Nerve Membrane Ion Channels as the Target Site of Insecticides

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Abstract, Most insecticides are potent neurotoxicants that act on various neuroreceptors and ion channels. However, the major target receptors are limited to sodium channels, GABA receptors, and nicotinic acetylcholine receptors. DDT and pyrethroids act similarly on sodium channels to keep them open leading to hyperexcitation. Indoxacarb inhibits sodium channels and certain subtypes of nicotinic receptors. Dieldrin, lindane and fipronil block GABA receptors. Imidacloprid modulates nicotinic receptors in a complex manner. Spinosad's major target site appears to be nicotinic receptors.

INTRODUCTION

An era of synthetic insecticides began in the middle of the 20th century when DDT was invented during the World War II. This was followed by the development of scores of potent insecticides such as lindane, parathion, malathion and dieldrin, to mention just a few examples. Such profound developments of insecticide industry stimulated studies of their mechanism of action especially when the toxicity to humans and non-target organisms and the development of insecticide resistance in insect populations became serious issues.

The history of the progress in the study of the mechanism of action of insecticides closely followed the steps of insecticide developments. It turned out that most insecticides are potent neurotoxicants, and interestingly, most of these neurotoxic insecticides act on three types of neuroreceptors and ion channels. This is exemplified by DDT and pyrethroids that act on voltage-gated sodium channels; lindane, dieldrin and fipronil that act on GABA receptors; and imidacloprid that acts on nicotinic acetylcholine (nACh) receptors. The reasons for this are not completely clear. However, it should be pointed out that

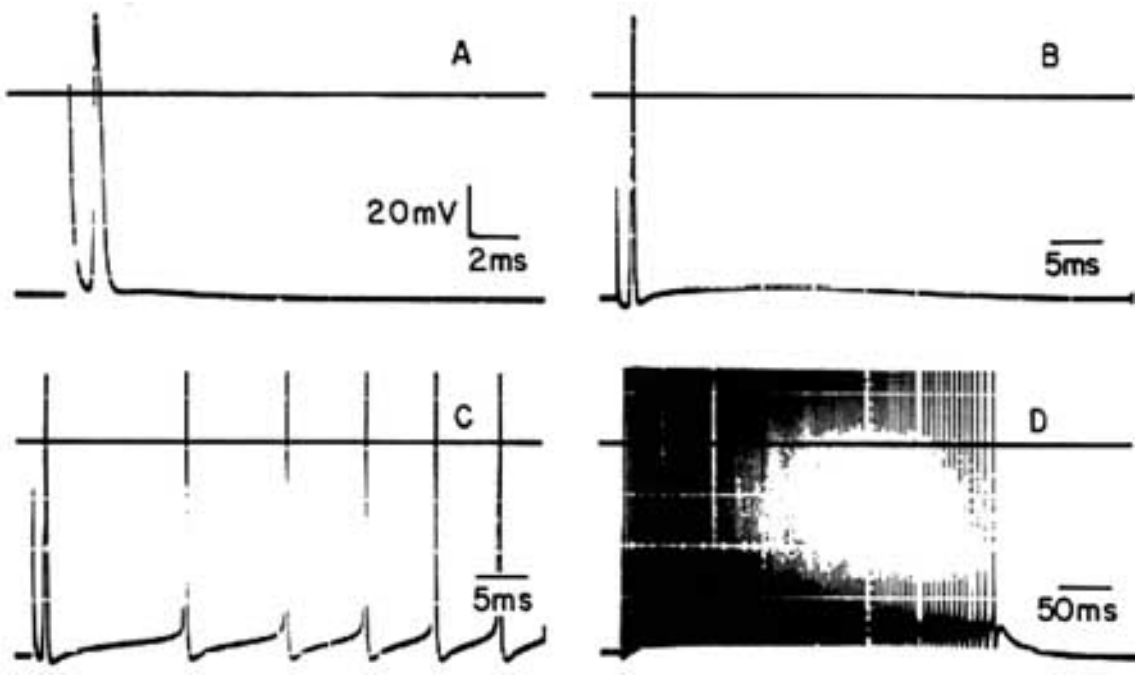

Fig. (1). Repetitive discharges induced by a single stimulus in a crayfish giant axon exposed to 10 μM tetramethrin. A. Control. B. 5 min after application of tetramethrin. C and D. 10 min after application. From Lund and Narahashi [75].

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none of the three receptors/channels is uniquely present in insects; they are common in both mammals and insects albeit their molecular structures are not identical between mammals and insects. This point turned out to be critical for understanding the selective toxicity of insecticides in mammals and insects.

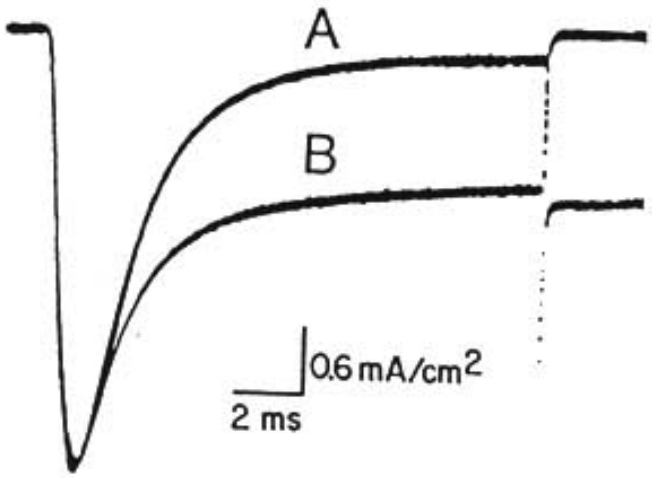

Fig. (2). Effects of 1 μM allethrin on the sodium current of a squid giant axon. The membrane was step depolarized to –20 mV from a holding potential of –100 mV in K-free external and internal perfusates. A. Control. B. The Peak transient sodium current remains unchanged, but the slow current and tail current are increased in amplitude and the latter decays very slowly. From Narahashi [76].

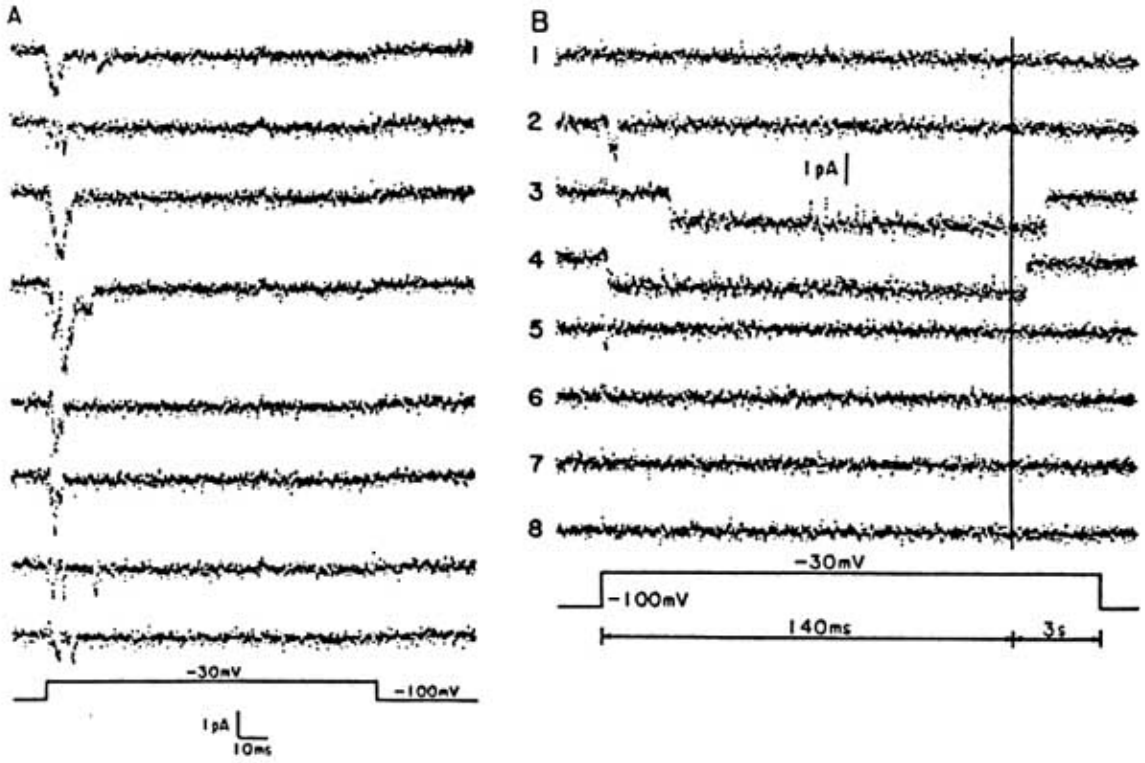

Fig. (3). Effects of deltamethrin on single sodium channel currents of a neuroblastoma cell (N1E-*115*). A. Currents from a cell before drug treatment in response to 140-ms depolarizing steps from a holding potential of –100 to –30 mV with a 3-s interpulse interval. B. Currents after exposure to 10 μM deltamethrin. The membrane patch was depolarized for 3140 ms from a holding potential of –100 to –30 mV. The interpulse interval was 3 s. The time scale changes during the voltage step as indicated in the Figure. From Chinn and Narahashi [18].

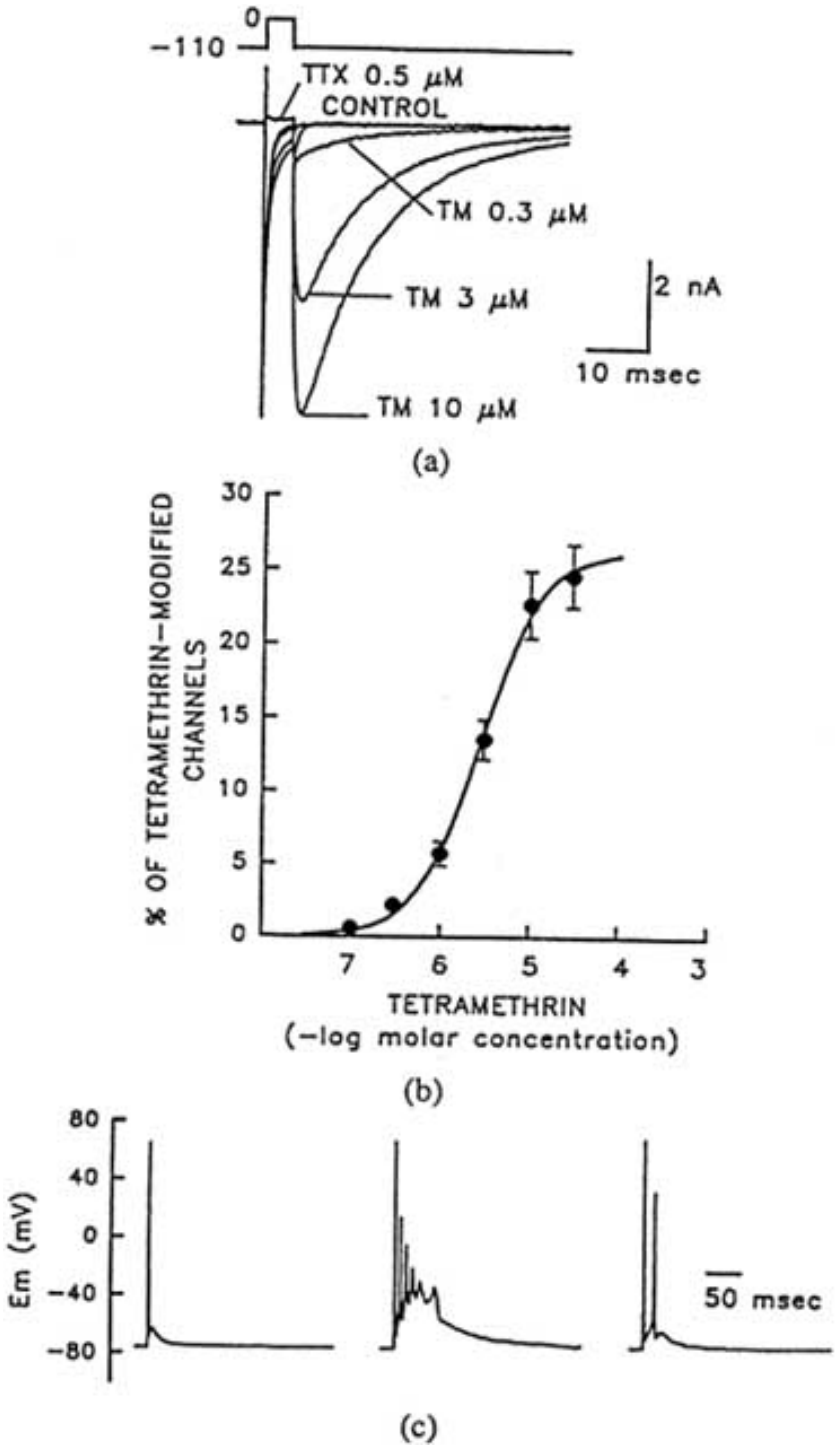

Fig. (4). Concentration-dependent effect of tetramethrin on TTX-S sodium currents of rat cerebellar Purkinje neurons. (a). Currents were evoked by a 5-msec step depolarization to 0 mV from a holding potential of –110 mV under control conditions and in the presence of tetramethrin (0.3 μM, 3 μM, and 10 μM). TTX (0.5 μM) completely blocked both the peak current and the tetramethrin-induced tail current. (b). The concentration-response relationship for induction of tail current. Each point indicates the mean ± S.E. M. (n = 6). Data were fitted by the Hill equation. The percentages of channels modified by tetramethrin are 0.62 ± 0.15%, 2.19 ± 0.36%, 5.75 ± 0.87%, 13.58 ± 1.*35*%, 22.77 ± 2.26%, and *24*.73 ± 2.11% at concentrations of 0.1, 0.3, 1, 3, 10, and 30 μM, respectively (n = 6). (c). Repetitive action potentials caused by 100 nM tetramethrin, the threshold concentration. From Song and Narahashi [22].

This mini-review gives the current progress of our knowledge of the mechanism of the action of insecticides on neuroreceptors and ion channels. A number of review articles have been published on this subject, and the readers are

encouraged to consult them for more comprehensive information, including older literature [1-10, 11, 12, 13, 14,15, 16, 17).

PYRETHROIDS AND DDT

Whereas pyrethroids and DDT are structurally different from each other, their mechanism of action has been shown to be very similar modulating the activity of sodium channels [10]. Therefore, their actions will be discussed together.

Our earlier studies showed that the depolarizing after-potential was prolonged by DDT and reached the threshold membrane potential to evoke repetitive after-discharges (Fig. **1**) [9]. Voltage clamp experiments using squid, crayfish and lobster giant axons clearly demonstrated that the sodium current was marked prolonged by DDT and pyrethroids (Fig. **2**), and patch clamp single-channel current recording revealed profound prolongation of channel open time (Fig. **3**) [9]. Similar studies were also performed by several other investigators [13].

While it is clear that the kinetics of both activation and inactivation mechanisms of sodium channels are slowed by pyrethroids based on single-channel and gating current measurements [18, 19], our recent study showed that slowing of the sodium current occurred in the presence of pyrethroids after the sodium inactivation had been removed by papain [20]. Thus, the sodium channel activation gate is critically important for pyrethroids to prolong the sodium current leading to hyperexcitation.

A method has been developed to measure the percentage of sodium channel population modified by various concentrations of pyrethroids [21]. In sodium channels of mammalian neurons, pyrethroids cause a large and slow sodium tail current to appear upon termination of depolarizing pulse (Fig. **4**). This tail current is generated in the pyrethroid-modified sodium channels, while the peak sodium current is generated in the normal sodium channels. Thus, the percentage of sodium channels modified by pyrethroids (M) can be calculated by the following equation.

$$M=\{[I_{tail}/(E_h-E_{Na})]/[I_{Na}/(E_t-E_{Na})]\} \times 100$$

where I_{tail} is the initial amplitude of the slowly decaying tail current, E_h is the potential to which the membrane is repolarized, E_{Na} is the equilibrium potential for Na^+, and E_t is the potential of the step depolarization.

In rat cerebellar Purkinje neurons, only a very small fraction (~1%) of sodium channel population needs to be modified by tetramethrin to cause repetitive discharges which in turn lead to hyperexcitation of the animals (Fig. **4**) [22]. This explains high potencies of pyrethroids in killing insects. Similarly small percentages of sodium channel modification were found to be caused by low concentrations of pyrethroids in rat dorsal root ganglion (DRG) neurons [20, 21].

Pyrethroids are divided into two groups, type I and type II, based on the absence and presence of an α-cyano moiety in the molecule, respectively. Although the symptoms of poisoning caused by these two types of pyrethroids in mammals are somewhat different, the major target site for

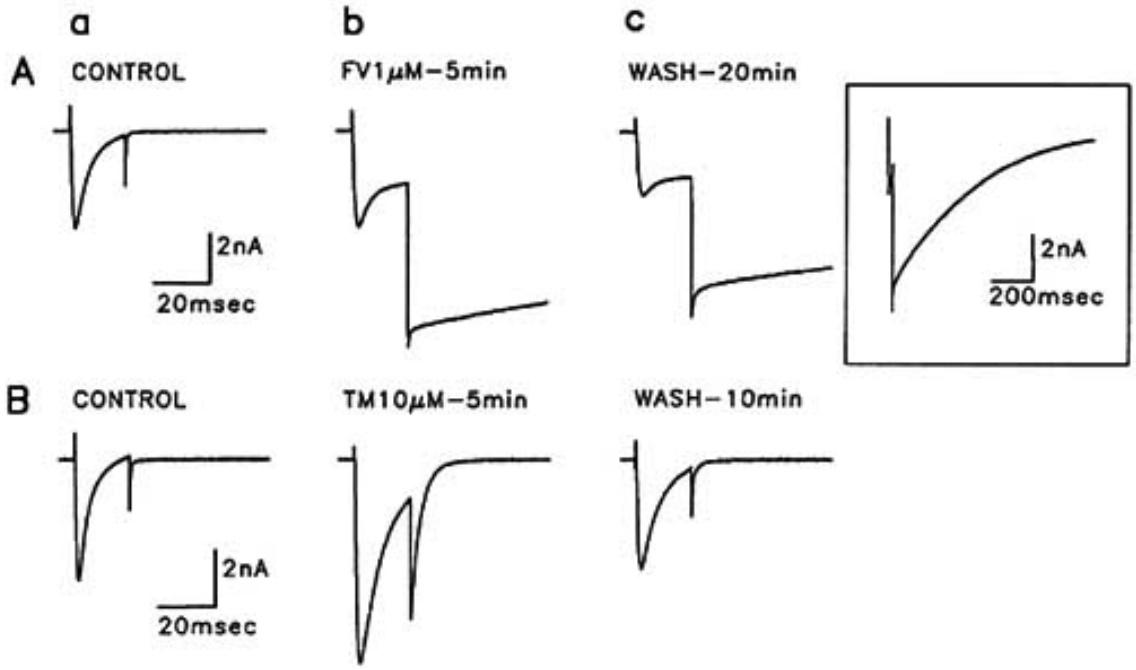

Fig. (5). Effects of 1 μM fenvalerate (FV) (A), and 10 μM tetramethrin (TM) (B) on TTX-R sodium currents in rat DRG cells. Currents were evoked by a 20-ms step depolarization to 0 mV from a holding potential of –90 mV before (a), during application of insecticide (b), and after washout with insecticide-free solution (c). Inset is the same as Ac, but with a slower time scale. From Song *et al*. [23].

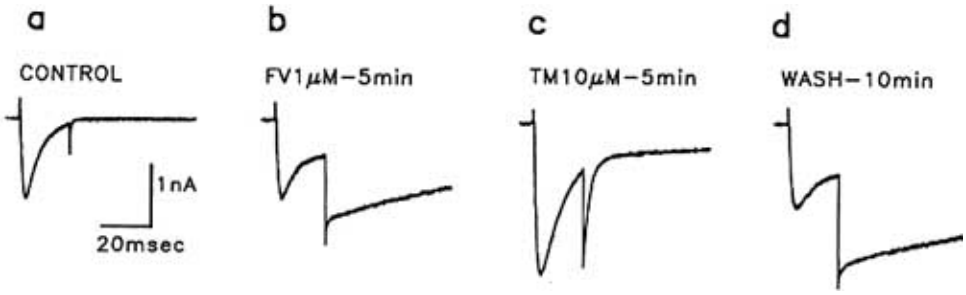

Fig. (6). Combined effects of 1 μM fenvalerate (FV) and 10 μM tetramethrin (FV) on TTX-R sodium current in DRG cells. Currents were evoked by a 20-ms step depolarization to 0 mV from a holding potential of –90 mV before (a), during application of fenvalerate (b) and of tetramethrin (c), and after washout with insecticide-free solution (d). From Song *et al*. [23].

both is the sodium channel. The prolongation of sodium current is more pronounced and the effect is less reversible in type II than type I pyrethroids. Intriguing interactions between type I and type II pyrethroids were discovered [23] in rat DRG neurons, which express both tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels [24]. In both types of sodium channels, tetramethrin (type I) prolonged the sodium tail current reversibly, and fenvalerate (type II) prolonged the current more markedly and irreversibly (Fig. **5**). When tetramethrin was applied to the fenvalerate-treated cell, the markedly prolonged tail current characteristic of fenvalerate action disappeared and was replaced by a shorter tail current characteristic of tetramethrin action (Fig. **6**). It appears that the two pyrethroids compete for binding to the same site in the sodium channel with tetramethrin having a higher affinity than fenvalerate. Single-channel analyses endorsed the competition [25].

Another unique feature of the action of DDT and pyrethroids is negative temperature dependence. It has long been known that these insecticides are more potent in killing insects at low temperature than at high temperature, and the most important factor for this phenomenon is the negative temperature dependence of nerve sensitivity to DDT and pyrethroids. Although the mechanism that underlies this phenomenon has been studied for a long time by several investigators, it has recently become clear that the most critical factor is the prolongation of sodium current, more accurately that of charge transfer (Fig. **7**). The Q_{10} value for the charge transfer was estimated to be 0.2 as opposed to that for the percentage of sodium channel modification, which was estimated to be 0.78 [9, 10, 22]. The prolongation of single sodium channel currents was also temperature dependent, being more profound at low temperature than at high temperature (Fig. **8**) [25].

Whereas pyrethroids are known to affect various neuroreceptors and ion channels other than sodium channels, it has become abundantly clear that the modification of sodium channels is most crucial in developing the primary symptoms of poisoning as represented by hyperexcitation, convulsions and tremors [10].

The selective toxicity of insecticides between mammals and insects is one of the most important aspects of insecticides. For pyrethroids it has become abundantly clear

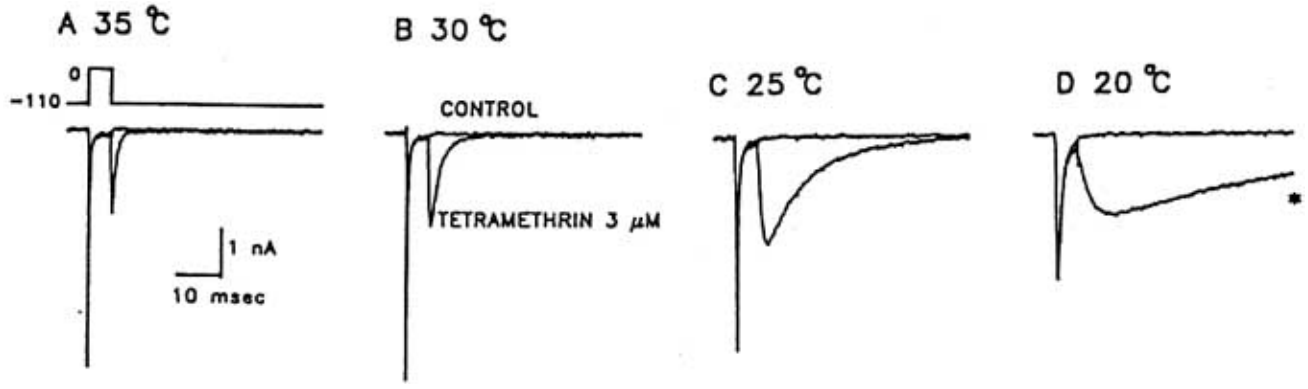

Fig. (7). Temperature-dependent effect of 3 μM tetramethrin on sodium currents recorded from a rat cerebellar Purkinje cell. The currents were evoked by a 5-msec step depolarization to 0 mV from a holding potential of –110 mV at various temperatures. The currents before and during application of tetramethrin are superimposed. *, Current recording is terminated before the tail current returns to the base-line. From Song and Narahashi [22].

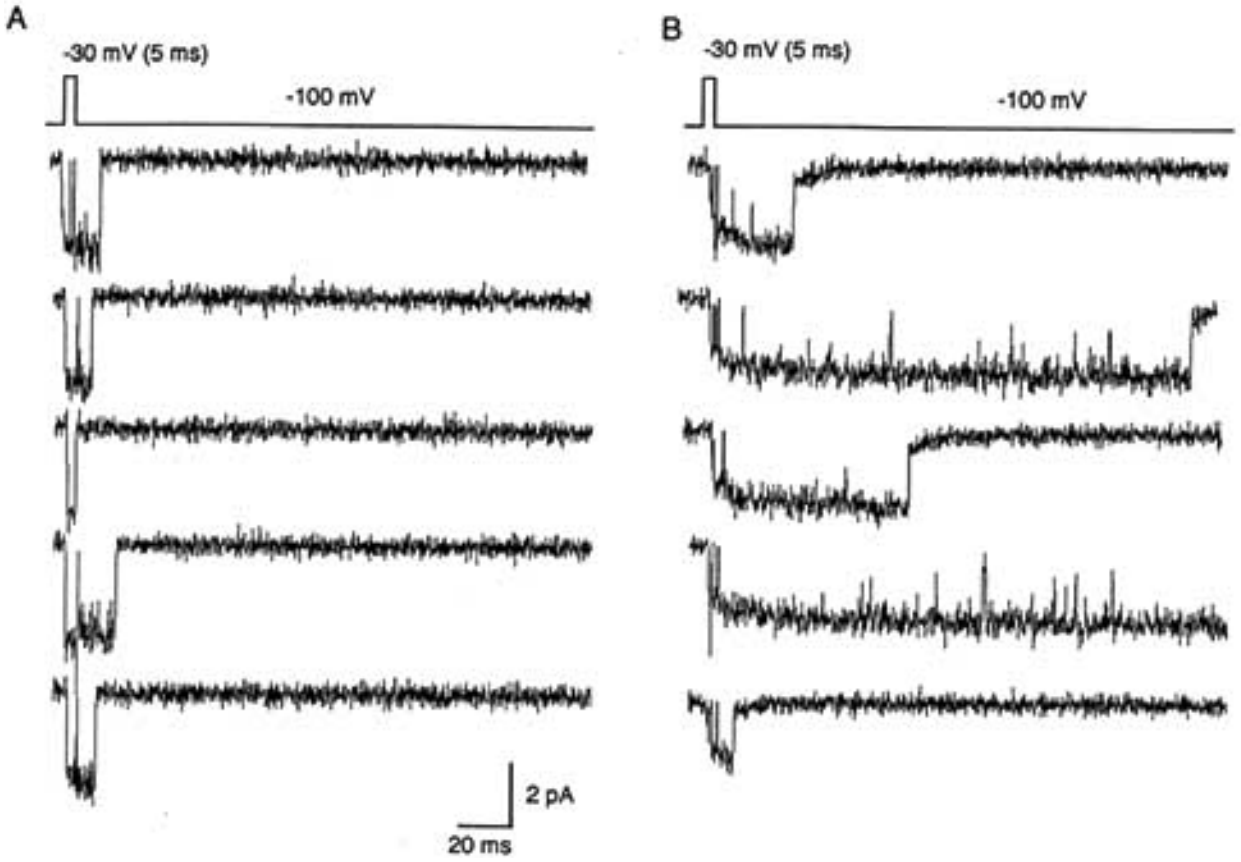

Fig. (8). Tetramethrin (10 μM) induces single-channel current openings upon repolarization in rat hippocampal neurons. Membrane patches were depolarized to –30 mV for 5 msec, and subsequently repolarized to –100 mV; A at 22°C; B at 12°C. A and B were from separate patch membranes. From Motomura and Narahashi [76].

that the difference in sodium channel sensitivity is the major factor for selective toxicity [10, 22]. When all factors are multiplied including nerve sensitivity to and detoxication of pyrethroids, the difference between mammals and insects amounts to several thousand fold, which is in the same order of magnitude as the difference in LD_{50}s.

Whereas detoxication of pyrethroids and DDT is in some cases an important mechanism of insect resistance to these insecticides, the target site resistance has been demonstrated to be a dominant fact for the so-called knockdown resistance (*kdr*) [26, 27]. Direct demonstration of the mechanism of target site resistance to DDT was made by Yamasaki and Narahashi [28] using resistant strains of houseflies. The identification of chromosomal genes for reduced nerve sensitivity to DDT was made in the mid-1960s [29]. However, it was not until in 1990s that mutations of the α subunit of sodium channels in various pyrethroid resistant insects were identified by taking full advantage of molecular biology and genetics techniques. Many mutations of amino acids are now known to occur in various insect species that gained resistance to pyrethroids [10]. A few such examples are, V410M in IS6 domain and transmembrane segment in *Heliothis virescens* [30, 31]; M918T in IIS4-S5 [32, 33] and L993F in IIS6 [32, 34] in *Musca domestica*; and L1014F in IIS6 in *Anopheles gambiae* [35].

INDOXACARB

There are a few other insecticides that act on sodium channels, the most notable of which is indoxacarb. However, the situation is complicated by the fact that this insecticide also interacts with other systems such as neuronal nicotinic acetylcholine receptors.

The oxadiazine compound indoxacarb is a new insecticide with potent insecticidal activity and low mammalian toxicity. It is metabolized to decarbomethoxyllated DCJW in insects. In insect nerve preparations, DCJW has been reported to block action potentials and sodium channels at nanomolar concentrations [36, 37], while indoxacarb itself is weak in blocking action [37]. In mammals, DCJW blocked sodium channels of rat dorsal root ganglion (DRG) neurons, but no detailed mechanism of action was elucidated [38].

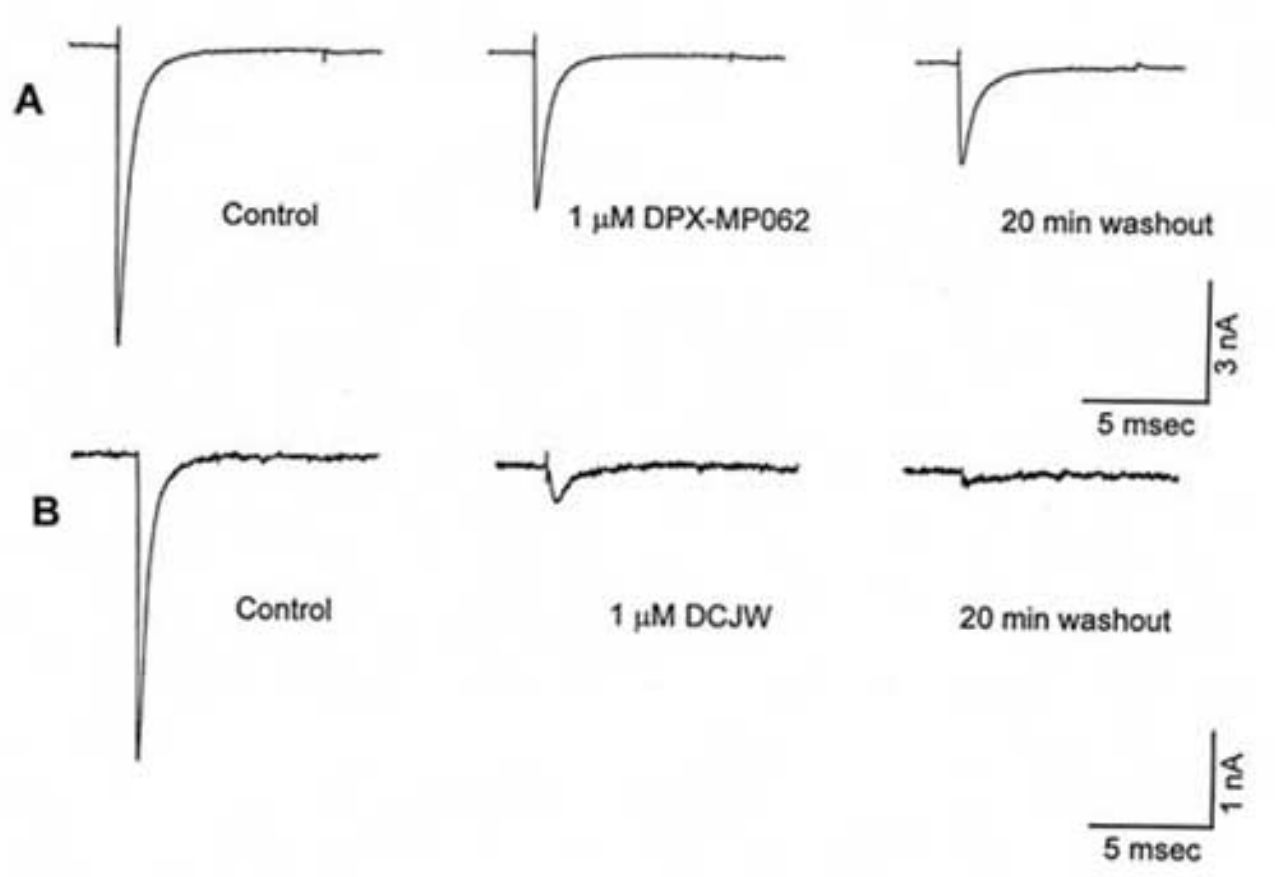

Fig. (9). Indoxacarb (DPX-MP062) and its metabolite DCJW block the TTX-S sodium channel of rat DRG neurons. DCJW is more potent than indoxacarb.

We studied indoxacarb block of sodium channels in rat DRG neurons (Fig. **9**) [39]. The steady-state sodium channel inactivation curve was located at more negative potentials in TTX-S than TTX-R channels, and indoxacarb and DCJW shifted the curve in the hyperpolarizing direction. This explains why at the resting membrane potential (-80 mV), TTX-S channels were blocked by both indoxacarb and DCJW more potently than TTX-R channels, and why indoxacarb and DCJW block was voltage dependent. Mammalian sodium channels were less sensitive to DCJW than insect sodium channels, but unlike insects, mammalian sodium channels were blocked by indoxacarb itself.

We also studied the effects of indoxacarb and its metabolite DCJW on ACh-activated currents in rat cerebral cortical neurons in primary culture. Rat cortical neurons are one of the few brain neuron preparations with which nicotinic AChRs are well characterized [40]. Indoxacarb was found to be a potent inhibitor of the α-bungarotoxin (α-BuTX)-sensitive, α7-type ACh-induced currents but exerted a potentiating effect on the α-BuTX-insensitive, α4β2-type currents (Fig. **10**). Contrary to the potent inhibitory action on the sodium channel in insects [36], DCJW suppressed α-BuTX-sensitive currents only slightly without much effect on α-BuTX-insensitive currents. However, the effects of indoxacarb on $GABA_A$ receptors were rather weak.

DIELDRIN AND HCH

It is now well established that dieldrin and hexachlorocyclohexane (HCH) block GABA receptors thereby causing hyperexcitation and convulsions in mammals and insects [10]. Our recent study has revealed that dieldrin has a dual action on the $GABA_A$ receptor of rat DRG neurons [41]. The GABA-induced current was first increased but later suppressed irreversibly during repetitive co-applications of GABA and dieldrin. Analysis of picrotoxin-dieldrin interaction experiments led to the conclusion that dieldrin acts on the picrotoxin site which is closely associated with the chloride channel of the receptor.

The $GABA_A$ receptor consists of five subunits forming a pentameric structure [42]. Dieldrin suppressed GABA-induced currents in human embryonic kidney (HEK) cells expressing α1β2, α1β2γ2S, and α6β2γ2S, but it

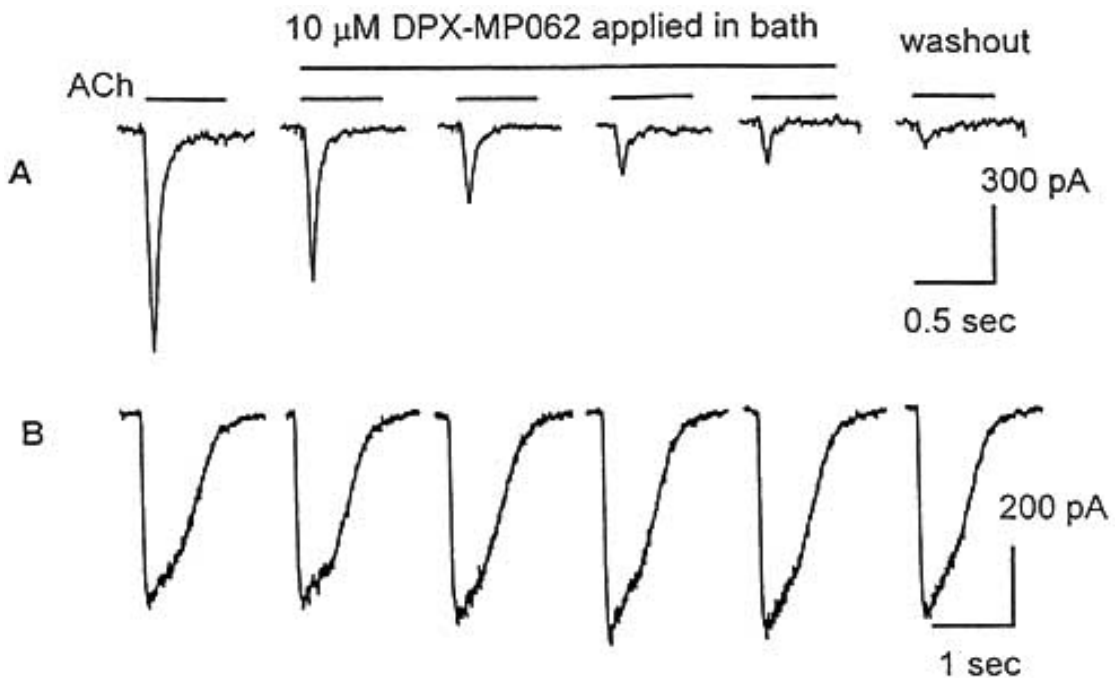

Fig. (10). Effects of indoxacarb (DPX-MP062) on different types of ACh-induced currents in rat cortical neurons. A. ACh (1mM) was applied for 0.5 sec at a 1 min interval. DPX-MP062 (10 μM) was applied to the bath after the current amplitude reached a steady level. The peak amplitude of rapidly decaying, ACh-evoked current, was irreversibly inhibited by DPX-MP062. B. The peak amplitude of slowly decaying 10 μM ACh-evoked current, was reversibly potentiated by DPX-MP062 at 10 μM. Holding potential was –70 mV. From Zhao *et al*. [77].

potentiated currents only in α1β2γ2S and α6β2γ2S HEK cells, indicating that dieldrin's potentiation of GABA currents requires γ2S subunit [41].

γ-HCH (lindane) mimics dieldrin in its $GABA_A$ receptor blocking action, and the effects of HCH isomers on the receptor reflect their behavioral effects on animals. It is well known that the γ-HCH is a strong stimulant, the α-HCH is a weak stimulant, the β-HCH is a weak depressant, and the δ-HCH is a strong depressant. γ-HCH had a weak potentiating and a strong inhibitory action on the $GABA_A$ receptor of rat DRG neurons, α-HCH and β-HCH had little or no effect on GABA-induced currents, and δ-HCH had a strong potentiating and inhibitory action [43].

The mechanisms of insect resistance to insecticides may be divided into two large categories, 1) Metabolic resistance in which metabolic enzymes are upgraded or mutated as the basis for resistance to insecticides. 2) Target site resistance in which the target sites, most of which are located in the nervous system, becomes less sensitive to insecticides. The mechanism of dieldrin resistance has been studied for a long period of time.

The first direct demonstration of target site resistance mechanism for dieldrin and lindane was reported by Yamasaki and Narahashi [44]. While the low nerve sensitivity to dieldrin was also reported more recently in resistant strains of *Drosophila* [45, 46], it was not until 1993 that a mutation in the *Drosophila* GABA receptor was found to be responsible for dieldrin resistance [47]. The cyclodiene resistance gene *Rdl* (resistance to dieldrin) was cloned from *Drosophila* resistant to cyclodienes and picrotoxinin. Single amino acid replacement from alanine to serine (A302S) occurs with the second membrane spanning domain which is the region to line the chloride channel pore. Subsequently, similar mutations of amino acids were discovered in several other insect species resistant to dieldrin, a single mutation A302S also occurred in *Aedes aegypti, Periplaneta americana, Musca domestica*, and *Tribolium castaneum*, and double mutations A302G and A302S were found in *Drosophila simulans* [48, 49, 50, 51, 52].

FIPRONIL

Fipronil is a phenylpyrazole compound and was developed as a useful insecticide in mid-1990s. One of the excellent aspects of fipronil as an insecticide is that it is effective against insects such as Colorado potato beetle and some cotton pests that have become resistant to the existing insecticides. Fipronil is much more toxic to insects than to mammals, and this is another advantage as an insecticide.

Fipronil has been found to block insect GABA receptor (*Rdl*). Wild-type *Rdl* of *Drosophila* was suppressed by t-butylbicyclophosphorothionate (TBPS), 4-n-propyl-4'-ethynylbicycloorthobenzoate (EBOB), picrotoxinin, and fipronil [53, 54]. Dieldrin-resistant *Drosophila melanogaster* and *D. simulans* were also resistant to fipronil

but to a much lesser extent, and the [^{3}H]EBOB binding to these resistant strains was less inhibited by fipronil as compared to the susceptible strains [52]. Mutant *Drosophila Rdl* (A302S) expressed in *Xenopus* oocytes was also less sensitive to fipronil than wild-type receptors [55].

We have performed detailed studies of fipronil modulation of $GABA_A$ receptors of rat DRG neurons [56]. Fipronil suppressed GABA-induced currents slowly and reversibly before channel opening as demonstrated by the experiments in which 1 μM fipronil suppressed the currents

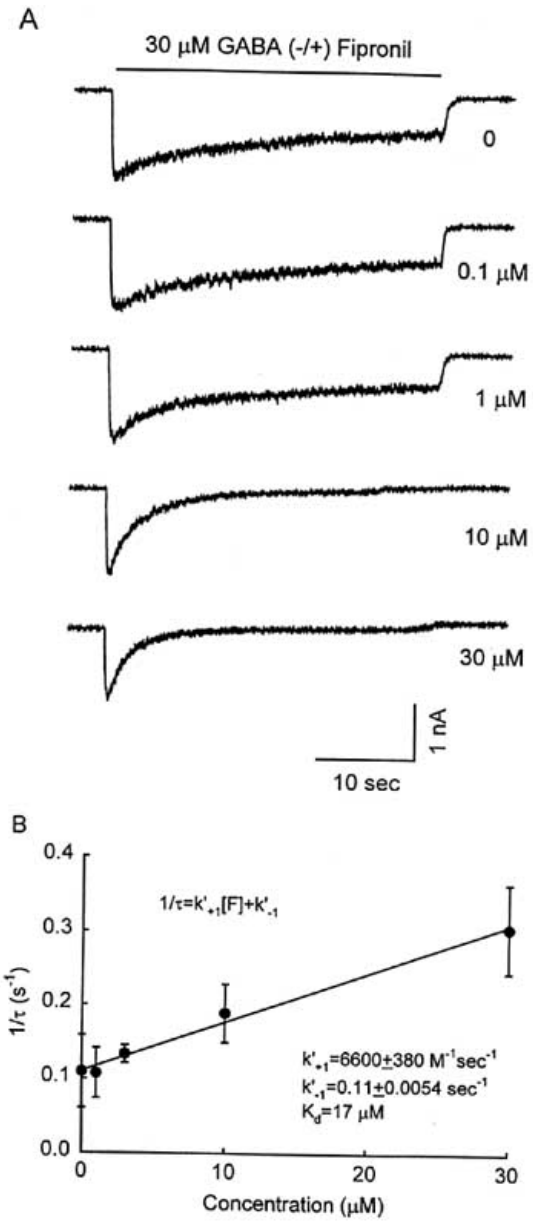

Fig. (11). Effects of fipronil at various concentrations on the decay phase of currents induced by long applications of GABA. A. GABA 30 μM was applied for 30 s to induce currents, and was coapplied with various concentrations of fipronil. The decay phase of currents was fitted with a single exponential function to obtain time constants. B. Reciprocal of the time constants ($1/\tau$) is plotted as function of fipronil concentration to calculate the association and dissociation rate constants for fipronil interaction with the activated receptor. Data points are best fitted to the solid line according to the equation $1/\tau = k'_{+1}[F] + k'_{-1}$. The correlation coefficient of 0.995 gives a significant level of $P < 0.05$. $k'_{+1} = 6600 \pm 380\ M^{-1}s^{-1}$; $k'_{-1} = 0.11 \pm 0.0054\ s^{-1}$ (mean ±S.D., n=3-7). From Ikeda *et al.* [*56*].

without GABA-induced activation. From the time course of fipronil acceleration of desensitization of GABA-induced currents, the rates of fipronil association with and dissociation from the GABA bound receptors were estimated to be 6600 M^{-1} sec^{-1} and 0.11 sec^{-1}, respectively (Fig. **11**). From the measurement of time course of GABA current block caused by preapplication of fipronil, the rates of fipronil association with and dissociation from the GABA-unbound receptors were estimated to be 673 $M^{-1}sec^{-1}$ and 0.018 sec^{-1}, respectively (Fig. **12**). Thus fipronil blocks GABA receptors at their resting state, and the GABA-induced activation of the receptors increases the association and dissociation rates of fipronil. Competitive experiments indicated that fipronil bound to the $GABA_A$ receptor site different from the picrotoxin binding site. Thus fipronil is different from dieldrin which binds to the picrotoxin binding site.

IMIDACLOPRID

A number of factors must be taken into consideration for developing new insecticides and for using the existing insecticides, mammalian toxicity and insecticide resistance

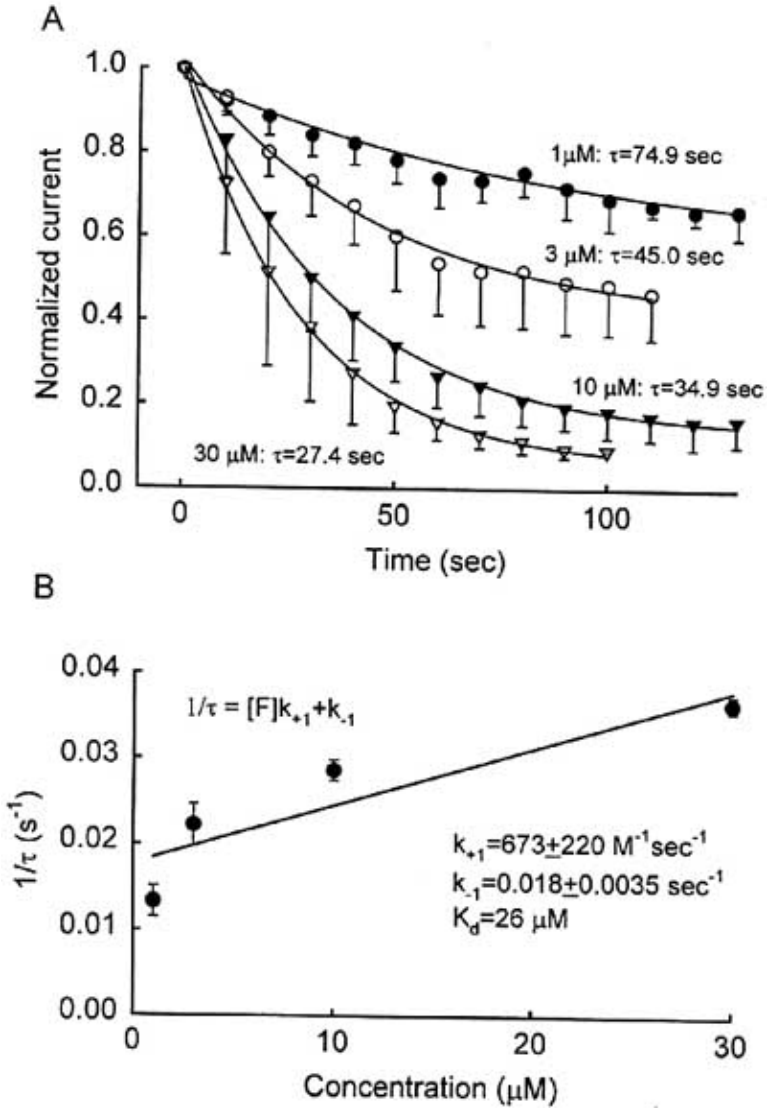

Fig. (12). Kinetics of fipronil suppression of the closed receptor. Decreases in currents by various concentrations of fipronil during bath application are plotted as a function of incubation period. GABA pulses (300 μM, 10 ms) were applied every 10 s to monitor the change in current amplitude. A. Time course of decrease in peak current amplitude by bath application of fipronil at 1, 3, 10, and 30 μM for 100 to 150 s. Changes in current amplitude relative to the first current amplitude are plotted as a function of incubation period for each fipronil concentration, and the plots are fitted with a single exponential function. B. Reciprocal of the time constants obtained from a single exponential fitting is plotted as a function of fipronil concentration. The solid line represents the relation $1/\tau = k_{+1}[F] + k_{-1}$. The fit was less satisfactory than would be expected as the correlation coefficient was 0.89 with a degree of freedom of 2. the association and dissociation rates for fipronil interaction with the closed receptor are calculated to be $k_{+1} = 673 \pm 220$ $M^{-1}s^{-1}$ and $k_{-1} = 0.018 \pm 0.00\mathit{35}$ s^{-1}, respectively. Ikeda *et al*. [*56*].

being among the most important. In order to cope with the situation, a new group of chemicals has been developed into commercial insecticides during the past 10 years or so, i.e. nitromethylene or chloronicotinyl insecticides. Imidacloprid exhibits a unique mechanism of action on nACh receptors. It binds to insect nACh receptors with a high affinity [57, 58, 59, 60, 61, 62, 63], and depolarizes nerve membrane and causes spontaneous discharges in cockroaches [63, 64, 65, 66]. Mammalian end-plate nACh receptors are less sensitive than those of locust neurons [67].

The effects of imidacloprid on nACh receptors were analyzed in detail using PC12 cells [38, 68]. Imidacloprid itself generated whole-cell currents with a low potency and efficacy. The imidacloprid-induced current was approximately 10% of the carbachol-induced current. As expected from a partial agonist, imidacloprid also suppressed carbachol-induced currents with a low potency. Single-channel analyses have disclosed an interesting feature of imidacloprid action. Application of ACh induced primarily main conductance (25.4 pS) currents and some low conductance (9.8 pS) currents, while imidacloprid primarily generated the low conductance currents (Fig. **13**). Co-application of ACh and imidacloprid generated both types of currents (Fig. **13**). The mean open time and burst duration of the main conductance current were decreased by the co-application of ACh and imidacloprid. These changes in single-channel behavior by imidacloprid can account for the changes in the whole-cell ACh receptor currents. Imidacloprid has both agonist and antagonist effects on the mammalian neuronal nicotinic ACh receptors.

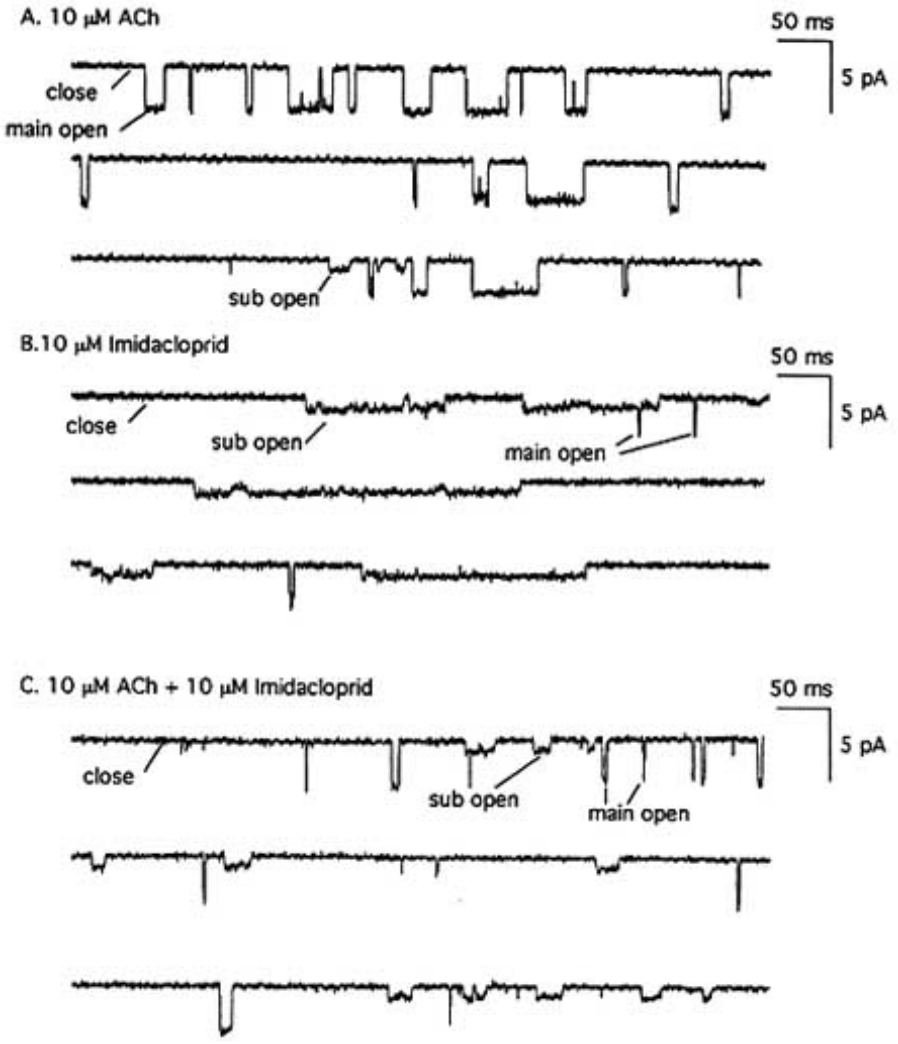

Fig. (13). Single-channel currents activated by 10 μM ACh, 10 μM imidacloprid and co-application of 10 μM ACh and 10 μM imidacloprid to cell-attached membrane patches of a PC12 cell clamped at a membrane potential 40 mV more positive than the resting potential. A. Currents induced by 10 μM ACh occurred during brief isolated openings or longer openings interrupted by a few short closures or gaps. Main conductance state currents were observed more frequently than subconductance state currents. B. Currents induced by 10 μM imidacloprid. Subconductance state currents were more frequently observed than main conductance state currents. C. Co-application of 10 μM ACh and 10 μM imidacloprid. Main conductance and subconductance state currents were induced, and channel openings were shortened. From Nagata *et al*. [38].

ACh receptor subunit specificity for imidacloprid action has recently been studied [69]. Imidacloprid was a partial agonist in generating currents in the recombinant chicken α4β2 subunit combination and in the hybrid receptor of *Drosophila* subunit (SAD) with the chicken β2 subunit, both expressed in *Xenopus* oocytes. However, imidacloprid was more potent on the SAD β2 subunit combination than on the α4β2 combination. Furthermore, imidacloprid was a weak potentiator of ACh-induced currents in the α4β2 receptors, whereas it was a weak antagonist of ACh-induced currents in the SAD β2 receptors.

Binding experiments indicated that imidacloprid, acetamiprid and nitenpyram had low to moderate potency at the α3 and α4β2 ACh receptors and were essentially inactive at α1 and α7 ACh receptors [70]. Insect ACh receptor subunits were also studied for imidacloprid action [71]. In the peach-potato aphid *Myzus persicae*, five α subunit cDNAs have been cloned, Mpα1, Mpα2, Mpα3, Mpα4, and Mpα5. Although the insect α subunits evolved in parallel with the vertebrate neuronal nACh receptors, the insect non-α subunits are different from vertebrate neuronal β and muscle non- α subunits. The aphid nACh receptor α subunit cDNAs were co-expressed with the rat β2 subunit in *Drosophila* S2 cells. The affinity of recombinant nACh receptors for [^{3}H]imidacloprid was α subtype dependent, being high in Mpα2 and Mpα3 subunits, but low in Mpα1 subunit.

SPINOSAD

Spinosad is a mixture of two active components, spinosyn A (85%) and spinosyn D (15%), which are produced by the soil actinomycete *Saccharopolyspora spinosa* [72, 73]. Spinosad is endowed with excellent properties for Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM). These include high potency on target insects, safety to beneficial insects, low toxicity in mammals and non-target animals, and lack of known cross-resistance. It was introduced for the control of lepidopteran insects in cotton, in 1997.

Spinosad is a potent neurotoxin. Spinosyn A, the major active component, caused involuntary muscle contractions and tremors by widespread excitation of neurons in the cockroach CNS [74]. The effective concentrations of spinosyn A to stimulate insect nervous systems are as follows, tobacco hornworm, EC_{50} of 5 nM; housefly, the minimal effective concentration of 3 nM and near-maximal stimulation at 10 nM; and cockroach, the minimal effective concentration of 10 nM and EC_{50} of 32 nM [74]. The major target site of spinosad is nACh receptors (Salgado, personal communication), but its effect on GABA-gated chloride channel is also suggested [73].

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