased intracellular Ca^{2+} levels in an additive manner [125]. The type I pyrethroids permethrin and cismethrin also produced additive effects on Ca^{2+} influx when combined with deltamethrin in binary combinations. In contrast, S-bioallethrin and tefluthrin (type I) and fenpropathrin (type II) in binary mixtures with deltamethrin caused less-than-additive effects on Ca^{2+} influx [125]. These studies suggested that pyrethroids which cause increased Ca^{2+} influx work together in mixtures to stimulate Ca^{2+} influx in an additive manner. However, those pyrethroids with weak stimulatory activity or inhibitory activity on influx do not synergistically modulate Ca^{2+} influx. The additive effects on Ca^{2+} influx did not predict the effects of the binary combinations of pyrethroids on glutamate release. A more-than-additive effect on glutamate release was observed when a subset of type I or type II pyrethroids was combined with deltamethrin, but none of these mixtures had produced more-than additive effects on Ca^{2+} influx [125].

7. Pyrethroid effects on $GABA_A$ receptors

GABA (γ -aminobutyric acid) is a small molecule inhibitory neurotransmitter utilized by nearly 1/3 of the mammalian brain's synapses. In contrast to excitatory neurotransmitters, inhibitory neurotransmitters prevent a neuron from firing an action potential [48]. There are three types of GABA receptors, A, B and C. GABA type A ($GABA_A$) and GABA type C ($GABA_C$) receptors are ligand-gated chloride ion (Cl^-) channels. They exist as pentamers containing various combinations of α, β, γ, δ, ρ, π, and ε subunits (**Figure 4**). The subunit composition of the GABA receptors determines the pharmacological and electrophysiological properties of the receptors [126]. Thus far 6 α_3, β, 3 γ, 1δ, 1Π, 1E, and 3 p subunits have been identified in the mammalian brain. They have distinct regional and cellular distributions, resulting in a diverse array of GABA receptors. However, the majority of $GABA_A$ receptors have 2 α, 2 β and 1 γ subunit. GABA binds $GABA_A$ receptors at the interface of the α and β subunits stimulating an influx of Cl^- that holds the neuron's membrane potential more negative than the threshold required to initiate an action potential.

In the1980s, $GABA_A$ receptors were postulated to play a role in type II pyrethroid poisoning signs. Diazepam, a benzodiazepine drug, delayed type II poisoning signs and reduced mortality in mice and cockroaches injected with deltamethrin or fenvalerate [127]. Benzodiazepines bind to $GABA_A$ receptors and enhance the inhibitory effects of GABA-induced activation of the receptor. Diazepam offered no protection from treatment with the

type I pyrethroids, allethrin and permethrin. Type II, but not Type I pyrethroids also inhibit binding of picrotoxin and t-butylbicyclophosphorothionate (TBPS) to rat brain membranes *in vitro* [128, 129]. Picrotoxin and its derivative TBPS antagonize[8] GABA-induced Cl^- flux by blocking the $GABA_A$ ion channel. Picrotoxin and TBPS binding were effectively inhibited with 5 μM deltamethrin, cypermethrin and fenvalerate, whereas 50 μM of the type I pyrethroids cismethrin and permethrin had no effect. Pyrethroids had no effect on the binding of benzodiazepines or muscimol (binds at the GABA site), suggesting that type II pyrethroids bind the $GABA_A$ receptor at the same site as picrotoxin/TBPS.

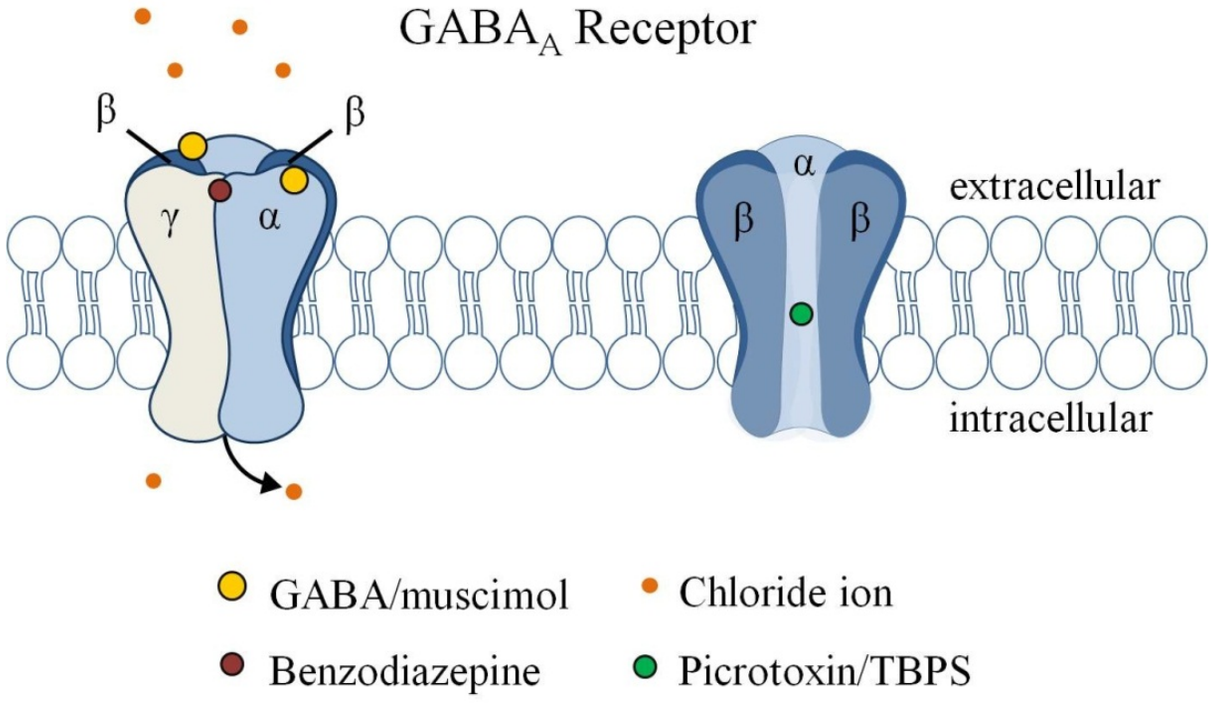

Figure 4. The $GABA_A$ receptor. This schematic depicts the most common $GABA_A$ receptor subunit composition and the binding sites of its ligand, GABA, and various drugs that influence the receptor's function. Modified from Motifolio Biomedical PowerPoint Toolkit Suite.

Functional evidence of type II pyrethroid inhibition of $GABA_A$ receptors arose from electrophysiology experiments using crayfish claw opener muscles [130]. Bathing crayfish claw opener muscles in a GABA bath causes a decrease in input resistance, indicating an activation of GABA receptors. $GABA_A$ receptor antagonists, such as picrotoxin, increase input resistance by blocking the ion pore. Several type II pyrethroids increased the input resistance of the claw opener muscle in a manner similar to picrotoxin. Furthermore, cypermethrin inhibited GABA-stimulated influx of radioactive $^{36}Cl^-$ into rat brain synaptosomes [131]. However, none of these experiments were conducted in the presence of TTX to block the action of pyrethroids on VGSCs. In the presence of TTX, the GABA induced inward Cl^- current of cultured rat sensory neurons was not affected by

[8] Antagonists are chemicals that bind a receptor and blocks action of the receptor. Agonists are chemicals that bind the receptor triggering a response.

deltamethrin [132]. Antagonism of $GABA_A$ receptors should decrease inhibition in the brain. However, cismethrin, fenvalerate or deltamethrin increased function of inhibitory neurons in the rat hippocampus [133]. Further evidence against a direct effect of pyrethroids on $GABA_A$ receptors came from trout brain synaptosome $^{36}Cl^-$ influx assays [134]. Deltamethrin, cypermethrin and permethrin caused a concentration-dependent decrease in GABA-dependent $^{36}Cl^-$ influx. This effect was completely inhibited by TTX, indicating that previously observed effects of type II pyrethroids on $GABA_A$ receptors are indirect and downstream of the effects on VGSCs.

8. Pyrethroid effects on voltage-gated Cl^- channels

Cl^- is the most abundant extracellular anion and its movement across cell membranes is involved in cell volume regulation, and acidification of intracellular compartments such as lysosomes [135-137]. In excitable cells, voltage-gated Cl^- channels (ClCs) act to maintain resting membrane potential. Comparatively little is known about the ClCs, but the channels are thought to function as dimers with two identical ion pores. In [134] an increase in basal uptake (GABA independent) of $^{36}Cl^-$ occurred upon pyrethroid administration. This pyrethroid-enhanced uptake was completely sensitive to TTX but only partially inhibited by a $GABA_A$ channel blocker, providing the first evidence that ClCs may be involved on pyrethroid action.

Deltamethrin decreased the probability of ClC opening in cultured mouse neuroblastoma cells in a concentration-dependent manner [138, 139]. This effect was initially thought to be type II specific as cypermethrin also decreased open channel probability. However, bioallethrin (type I) also decreased the probability of ClC opening, whereas esfenvalerate and cyhalothrin (type II) had no effect [140]. Therefore the ability of a pyrethroid to modify ClC is not solely determined by the presence of an α cyano group. The Cl^- channel agonist, ivermectin, increased the probability of ClC opening in deltamethrin-treated cultured mouse neuroblastoma cells [141]. *In vivo* experiments demonstrated that ivermectin decreased salivation and muscle twitching. Thus, ClCs are biologically relevant sites of action for certain pyrethroids that contribute to some of the intoxication signs.

9. Conclusion

Pyrethroid insecticides are synthetic analogs of pyrethrin, the natural insecticides produced by *Chrysanthemum* species. Pyrethroid insecticides are divided into two groups based on the absence (type I) or presence (type II) of a cyano group. Both types disrupt action potentials in insects by prolonging the open time of VGSCs in a concentration and temperature dependent manner by delaying channel inactivation and deactivation. As the type II pyrethroids hold VGSCs open longer, they allow greater influx of Na^+ resulting in neuron depolarization and conduction block. Pyrethroids similarly affect mammalian VGSCs and those effects are thought to underlie the signs associated with pyrethroid intoxication. Developing mammals are more sensitive to pyrethroids than adults, but the molecular mechanisms for this increased sensitivity is unknown. The developmental neurotoxicity of

pyrethroids may be due to effects on VGSC isoforms that are only expressed during development. There is also evidence that pyrethroid insecticides target mammalian VGCCs and ClCs. Pyrethroid appear to cause VGCC sub-type specific effects, although there are conflicting data present in the literature. Some pyrethroids are capable of antagonizing ClCs, and this action appears to be partly responsible for the hypersalivation and motor dysfunction seen in pyrethroid intoxication. There is currently little understanding as to how or if pyrethroid modification of VGCCs and ClCs contribute to mammalian toxicity both in adult and developing animals. Furthermore, as most mammals are exposed continually to a mixture of pyrethroids it remains to be seen how the combined effects of different pyrethroids affect neuronal function long-term.

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