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Differential actions of insecticides on target sites: basis for selective toxicity

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Abstract

Whereas the selective toxicity of insecticides between insects and mammals has a long history of studies, it is now becoming abundantly clear that, in many cases, the differential action of insecticides on insects and mammalian target receptor sites is an important factor. In this paper, we first introduce the mechanism of action and the selective toxicity of pyrethroids as a prototype of study. Then, a more detailed account is given for fipronil, based primarily on our recent studies. Pyrethroids keep the sodium channels open for a prolonged period of time, causing elevation of the depolarizing after-potential. Once the after-potential reaches the threshold for excitation, repetitive after-discharges are produced, resulting in hyperexcitation of intoxicated animals. Only about 1% of sodium channels needs to be modified to produce hyperexcitation, indicating a high degree of toxicity amplification from sodium channels to animals. Pyrethroids were > 1000-fold more potent on cockroach sodium channels than rat sodium channels, and this forms the most significant factor to explain the selective toxicity of pyrethroids in insects over mammals. Fipronil, a phenylpyrazole, is known to act on the γ -aminobutyric acid receptor to block the chloride channel. It is effective against certain species of insects that have become resistant to most insecticides, including those acting on the γ -aminobutyric acid receptor, and is much more toxic to insects than to mammals. Recently, fipronil has been found to block glutamate-activated chloride channels in cockroach neurons in a potent manner. Since mammals are devoid of this type of chloride channel, fipronil block of the glutamate-activated chloride channel is deemed responsible, at least partially, for the higher selective toxicity to insects over mammals and for the lack of cross-resistance.

Keywords

fipronil; GABA receptor; glutamate-activated chloride channel; pyrethroid; selective toxicity; sodium channel

Introduction

Most insecticides are much more toxic to insects than to mammals. In many cases, the mechanism of this selective toxicity lies in differential actions on the target receptors/channels. However, there are some exceptions, for example, the selective toxicity of malathion is due primarily to the difference in detoxifying enzymes between mammals and insects.

With the exception of organophosphates and carbamate insecticides which inhibit cholinesterases, most other insecticides act directly on neuronal receptors and/or ion channels, thereby causing symptoms of poisoning. In this paper, we introduce the mechanism of action

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including the selective toxicity of pyrethroids, one of the most well-established cases among various insecticides. The major part of this paper deals with the selective toxicity of fipronil, for which a unique target site has been disclosed as relating to the selective toxicity.

Pyrethroids

Early studies showed that pyrethroids and dichlorodiphenyltrichloroethane (DDT) exerted a similar action on the nervous system causing hyperexcitation as represented by repetitive discharges.^{1,2} Extensive voltage clamp experiments using giant axons of lobster, crayfish, and squid have clearly demonstrated that the sodium channel current is greatly prolonged leading to elevation of the depolarizing after-potential which, in turn, generates repetitive action potentials once it reaches the threshold for excitation.²⁻⁵ Since the whole-cell sodium channel current under voltage clamp conditions is a result of the opening and closing of many sodium channels contained in the preparation, patch clamp recording of single sodium channels is required to elucidate the mechanism of action of pyrethroids. Single sodium channel currents have been shown to be kept open for a prolonged period of time in pyrethroid-intoxicated cells.⁶ We have also developed a method by which the percentage of sodium channels that are modified by pyrethroids from normal short opening to prolonged opening can be measured.⁷ In rat cerebellar Purkinje neurons, pyrethroid modification of only 0.6% of sodium channel population was required to produce repetitive discharges which, in turn, would cause hyperactivity in animals (Figure 1).⁸ Thus, pyrethroid toxicity is greatly amplified from the sodium channel level to animal level.

The selective toxicity of pyrethroids in insects over mammals presents an interesting case. When the *in vitro* potency was compared, allethrin was much more potent on cockroach sodium channels than rat tetrodotoxin-sensitive sodium channels (Figure 2). Allethrin at 100 nM modified 14% of sodium channels in cockroach neurons, whereas allethrin at 100 μ M modified only 4% of sodium channels in rat neurons. Thus, the difference in allethrin sensitivity is little over 1000-fold. Another important factor involved in pyrethroid action is temperature effect. Pyrethroids as well as DDT are much more potent as insecticides at low temperatures than at high temperatures, and this negative temperature dependence has been shown to be due to the negative temperature dependence of sodium channel sensitivity to pyrethroids and DDT.^{1,2,4,5,9-11} The increase in sodium channel sensitivity to pyrethroids when the temperature is lowered has been demonstrated to be due to increases in the time constant and the amount of charge during the tail sodium current.⁸ This temperature dependence becomes an important factor as there is approximately a 10°C difference in body temperature between mammals and insects. The rate of detoxification of pyrethroids should also be considered. These differences between mammals and insects are summarized in Table 1, which gives an overall difference in pyrethroid sensitivity of 15000-fold. The difference in lethal dose 50 (LD₅₀) between mammals and insects, which amounts to several thousand fold or more, depends on the kind of pyrethroids, the species of insects and mammals, and the method of pyrethroid administration.

Fipronil

Fipronil is a new insecticide developed in the mid-1990s. It not only exhibits a high selective toxicity in insects over mammals, but is also effective against some of the dieldrin-resistant strains of insects. Fipronil is more potent in houseflies than in mice as a toxicant and in competing with 4'-ethynyl-4-*n*-[2,3-³H₂]propylbicycloorthobenzoate ([³H]EBOB) binding.¹² The LD₅₀ values of fipronil are 0.13 and 41 mg/kg for housefly and mouse, respectively, and the receptor inhibitory concentration 50 (IC₅₀) values are 6.3 and 1010 nM for housefly and mouse, respectively. Fipronil blocks insect γ -aminobutyric acid (GABA) receptors, which are different in molecular structure and pharmacological profiles from vertebrate GABA_A

receptors. Insect GABA receptors are not blocked by bicuculline,¹³⁻¹⁸ and are not potentiated by benzodiazepines and barbiturates.¹⁸

Fipronil block of GABA receptors

In rat dorsal root ganglion (DRG) neurons in primary culture, fipronil suppressed the GABA-induced whole-cell currents reversibly in both closed and activated states.¹⁹ The IC_{50} value for fipronil block of the $GABA_A$ receptor was estimated to be $1.669 \pm 0.14 \mu M$ for the closed receptor and $1.61 \pm 0.14 \mu M$ for the activated receptor. Both the association and dissociation rate constants of fipronil for the activated $GABA_A$ receptor are approximately ten times as large as those for the closed receptor. Fipronil and picrotoxinin, a $GABA_A$ receptor blocker, did not compete for the same binding site to block the receptor. It is concluded that although fipronil can bind to the $GABA_A$ receptor without activation, receptor activation facilitates fipronil binding to and unbinding from the channel.

Fipronil interactions with $GABA_A$ receptor single channels were also analysed using rat DRG neurons.²⁰ There were three components of open time of single channels. After application of $10 \mu M$ fipronil, the proportion of the fastest open time component increased slightly and that of the slowest component decreased slightly. Thus, the mean open time was decreased from 11.4 to 7.8 ms by fipronil. The closed time was distributed in four components. Fipronil $10 \mu M$ prolonged the slowest time constant resulting in a prolongation of the mean closed time from 29.7 to 52.8 ms. Therefore, the frequency of channel openings was reduced. In conclusion, the fipronil suppression of GABA-induced whole-cell currents is caused, in part, by decreases in the channel open time and in the frequency of channel openings.

We then studied the mechanism of the highly selective toxicity of fipronil in insects over mammals. One possible mechanism of the selective toxicity is that insect GABA receptors are more sensitive than mammalian $GABA_A$ receptors to fipronil. We examined the differential action of fipronil on GABA-gated chloride channels in insects and compared them with the data on mammalian $GABA_A$ receptors.²¹ Neurons were acutely dissociated from the American cockroach thoracic ganglia, and whole-cell currents evoked by GABA were recorded. Experiments showed that GABA-evoked currents were carried by chloride ions, and blocked by picrotoxinin, but not by bicuculline, which is known to block mammalian $GABA_A$ receptors. Fipronil inhibited insect GABA currents which an IC_{50} value of 28 nM, and was 59 times more potent on cockroach GABA receptors than on rat $GABA_A$ receptors. It was concluded that the higher toxicity of fipronil in insects than in mammals is due partially to the higher sensitivity of GABA receptors.

Fipronil block of glutamate-activated chloride channels in insects

Whereas glutamate acts as an excitatory synaptic transmitter in mammals, it plays a role as an excitatory as well as an inhibitory transmitter in insects. Excitation at the neuromuscular junctions of insects is transmitted by glutamate receptors which become cation permeable when activated by glutamate. Inhibition at the central synapses of insects is transmitted by both glutamate and GABA receptors. Both of these inhibitory post-synaptic receptors become permeable to chloride ions when activated by glutamate or GABA. The glutamate-activated chloride channels (GluCl_s), which are found in *C. elegans* as well as insects, have been cloned and some physiological and pharmacological characteristics have been studied.²²⁻²⁴ Due to their unique and exclusive presence in invertebrates, GluCl_s are deemed a good target site of insecticides to provide a high degree of selective toxicity in insects over mammals. We will have to first characterize the electrophysiological and pharmacological properties of GluCl_s before studying the effect of fipronil.

Glutamate evoked a desensitizing current in neurons isolated from cockroach thoracic ganglia with an effective concentration 50 (EC_{50}) of 36.8 μ M.²⁵ The similarity between the reversal potential and the calculated chloride equilibrium potential indicated that glutamate-induced currents were carried by chloride ions. Fipronil suppressed the glutamate-induced peak currents in a dose-dependent manner with an IC_{50} of 0.73 μ M. Picrotoxinin at 100 μ M slightly suppressed glutamate-induced currents to 87.8% of the control. DL-2-Amino-5-phosphonopentanoic acid (APV) and 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), mammalian glutamate receptor antagonists, were without effect on glutamate-induced Cl^- currents.

Further analyses indicated that two GluCl_s were distinguished based on pharmacology and current kinetics (Figure 3).²⁶ Both types of GluCl_s were sensitive to glutamate and ibotenic acid. The non-desensitizing GluCl subtype was elicited by glutamate with an EC_{50} of 115.8 μ M and was also sensitive to the agonist ibotenic acid with an EC_{50} of 42 μ M. The desensitizing and non-desensitizing currents were carried by chloride ions, and occurred either separately or in combination in individual neurons. The GluCl_s were also found to coexist with and function independently of the GABA-activated chloride channels. The desensitizing and non-desensitizing GluCl_s exhibited different sensitivities to picrotoxinin. The desensitizing GluCl_s were blocked only 8% by 100 μ M picrotoxinin, whereas the non-desensitizing GluCl_s were potently blocked by picrotoxinin with an IC_{50} of 4.1 μ M. Fipronil at 1 μ M inhibited the desensitizing currents by 56% and the non-desensitizing currents by 98%. It is concluded that the two types of GluCl_s found in cockroach neurons exhibit significantly different electrophysiological and pharmacological characteristics.

To better elucidate the mechanism of its selective toxicity between insects and mammals and activity against dieldrin-resistant insects, we further studied the fipronil action on GluCl_s.²⁷ Fipronil inhibited the desensitizing and non-desensitizing GluCl_s with IC_{50} values of 801 and 10 nM, respectively (Figure 4). Kinetic analysis revealed that fipronil block required channel opening. Recovery of the desensitizing current from fipronil block also required channel opening, whereas recovery of non-desensitizing current from block was also independent of channel opening. The high potency of fipronil against the non-desensitizing current was due to a slow unblocking rate constant. In addition, when the nondesensitizing GluCl_s were occupied by picrotoxinin, the receptors became less sensitive to fipronil block. This is in sharp contrast with the results of rat DRG GABA_A receptor in which fipronil and picrotoxinin bind to two independent sites. It is concluded that GluCl_s are a critical target for fipronil, especially for the selective toxicity between mammals and insects, and that fipronil block of GluCl_s may play a role in the lack of the cross-resistance with dieldrin.

Acknowledgements

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Figure 1. Concentration-dependent modification of sodium channels by tetramethrin. The percentage of sodium channel modification was calculated from whole-cell tail current and peak current in rat Purkinje neurons (Song and Narahashi).⁸

Figure 2.

Allethrin modulates tetrodotoxin-sensitive sodium channels > 1000 times more potently in cockroach neurons than in rat dorsal roof ganglion neurons. Rat data from Ginsburg and Narahashi.²⁸ Cockroach data from Narahashi.²⁹

Figure 3.

Two components of glutamate-activated chloride currents in cockroach neurons.

Figure 4.
Fipronil blocks slow non-desensitizing glutamate-induced current more potently than fast desensitizing current.

Table 1

Mechanism of selective toxicity of pyrethroids

Selectivity factors	Mammals	Insects	Estimated differences
Potency on nerve Na ⁺ channels			
Due to temperature dependence	Low (37°C)	High (25°C)	5
Due to intrinsic sensitivity	Low	High	1000
Detoxication rate	High	Low	3
Overall difference = 15 000			