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Why Insecticides are More Toxic to Insects than People: The Unique Toxicology of Insects*

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The unique toxicology of insects provides the safety mechanisms for the major insecticides. The selectivity of insecticidal nerve poisons is attributable to structural differences in binding subsites (acetylcholinesterase and nicotinic receptor) or receptor subunit interfaces (γ-aminobutyric acid receptor) or transmembrane regions (voltage-sensitive sodium channel) supplemented by metabolic activation and detoxification. Slow action limits the use of the remarkably selective insecticides acting at juvenile hormone and ecdysone receptors and inhibiting chitin biosynthesis. The δ-endotoxin of Bacillus thuringiensis induces midgut lysis and death in insects by a mechanism not applicable in mammals. Future pest management will rely on continuing advances in insect toxicology.

Keywords: acetylcholinesterase, Bt endotoxin, chitin synthesis, γ-aminobutyric acid receptor, nicotinic acetylcholine receptor, voltage-sensitive sodium channel.

1. INTRODUCTION

Insects are unique in their diversity (a million different kinds, >70% of all species), adaptation for small size, chitinous exoskeleton, high birth rate, short generation time and complete metamorphosis. There is a continuing need for new, safe, effective and economical insecticides for crop protection and public health. Almost everyone is exposed to insecticides and depends on them being safe. Lead compounds as natural products or from screening synthetics are optimized by chemists for potency and selectivity while toxicologists study their mode of action and safety. There are three general types of selective toxicity mechanisms. First, similar targets are involved in insects and mammals for nerve and bioenergetic toxins, but significant differences in enzyme or receptor structures confer selectivity. Second, there are unique targets in insects for chitin synthesis, metamorphosis hormone receptors and midgut membrane receptors. Third, there can be selective bioactivation by insects and detoxification by mammals. Insect systems are relatively simple compared to the highly specialized organs of mammals. Insect hemolymph bathes the entire hemocoel, including the nervous system, and tracheoles lead directly from the outside into the nerves. Why are the current insecticides more toxic to insects than people? Are there lessons for the future? The answers lie in the unique toxicology of insects.

2. NERVOUS SYSTEM

2.1. General Aspects

Neurotoxicants are about 90% of the synthetic insecticide market for several reasons. They act quickly to stop crop damage and disease transmission. There are many ultrasensitive sites and even a small disruption may ultimately prove to be lethal. In insects a lipoidal sheath protects the nerve from ionized toxicants but not from lipophilic insecticides. The nerve is poor in detoxification mechanisms providing prolonged toxicant effects. There are four principal nerve targets for insecticides, i.e. acetylcholinesterase (AChE), the nicotinic acetylcholine (ACh) receptor (nAChR), the γ-aminobutyric acid (GABA)-gated chloride channel and the voltage-sensitive sodium channel with a market share in 1999 of 52, 12, 18 and 18%, respectively.

2.2. Acetylcholinesterase

Discovery and Development. The organophosphates (OPs) and methylcarbamates (MCs) started from fluorophosphorus compounds and physostigmine which are much more toxic to mammals than insects. By evaluating tens of thousands of analogs it was possible to discover acceptable levels of selective toxicity. Ten to 20 major OPs and MCs account for about half of the pesticide market.
ACH-MEDIATED EXCITATORY SYNAPSE

ACHE ACTIVE SITE

insect (shown)
3-CH₃ increases affinity
subsite open

mammal
3-CH₃ reduces affinity
subsite blocked

Mode of Action. ACh is the principal excitatory neurotransmitter of the central and peripheral nervous systems of mammals but only the central nervous system of insects. AChE is concentrated in synaptic regions of cholinergic nerve endings and is responsible for the rapid hydrolysis of ACh (Fig. 1). AChE inhibitors cause ACh to accumulate resulting in excessive stimulation of cholinergic receptors. Insect and mammalian AChE have an active center gorge for ACh hydrolysis consisting at its base of a catalytic triad (His, Ser, Glu), choline subsite (Trp), acyl pocket and a peripheral site at the rim. OPs phosphorylate at serine for essentially irreversible inhibition (in the absence of a reactivator such as pralidoxime which acts as an antidote in mammals) whereas MCs act as both competitive AChE inhibitors and methylcarbamoylating agents. Resistance in some cases is associated with multiple mutations in the active site, conferring cross resistance to many OPs and MCs.

Unique Toxicology. There is considerable inhibitor specificity between the AChE of insects and mammals contributing to selective toxicity. The classical example is that of methyl parathion and the much more selective 3-methyl analog fenitrothion where addition of the methyl substituent increases the affinity for insect and decreases for mammalian AChE (Fig. 1). Reactivation rates for inhibited AChE may also vary for insects and mammals. Mammals have a second related enzyme, butyrylcholinesterase, abundant in blood plasma, which reacts with OP and MC toxicants and provides a protection not available to insects.

2.3. Nicotinic Acetylcholine Receptor

Discovery and Development. Nicotine, the first botanical insecticide, is more toxic to mammals than to most insects and systematic changes in its structure did not greatly improve its potency or safety. Neonicotinoids were introduced in 1990 following screening new types of structures, optimization of a lead compound to give nithiazine, then further optimization for potency and stability resulting in imidacloprid, which is more toxic to insects than to mammals at the organism, nerve and receptor level. Nicotine and imidacloprid have some structural similarities and act at the same type receptor but in a different way. Neonicotinoids control most sucking insects and some lepidopterous and coleopterous pests.

Mode of Action. ACh is the endogenous agonist and excitatory neurotransmitter of the cholinergic system (Fig. 2). ACh released from the presynaptic membrane interacts with the binding site located at the extracellular domain of the nAChR/ion channel complex (Fig. 1). A conformational change of the receptor molecule then leads to channel opening, influx of extracellular Na⁺ and efflux of intracellular K⁺. The nAChR is responsible for rapid neurotransmission in the insect central nervous system. The neonicotinoids and related compounds act as agonists on multiple nAChR subtypes in both mammals and insects with differential selectivity conferred by only minor structural changes. The binding in mammalian neuronal receptors is at the a4/β2 interface and a similar site is probably also involved in insects. Resistance to neonicotinoids has developed in white flies and Colorado potato beetle apparently due to both cases to metabolic rather than target site changes.

Unique Toxicology of Insects. Imidacloprid is not protonated, but the electronegative (δ⁻) nitroimine tip may bind to a lysine or arginine residue in a subsite of the insect nAChR. Nicotine in contrast is protonated at physiological pH and undergoes cation-π interaction with Trp at an nAChR subsite in mammals. Thus, binding subsite
2.4. GABA-Gated Chloride Channel

Discovery and Development. The polychlorocycloalkane (PCCA) insecticides originated from chlorination of benzene, camphene and cyclopentadiene, to obtain lindane and its isomers, toxaphene, and the cycloclaines (such as α-endosulfan), respectively, each with 6–8 chlorines/molecule. They are very toxic to most insects and to fish and vary in mammalian toxicity from moderate to high. They were joined in 1993 by fipronil of greatly different structure (a 1-phenylpyrazole with 8 halogens) but of lower acute toxicity to mammals, yet similar or identical action.

Mode of Action. The PCCA insecticides and fipronil act on the GABA-gated chloride channel to block GABA-induced signals and chloride flux on binding to a non-competitive blocker or insecticide binding site (Fig. 3). GABA is the principal inhibitory neurotransmitter of insects and mammals. Several billion pounds of PCCAs were used in crop protection before their mode of action was established. Cross resistance among the various classes of PCCAs was the first indication of a common mode of action. More pest species have been selected for resistance to insecticides acting at the GABA-gated chloride channel than any other single mechanism. Cross resistance extends to fipronil but not to avermectin analogs binding at different
sites. PCCA resistance in most cases is due to a single point mutation in their GABA receptor, first established in Drosophila (Ala-277 replaced by Ser in the ion channel lining of the transmembrane 2 region).

Unique Toxicology of Insects. Target-site selectivity is evident for the major GABAergic insecticides with higher potency on the native house fly or Drosophila receptor than the less sensitive native human brain receptor. This selectivity is not present for the human expressed β3 homeric receptor (Fig. 3) which is just like the native house fly and Drosophila receptors in sensitivity and general amino acid sequence. The insect versus human target-site specificity is much greater for lindane and fipronil than for α-endosulfan. These selectivity relationships are attributable to the α subunit in mammals, but not insects, modulating the binding site at the α/β subunit interface to reduce its overall sensitivity to some chloride channel blockers.

2.5. Voltage-Sensitive Sodium Channel
Discovery and Development. The pyrethrins (from pyrethrum flowers), pyrethroids and DDT are very different in structure and origin, yet act in the same way. They have a long history of safe use relative to human poisoning. Some highly insecticidal pyrethroids have exceptionally low toxicity to mammals (e.g. bioresmethrin) and fish (e.g. etofenprox and silafluoufen).

Mode of Action. Neurotoxins acting at insect voltage-sensitive sodium channel recognition sites block sodium transport, enhance sodium channel inactivation or prolong the time course of sodium channel activation. The pyrethrins and DDT prolong the course of the sodium current during depolarization and induce a residual slow-acting current (“tail current”). Some Type I-acting pyrethroids (such as cismethrin) bind to resting or inactivated channels, shifting the voltage dependence of activation to more negative potentials and causing a slowly-activating sodium-current responsible for repetitive activity. Other pyrethroids, such as deltamethrin and related compounds that exhibit Type II action, induce profound use-dependent modification of sodium currents, implying preferential binding to activated sodium channel states. Type II action recruits increasing numbers of sodium channels into permanent open states which results in use-dependent depolarization, inactivation of unmodified channels and block of conduction. This is the most sensitive target in insects to any insecticide. The toxicity is greatly amplified from channel modulation to hyperactive symptoms in insects. The first dramatic case of selection for insecticide resistance was when DDT lost its effectiveness for controlling house flies. It was surprising to observe cross resistance of DDT and the pyrethrins which was confirmed by electrophysiological studies. This was further indication of a common mode of action. The insect sodium channel protein consists of four homologous domains each with six transmembrane segments. Resistance to pyrethrins and DDT in Musca is conferred by the L1014F mutant alone (kdr) or with M918T (super-kdr) in domain II of the α subunit. Mutations at these and other sites in the channel proteins confer resistance and cross-resistance that can greatly reduce the effectiveness of all pyrethroids. The lipid amide and oxadiazine insecticides act at other sodium channel sites without cross resistance, but of these only the oxadiazine indoxacarb has proven effective and safe in practice.

Unique Toxicology of Insects. The higher sensitivity of insects relative to mammals is proposed to be due to several features. First, the insect target is intrinsically more sensitive than that of mammals. Second, the target has a negative temperature coefficient, i.e. the insecticides are more effective at the ambient temperature of insects (e.g. 15–20°C) than people (37.5°C). Other selectivity factors are lipophilicity for selective pickup by insects and enzymatic detoxification. The unique toxicology of these voltage-dependent sodium channels is a major factor in pyrethroid and DDT potency on insects and safety for humans.

2.6. Other Nerve Targets
The neuromuscular junction in insects is mediated by glutamate and in mammals by ACh, prompting a continuing effort to use this unique difference to develop selective glutamate agonists or noncompetitive blockers of glutamate receptors as practical insecticides. In addition, the glutamate-gated chloride channel is also of interest as one of the targets for avermectins. The octopamine receptor is unique to insects and potent agonists (0.6% of the insecticide market) are effective in control (e.g. amitraz), but further development in this area requires the potency of chloridimeform without its toxicological problems.

3. GROWTH, METAMORPHOSIS AND BIOENERGETICS

3.1. General Aspects
Chitin is unique to insects, fungi and some yeasts. The chitinous exoskeleton requires a periodic molt (digesting and resynthesizing chitin) to develop and expand. Metamorphosis is synchronized by two opposing hormonal systems which are absolutely unique to insects compared with people and are therefore ideal targets for selective control chemicals. However, these hormones act slowly in affecting growth and metamorphosis. Disruption of their action is also slow in stopping damage or killing the pest. Therefore, despite many ideal properties, the inhibitors of chitin synthesis and hormone analogs or agonists play only a small role in pest control.

3.2. Chitin Synthesis and Hydrolysis
Chitin is made by chitin synthetase-catalyzed polymerization of uridine diphospho-N-acetylglucosamine. Chitin biosynthesis in insects and fungi can be prevented by direct inhibitors of chitin synthetase (none of which are commercial insecticides) and in insects (but not fungi) by
compounds (benzoylphenylureas) that block an unknown step in the polymerization process. The benzoylphenylureas
(e.g. diflubenzuron and lufenuron) constitute 3% of the insecticide market. Analogous targets are not present in mammals, providing a high degree of safety. Chitin is degraded by chitinase which is insecticidal when expressed in plants.

3.3. Juvenile Hormone and Ecdysone Receptors

Juvenile hormone (JH) induces a multitude of physiologi-
cal effects in insect larvae, suggesting action as a lipid sig-
naling system rather than through a single JH receptor. JH
synthesis in the corpus allatum can be blocked by the
naturally-occurring precocenes and fluoromevalonate but not
practically on important pests. Potent inhibitors of JH ester-
ase and JH epoxide hydrolase do not give practical pest
control. Synthetic JH analogs of greatly varied types serve
as agonists at very low concentrations in vitro and doses in
vivo. Some of these compounds are used practically but on
a small scale in niche markets. Ecdysone binding to its
receptor leads to molting with digestion of the exoskeleton
and organogenesis with development of adult characteristics.
Ecdysone is made from dietary sterols (e.g. cholesterol
and sitosterol) in steps that can be effectively blocked, but
this has not led to practical pest control. Most important
currently was a serendipitous discovery of potent synthetic
tert-butyl dibenzoylhydrazine ecdysone agonists that are
very effective in blocking development and controlling
lepidopterous larvae at a target without direct analogy in hu-
mans. This is currently a small (0.4%) segment of the insect
control market.

3.4. Bioenergetics

The principal bioenergetic precursors and metabolic path-
ways are the same in insects and humans. Insecticidal and
miticidal Complex I inhibitors and uncouplers of oxidative
phosphorylation (2% of the insecticide and miticide market)
are limited by low selectivity between insects and mam-
mals. Selected thioureas (and their carbodiimide metabo-
lites) and organotins are potent respiratory inhibitors at
other sites but also lack the desired selective action.

4. Bt ENDOTOXIN MIDGUT RECEPTORS

Discovery and Development. Bacillus thuringiensis (Bt),
a gram-positive bacterium first isolated from the silkworm
in Japan in 1902, has been used for decades as a mixture of
living spores and endotoxin crystalline (Cry) protein for
control of many lepidopterous and a few coleopterous pests
(about 1% of worldwide pesticide sales). The first Bt Cry
protein toxin gene was identified and cloned in 1981, fol-
lowed quickly by an expanding set of related proteins. The
timing was propitious since the delivery systems provided
by molecular biology made it possible to use proteins as
pest control agents. A revolution in pest control, both eco-
nomic and political, resulted and continues from expression
of these insect-specific and human-safe Bt δ-endotoxins in
major crops. Genetically-modified corn and cotton are pro-
tected from major pests without requiring extensive addi-
tional use of synthetic chemical insecticides, a procedure so
effective that 70% of the cotton in the U.S. is protected by
expressed Bt δ-endotoxin, e.g. Cry1Ac, and Cry1Ac plus
Cry2A.

Mode of Action. Bt produces parasporal, proteinaceous,
crystal inclusion bodies during sporulation. The Cry pro-
teins are solubilized and gut proteases convert the original
δ-endotoxin protoxin (130–140 kDa) into toxic cores of 60–
70 kDa. In lepidopterous insects these hydrolyzed toxins
bind to the surface of the insect’s midgut columnar epithelial
cells with high-affinity, undergo further proteolysis,
then insert into the cellular membrane and aggregate form-
ing pores. These pores are cation-selective channels result-
ing in loss of osmotic regulation thereby lysing the midgut
epithelium. Disruption of the epithelial layer leads to an
increase in potassium concentration and pH in hemolymph
from the highly alkaline (pH 10–11) midgut lumen.
Ultimately, the affected cells are destroyed and the insects
eventually die from gut paralysis, feeding inhibition, and
starvation or septicemia. Different toxins bind to distinct re-
ceptors and this explains the selectivity of Bt strains.
Different genes are used to produce insecticidal proteins that
provide seasonal protection of crops. In some cases the trun-
cated genes are used so that the insect-active toxin is pro-
duced directly in the crop. The principal biochemical
mechanism for resistance to Cry toxins involves altered
binding to receptors in the midgut, but changes in process-
ing of the toxins and rapid repair of damaged cells have also
been implicated. Although resistance development has been
observed in the field with lepidopterous pests, the use
of toxin mixtures may allow the sustained effectiveness of Bt
in agriculture and public health programs.

Unique Toxicology of Insects. Bt preparations are con-
sidered to be nontoxic to humans and are tolerance exempt
in the U.S. on all raw agricultural commodities. Mammals
are not affected by Cry toxins because these proteins are la-
bile in the acidic pH of the stomach. In contrast to insects,
mammals lack the specific receptors required for binding,
which leads to the subsequent cascade of biochemical
events and insecticidal activity.

5. INSECTICIDE ACTIVATION AND
DETOXIFICATION

5.1. General Aspects

Every animal has a set of detoxifying enzymes for dietary
constituents that are also effective for some but not all
xenobiotics. These enzymes recognize and modify func-
tional groups for reduced reactivity and added polarity
(phase I) and conjugation for excretion (phase II). Phase I
reactions oxidize, reduce or hydrolyze and may result in
bioactivation. These systems are fundamentally the same in
insects and people. Insecticides are largely lipophilic com-
pounds so systems of particular importance are those that
handle apolar molecules. As insecticides are developed with progressively higher potency they are used in smaller amounts which are more easily detoxified. A principal type of selective toxicity involves bioactivation in insects and detoxification in mammals. Genetic selection in insects for metabolic resistance to one insecticide will potentially confer resistance to all other insecticides metabolized by the same enzyme, regardless of their toxicological target—a particularly disastrous type of cross resistance. Metabolic resistance and cross resistance usually involves a single limiting enzyme (mutated or overexpressed) acting at a specific site on the insecticide.

5.2. Cytochrome P450s

Cytochrome P450s (CYP450s) are principal activation and detoxification enzymes. They are a family of genes and enzymes in insects and humans, each with a characteristic substrate specificity (detoxification potential). They activate many types of insecticides including phosphorothionates by oxidative desulfuration to the corresponding oxons. CYP450s can be induced by dietary constituents and inhibited by synergists (e.g. piperonyl butoxide). The principal CYP450 in human liver for metabolizing many insecticides is CYP3A4 and in resistant Drosophila is CYP6G1, each with a broad substrate specificity.

5.3. Hydrolases and Transferases

Many hydrolases and transferases contribute to selective toxicity and resistance mechanisms. There are specific OP hydrolases and in addition carboxylesterases hydrolyze the pyrethroids and some OPs. Glutathione S-transferases detoxify DDT and some OPs. The unique aspect of insect toxicology is sometimes a single enzyme of one of these types conferring unusual toxicity relationships.

6. UNIQUE TOXICOLOGY OF INSECTS

Discoveries on the unique toxicology of insects come from advances in five areas. Historically, and probably for the foreseeable future, the seminal discovery is most often from novel chemistry giving an insecticide of unknown mode of action. Alternatively advances in biology provide new methods of searching for novelty. The study of target sites at organismal, cell, receptor or enzyme and molecular levels reveals differences for directed probe design. Defining insecticide metabolism in pests and people reveals unique aspects that can be used to achieve selective toxicity. The fifth (and possibly most important) factor is serendipity and particularly the ability to recognize the unusual and unexpected.

The unique toxicology of insects versus mammals is sometimes due to major morphological and physiological adaptations. More often it is attributable to specific differences in binding subsites comparing susceptible and resistant insects with mammals. Ultimately, a single amino acid change at the right time and place can be the crucial item for selective toxicity from target site or metabolic specificity. Considerable progress has been made in discovering novel targets resulting from new chemical classes now under development. The unique toxicology of insects provides the basis for the safety of the current major insecticides and novel and unexploited targets for future pest management.

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RELEVANT LITERATURE


