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Serine hydrolase targets of organophosphorus toxicants

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Abstract

Acetylcholinesterase (AChE) is one of several hundred serine hydrolases in people potentially exposed to about 80 organophosphorus (OP) compounds important as insecticides or chemical warfare agents. The toxicology of OPs was interpreted until recently almost solely on the basis of AChE inhibition. It is assumed that each serine hydrolase has a specific function and proposed that every OP compound has a unique inhibitory profile. This review considers the progress in sifting the expanding list of potential serine hydrolase toxicological targets. About 50 serine hydrolase targets have been recognized but only a few studied thoroughly. The toxicological relevance of known secondary OP targets is established mainly from observations with humans (butyrylcholinesterase and neuropathy target esterase–lysophospholipase) and studies with mice (cannabinoid CB1 receptor, carboxylesterase, lysophospholipase and platelet activating factor acetylhydrolase) and hen eggs (arylformamidase or kynurenine formamidase). Pesticides most commonly shown to inhibit these targets in experimental vertebrates are chlorpyrifos and tribufos. Generally the levels of environmental and occupational OP pesticide exposure are well below those causing in vivo inhibition of secondary serine hydrolase targets. Although exposure to OP insecticides is decreasing from stricter regulations and the development of resistant pest strains, it will continue to some degree for decades in the future. Only two OPs are used as pharmaceuticals, i.e. echothiophate as an ophthalmic for treatment of glaucoma and metrifonate as an anthelmintic for *Schistosoma* (and formerly as a candidate drug for improved cognitive function in Alzheimer's disease). In safety evaluations, knowledge on known OP targets must be balanced against major gaps in current understanding since more than 75% of the serine hydrolases are essentially unknown as to OP targeting and relevance, i.e. it is not clear if they play a role in OP toxicology.

Keywords: Acetylcholinesterase; Chemical warfare agent; Insecticide; Neurotransmitter receptor; Organophosphorus; Serine hydrolase

1. Introduction

Organophosphorus (OP) compounds were discovered as toxicants for people in 1932 and for insects in 1937. They evolved into the principal chemical warfare agents (sarin, soman and VX optimized for lethality) and the major class of insecticides (including chlorpyrifos, acephate, and malathion usually with selective toxicity to insects compared with mammals) (Fig. 1). The 76 commercial OP pesticides consist of 58 insecticides, seven herbicides, five fungicides, four nematicides, and two plant growth regulators [2]. They have a wide diversity of chemical substituents and biochemical targets. The use of glyphosate herbicide in the United States exceeds that of all other OP pesticides combined. The amount of OP insecticide use is declining, but their importance will probably continue for decades since they are effective and inexpensive; replacements will come from other chemical classes that work in different ways.

Acetylcholinesterase (AChE) was shown to be the target for the OP toxicants in 1940 for mammals and a few

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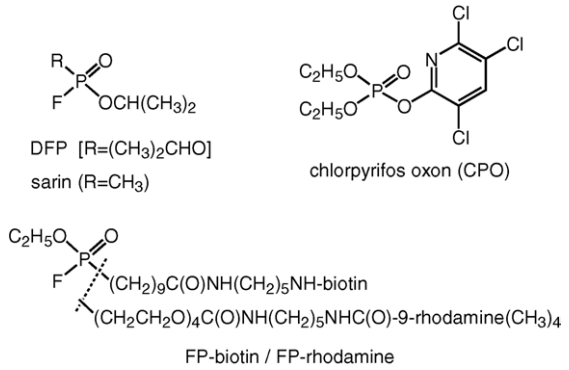

Fig. 1. Five OP toxicants and probes important in evaluating the significance of serine hydrolase targets. For structures and properties of other OP pesticides and toxicants referred to see The Merck Index [1] or The Pesticide Manual [2]. DFP and the insecticide metabolite CPO potently inhibit a broad range of serine hydrolases in vitro and/or in vivo. FP-biotin is used for serine hydrolase isolation and characterization and the tetramethylrhodamine fluorescent derivative for quantitation.

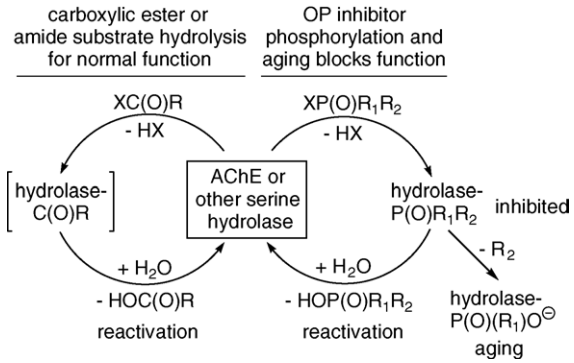

Fig. 2. AChE is one of several hundred serine hydrolases with catalytic triads containing serine and histidine usually along with glutamate or aspartate. Hydrolysis of the neurotransmitter acetylcholine $[(CH_3)_3{}^+NCH_2CH_2OC(O)CH_3$ shown generically as XC(O)R] is a critical step in nerve function. Phosphorylation of AChE blocks acetylcholine hydrolysis temporarily with reactivation and "permanently" with aging. Less than 25% (50 from more than 200) of the serine hydrolases are of known function.

years later for insects. It plays a central and critical role in the nervous system as detailed by Giacobini [3] and at the VIIIth International Meeting on Cholinesterases (this volume). AChE is also the target for the insecticides and nematicides which are mostly dimethyl and diethyl phosphates acting directly or phosphorothionates requiring oxidative bioactivation. Inhibition of brain or neuromuscular AChE activity to the extent of 70–90% is usually lethal. This is achieved with some OPs in either mammals or insects at doses as low as 10–1000 μg/kg. They are simple, potent and essentially irreversible AChE inhibitors, but that's not all there is to OP toxicology (see [4], and citations therein).

There are several hundred serine hydrolases in people and more than 600 in *Drosophila* based on genomic evidence (Table 1) [5–8]. AChE is but one of this long list and there are clearly many other potential OP targets. The apparent number of serine hydrolases will change with further knowledge, but the concepts considered here will still hold. The serine hydrolase reaction with OPs is shown on a generic basis in Fig. 2. It consists of phosphorylation of the active site serine, sometimes followed by reactivation or aging.

Table 1
Serine hydrolases in people and *Drosophila* classified by type based on genomic evidence [5–8]

Species	Gene products	
	Total	Hydrolases
Number		
Human	22500	1227[a]
Drosophila	13601	649[b]
Percent		
Human	100	5
Drosophila	100	5

[a] Includes 150 serine proteases.
[b] Includes 377 serine proteases plus 272 candidate serine hydrolases (140 esterases/lipases/thioesterases, 66 lipases, 38 carboxylesterases and 28 alpha/beta hydrolases).

2. Identification and analysis of OP-sensitive serine hydrolases

Three principal methods have been used to recognize OP-sensitive serine hydrolases. The first is to assay established enzymes (e.g. AChE, butyrylcholinesterase (BChE), chymotrypsin and arylformamidase (AFMID) (also known as kynurenine formamidase)) for sensitivity to diisopropyl fluorophosphate (DFP) and other OPs [4,9]. They were found to vary in OP-sensitivity from nanomolar to high micromolar ranges. The second procedure is to label tissue preparations with OP inhibitors for target isolation and identification by protein sequencing. This was used for neuropathy target esterase–lysophospholipase (NTE–lysoPLA), AFMID and acylpeptide hydrolase (APH). A third more generic method involves reaction with FP-biotin or an analog to introduce the phosphoryl substituent and biotin for isolation with avidin [10,11]. Serine hydrolases in sufficient amount and purity are characterized by LC–MALDI–TOF MS, comparing with deduced sequences from genomic data. This has led to a recent surge of new candidate targets. Replacing the biotin moiety with rhodamine or other fluorescent derivative allows