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Each Lipase Has a Unique Sensitivity Profile for Organophosphorus Inhibitors

Gary B. Quistad, Shannon N. Liang, Karl J. Fisher, Daniel K. Nomura, and John E. Casida[1]

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720–3112, USA

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Lipases sensitive to organophosphorus (OP) inhibitors play critical roles in cell regulation, nutrition, and disease, but little is known on the toxicological aspects in mammals. To help fill this gap, six lipases or lipase-like proteins are assayed for OP sensitivity *in vitro* under standard conditions (25°C, 15 min incubation). Postheparin serum lipase, lipoprotein lipase (LPL) (two sources), pancreatic lipase, monoacylglycerol (MAG) lipase, cholesterol esterase, and KIAA1363 are considered with 32 OP pesticides and related compounds. Postheparin lipolytic activity in rat serum is inhibited by 14 OPs, including chlorpyrifos oxon (IC_{50} 50–97 nM). LPL (bovine milk and *Pseudomonas*) generally is less inhibited by the insecticides or activated oxons, but the milk enzyme is very sensitive to six fluorophosphonates and benzodioxaphosphorin oxides (IC_{50} 7–20 nM). Porcine pancreatic lipase is very sensitive to dioctyl 4-nitrophenyl phosphate (IC_{50} 8 nM), MAG lipase of mouse brain to *O*-4-nitrophenyl methyldodecylphosphinate (IC_{50} 0.6 nM), and cholesterol esterase (bovine pancreas) to all of the classes of OPs tested (IC_{50} < 10 nM for 17 compounds). KIAA1363 is sensitive to numerous OPs, including two *O*-4-nitrophenyl compounds (IC_{50} 3–4 nM). In an overview, inhibition of 28 serine hydrolases (including lipases) by eight OPs (chlorpyrifos oxon, diazoxon, paraoxon, dichlorvos, and four nonpesticides) showed that brain acetylcholinesterase is usually less sensitive than butyrylcholinesterase, liver esterase, cholesterol esterase, and KIAA1363. In general, each lipase (like each serine hydrolase) has a different spectrum of OP sensitivity, and individual OPs have unique ranking of potency for inhibition of serine hydrolases.

Key Words: **chlorpyrifos oxon; cholesterol esterase; lipase; lipoprotein; organophosphorus.**

Organophosphorus (OP) toxicology research has evolved from evaluating acetylcholinesterase (AChE) inhibition (the primary effect) to consideration of multiple secondary targets. There are several hundred serine hydrolases, including lipases, in humans. Although at least 50 are known to be OP targets, only about 20 have been systematically evaluated for OP sensitivity and relevance, leading to AChE, neuropathy target esterase-lysophospholipase (NTE-LysoPLA), butyrylcholinesterase (BChE), platelet-activating factor acetyl hydrolase (PAF-AH), monoacylglycerol (MAG) lipase, fatty acid amide hydrolase (FAAH), and carboxylesterase as most important to mammalian toxicology (Casida and Quistad, 2004, 2005; Quistad *et al.*, 2006).

Lipases are serine hydrolases which cleave lipids essential as energy sources (e.g., triacylglycerols) and signaling molecules (e.g., 2-arachidonoylglycerol) (Dinh *et al.*, 2002; Wong and Schotz, 2002). Lipoprotein lipase (LPL, EC 3.1.1.34) and hepatic triglyceride lipase (H-TGL, EC 3.1.1.3) are the major lipolytic enzymes for metabolism of lipoproteins in circulation (Jackson and McLean, 1991). These enzymes, which play key roles in lipid metabolism and transport, are anchored to the plasma membrane of endothelial cells and are released after intravenous heparin administration—thus the term postheparin plasma lipolytic activity (Mead *et al.*, 2002). The major tissue sources of LPL are adipose and muscle, while H-TGL is synthesized by hepatocytes. Bovine milk is a major source of LPL (Bengtsson-Olivecrona and Olivecrona, 1991) and related LPLs have been isolated from *Pseudomonas* (Sugiura *et al.*, 1977). Human, rat, and bovine LPL have 447–450 amino acids and are highly homologous to each other. Human LPL is 47% homologous to human H-TGL (Jackson and McLean, 1991; Raisonnier *et al.*, 1995) and 28% homologous to porcine pancreatic lipase (EC 3.1.1.3) (Wion *et al.*, 1987), a key enzyme of dietary triacylglycerol uptake (Hadváry *et al.*, 1988). Amino acid sequences near the active site serine are very similar for each of these mammalian lipases (Jackson and McLean, 1991; Raisonnier *et al.*, 1995). MAG lipase modulates the pharmacological activity of 2-arachidonoylglycerol, the principal natural ligand acting at the CB1 cannabinoid receptor (Sugiura and Waku, 2000). Rat brain MAG lipase (EC 3.1.1.23) is a 33-kDa protein and is unique among lipases by using only monoacylglycerols as substrates (Dinh *et al.*, 2002; Karlsson *et al.*, 2000). Cholesterol esterase (EC 3.1.1.13) is synthesized in the pancreas and transported into the intestine, where it

[1] To whom correspondence should be addressed at Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720–3112. Fax: (510) 642-6497. E-mail: ectl@nature.berkeley.edu.

TABLE 1
Enzymes Studied and Assays Used

Enzyme		Assay		
Name	Source	Substrate	Product	Reference
Postheparin lipolytic activity	rat serum	*O,O,S*-tributyrin ^{14}C-triolein	dibutyrin (SH) ^{14}C-oleic acid	this study
Lipoprotein lipase	bovine milk	*O,O,S*-tributyrin	dibutyrin (SH)	this study
	Pseudomonas	*O,O,S*-tributyrin	dibutyrin (SH)	this study
Pancreatic lipase	porcine	*O,O,S*-tributyrin	dibutyrin (SH)	this study
MAG lipase	mouse brain	^{14}C-oleyl-monoolein	^{14}C-oleic acid	Quistad *et al.*, 2006
Cholesterol esterase	bovine pancreas	4-nitrophenyl butyrate cholesteryl ^{14}C-oleate	4-nitrophenol ^{14}C-oleic acid	DiPersio *et al.*, 1990
KIAA1363	mouse brain	^{3}H-CPO binding	^{3}H-CPO-protein	Nomura *et al.*, 2005
Long-chain acyl-CoA hydrolase	mouse brain	palmitoyl-CoA	CoASH	Yamada *et al.*, 1996
Urokinase-type plasminogen activator	human urine	pGlu-Gly-Arg-*p*-nitroanilide	*p*-nitroaniline	Liu and Gurewich, 1995

catalyzes fat and vitamin absorption; this 74-kDa enzyme from porcine pancreas is quite different from other lipases (DiPersio *et al.*, 1990). KIAA1363 is a serine hydrolase with lipase homology, associated with cancer cell invasiveness (Jessani *et al.*, 2002) and is the principal enzyme in mouse brain for detoxifying low levels of chlorpyrifos oxon (CPO), the active metabolite from the major insecticide chlorpyrifos (Nomura *et al.*, 2005).

Inhibition of lipase activity could disrupt lipid homeostasis, thereby causing pathological conditions in mammals. For example, cardiac-specific gene knockout of LPL leads to defective triacylglycerol metabolism in plasma (Augustus *et al.*, 2004). OP compounds in general, and pesticides in particular, are potential inhibitors of lipases, but a systematic investigation of OP potency and selectivity is lacking. This investigation partially fills the gap by determining the sensitivity and structure-activity relationships (SAR) for 32 OP compounds inhibiting six lipases, lipoprotein lipases, or lipase-like enzymes.

MATERIALS AND METHODS

Enzymes and chemicals. Swiss-Webster mouse brains were from Pel-Freez (Rogers, AR). The following purified enzymes and substrates were from Sigma (St. Louis, MO): LPL (bovine milk, *Pseudomonas*), pancreatic lipase (porcine), unpurified plasma for BChE (mouse), liver esterase (a carboxylesterase) (porcine), cholesterol esterase (bovine pancreas), urokinase-type plasminogen activator (human urine), 4-nitrophenyl butyrate, palmitoyl CoA, and pGlu-Gly-Arg-*p*-nitroanilide. ^{14}C-Mono- and ^{14}C-triolein were synthesized from ^{14}C-oleic acid (PerkinElmer Life Sciences, Boston, MA). 3-Mercaptopropane-1,2-diol tributyrate (*O,O,S*-tributyrin; *O,O,S*-TB) was made from 3-mercaptopropane-1,2-diol and butyric acid anhydride.

Enzymes studied and assays used. Table 1 summarizes the enzyme sources and assays. The test conditions in every case gave linearity of product formation with enzyme level and time. In this investigation, candidate inhibitors were added in dimethyl sulfoxide (DMSO) (5 µl), and the mixture was incubated for 15 min before adding the substrate to determine activity. Assays were conducted at 25°C for comparison to previous data from this laboratory. Appropriate controls were included for the DMSO vehicle. Tributyrin was used as a convenient substrate for early studies with lipases (Aldridge and Reiner, 1972). Subsequently, *O,O,S*-TB was even more useful since it allowed colorimetric assay of pancreatic lipase activity (Kurooka *et al.*, 1976).

Postheparin serum lipolytic activity assay. Sprague-Dawley rats (*ca.* 220 g) were treated iv with heparin (0.25 ml of a 1 mg/ml solution in 145 mM NaCl) and blood withdrawn after 5 min (Fielding, 1969). Postheparin rat serum (10 µl) was added to 100 mM Tris (pH 8.8, 25°C) (185 µl). After the inhibition reaction, 5,5'-dithiobis(2-nitrobenzoic acid) (50 µl, 1.76 mg/ml) and *O,O,S*-TB (50 µl, 0.15 mg/ml emulsion in 100 mM Tris, pH 7.7, 25°C) were added, with kinetic assay at 412 nm for 15 min at 25°C using a microplate reader with 96-well plates (Versamax, Molecular Devices, Sunnyvale, CA).

For comparison, ^{14}C-triolein was examined as a possibly equivalent substrate. Postheparin rat serum (10 µl) was added to 100 mM Tris (pH 8.8, 25°C) (185 µl) containing 3 µM lecithin and 0.01% Triton X-100 in 4-ml glass vials. Following the inhibition reaction, ^{14}C-triolein (38 µM final concentration, 50,000 dpm) was added in DMSO (5 µl). After vortexing for 10 s, samples were incubated 1 h at 25°C. The reaction was stopped by addition of chloroform:methanol:hexane, 1.25:1.4:1 (2.5 ml) and 200 mM K_2CO_3 (0.83 ml). Vials were capped, mixed, centrifuged, and potassium ^{14}C-oleate was extracted into the upper aqueous phase, 1.5 ml of which was quantified by liquid scintillation counting. Under these conditions control preparations hydrolyze 2% of the ^{14}C-triolein.

Other lipase assays. Lipoprotein and pancreatic lipase activities were determined as described for postheparin serum, but using LPL (bovine milk and *Pseudomonas*) and porcine pancreatic lipase (2, 6 and 50 units per well, respectively). MAG lipase activity was monitored as release of ^{14}C-oleic acid from 1(3)-^{14}C-oleoyl-monoolein using mouse brain homogenate (Quistad *et al.*, 2006). Cholesterol esterase activity was determined as described, with 4-nitrophenyl butyrate as substrate (no addition of taurocholate) (DiPersio *et al.*, 1990). As validation of this colorimetric method, similar IC_{50} values are obtained for CPO, ethyl octylphosphonofluoridate (EOPF), and isopropyl dodecylphosphonofluoridate (IDFP) using cholesteryl ^{14}C-oleate and taurocholate (data not shown) (DiPersio *et al.*, 1990). KIAA1363 was assayed as ^{3}H-CPO binding in mouse brain membranes as described (Nomura *et al.*, 2005).

Other serine hydrolase assays. Inhibition by eight OPs was compared for 28 serine hydrolases (including lipases) under similar conditions (15-min inhibition, 25°C). For comparisons of potency *in vitro*, some of the data used are from previous publications of this laboratory. Missing IC_{50} values were obtained by the same methods: AChE, mouse brain (Quistad *et al.*, 2005b); BChE, mouse plasma (Quistad *et al.*, 2005b); liver esterase (Est-L), porcine, using 4-nitrophenyl butyrate as substrate (Quistad and Casida, 2000); brain esterase (Est-B), mouse, using the 1200 × *g* supernatant of a 20% w/v

homogenate in 100 mM phosphate, pH 7.4 and 4-nitrophenyl butyrate (as above); long-chain acyl-CoA hydrolase, mouse brain homogenate as for Est-B and palmitoyl-CoA as substrate (Yamada *et al.*, 1996); urokinase-type plasminogen activator (u-PA), from human urine with p-Glu-Gly-Arg-*p*-nitroanilide as substrate (Liu and Gurewich, 1995).

Analysis of data. The concentration of compound inhibiting 50% of enzyme activity (IC_{50}) was derived with two to three levels (above and below the IC_{50}, each in at least triplicate) in the range of 15–85% enzyme inhibition. Results are reported as the mean ± SD of the individual IC_{50} values for each OP ($n = 3$–6).

RESULTS

Sensitivity Profiles

Candidate inhibitors are compared on the basis of SAR and potency. $IC_{50} \leq 200$ nM (potent); 200 nM–10 μM (moderate); >10 μM (weak).

Postheparin Serum Lipolytic Activity

Assays with O,O,S-tributyrin. Postheparin lipolytic activity in rat serum can be measured with either O,O,S-TB or ^{14}C-triolein, which give similar IC_{50} values for CPO (80 ± 13 and 112 ± 41 nM, respectively). O,O,S-TB is used routinely in this investigation because of the ease of colorimetric assay. The lipolytic activity in rat serum increases 57% 5 min after iv treatment with heparin, and this postheparin lipolytic activity predominantly is from lipase(s) and not BChE, since the BChE-specific inhibitor ethephon (Haux *et al.*, 2000) at 300 μM reduces hydrolysis only 10% for O,O,S-TB versus 90% for butyrylthiocholine (Supplementary Table 1). A related inhibition study with ethephon showed that O,O,S-TB is not a suitable substrate for lipase activity in human plasma (preheparin), since it is hydrolyzed by BChE. In fact, purified human serum BChE cleaves O,O,S-TB, although about 15-fold slower than butyrylthiocholine (standard assay conditions referred to earlier; data not shown).

OP SAR (Table 2). Five 4-nitrophenyl phosphates (including paraoxon) with C_2–C_8 alkoxy substituents (**1–5**) are potent inhibitors (IC_{50} 50–170 nM). Three analogs with longer (C_{12}) substituents (**6–8**) are less active. Three fenitrothion oxon analogs (ethyl, pentyl, and isopropyl) (**9–11**) have similar potency (IC_{50} 57–76 nM), while the pentyl substituents (**12**) double the potency of dichlorvos (**13**). CPO (**17**) and two analogs (pentyl and isopropyl) (**14, 16**) also have similar activity (IC_{50} 78–94 nM). Four fluorophosphorus compounds (**18–21**) are potent (IC_{50} 97–180 nM) while **22** and a methylphosphonate with a long (C_{13}) chain (**24**) are moderately active (IC_{50} 550–670 nM), but two alkylphosphonates with shorter (C_5 and C_8) chains (**27, 28**) have reduced activity (IC_{50} 4–11 μM). Three benzodioxaphosphorin oxides (BDPOs) (**29–31**) and diazoxon (**32**) are potent inhibitors (IC_{50} 67–200 nM).

Lipoprotein Lipase

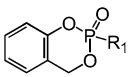
Bovine milk OP SAR (Table 2). Fourteen phosphates, including paraoxon, dichlorvos, CPO, diazoxon, fenitrothion-ethyl analogs, and diisopropyl fluorophosphate (DFP), have moderate to low potency ($IC_{50} > 1$ μM). However, several fluorophosphorus compounds are very potent inhibitors. Thus, EOPF (**19**) and a methylphosphonate with a C_{12} substituent (**25**) are the most potent inhibitors examined (IC_{50} 7–9 nM), and four others (**18, 23, 24, 26**) are also very active (IC_{50} 18–64 nM). Activity is retained for methylphosphonates with long chains (C_{11}–C_{14}) (**23–26**), but diminishes for C_8 (**27**). O-octyl (**30**) and heptyl (**31**) BDPO are very active (IC_{50} 15 nM), but the S-pentyl analog (**29**) is 28-fold less active.

Pseudomonas OP SAR (Table 2). In the 4-nitrophenyl series, dioctyl (**4**) is most potent (IC_{50} 150 nM). Dipentyl and didodecyl analogs (**2, 8**) are 53- and 31-fold less active, respectively, whereas diisopropyl (**3**) and diethyl (paraoxon) (**5**) are inactive. Replacement of one octyl with isopropyl (**1**) in the dioctyl compound (**4**) reduces LPL inhibitory activity 80-fold. Although fenitrothion-ethyl oxon (**9**) is potent for postheparin serum lipolytic activity, it is inactive for *Pseudomonas* LPL, but its dipentyl analog (**10**) is weakly active (IC_{50} 27 μM). In the trichloropyridinyl series, CPO (**17**) is moderately active (IC_{50} 2.2 μM). The dipentyl analog (**14**) is 16-fold more active, but dibutyl (**15**) is similar (IC_{50} 2.5 μM), and diisopropyl (**16**) is inactive. For fluorophosphorus compounds, DFP (**20**) is weakly active (IC_{50} ca. 100 μM), and its dipentyl analog (**21**) is inactive. EOPF (**19**) has moderate activity (IC_{50} 3.5 μM), but IDFP (**18**) is inactive. Methylphosphonates are active (IC_{50} 160–170 nM) for C_{12}–C_{14} alkoxy groups (**23–25**), decreasing 4-fold for C_{11} (**26**) and >30-fold for C_8 (**27**). The S-pentyl, O-octyl, and heptyl BDPOs (**29–31**) tested are moderately active (IC_{50} 2–6 μM). Dichlorvos (**13**), its dipentyl analog (**12**), and diazoxon (**32**) are inactive. Thus, five OP compounds are potent inhibitors of *Pseudomonas* LPL (IC_{50} 140–170 nM). In general, longer alkoxy groups, C_5–C_{14}, are preferred whereas branched isopropyl groups greatly reduce activity.

Other Lipases

Pancreatic lipase OP SAR (Table 2). The only highly potent inhibitor discovered is dioctyl 4-nitrophenyl phosphate (**4**) (IC_{50} 8 nM). Activity is very sensitive to alkyl chain length with a >2000-fold decrease for the following changes: one *n*-C_8 to *i*-C_3 (**1**); two *n*-C_8 to two *n*-C_5 (**2**) or *n*-C_{12} (**8**). Eight oxon analogs in the fenitrothion, dichlorvos and chlorpyrifos series (**9–16**) are almost inactive, although CPO itself (**17**) has weak activity (IC_{50} 20 μM). In the fluorophosphorus and BDPO groups, four alkyl methylphosphonates (C_{11}–C_{14}) (**23–26**) are fairly strong inhibitors (IC_{50} 210–650 nM) with C_{12} (**25**) most active, while the other compounds are less active.

TABLE 2
Inhibition of Lipase Activity by OP Pesticides and Related Compounds

Compounds[a]			IC_{50}, nM[b]						
			Postheparin serum	Lipoprotein		Pancreatic porcine	MAG	Cholesterol esterase	KIAA 1363
R_1	R_2		Rat	Bovine milk	*Pseudomonas*		Mouse brain	Bovine pancreas	Mouse brain
R_1R_2 P(O)OC_6H_4-4-NO_2									
1 *n*-$C_8H_{17}O$	*i*-C_3H_7O		50 ± 3	34,000 ± 11,000	12,000 ± 580	16,000 ± 1,800	5,300 ± 4,200	1.2 ± 0.1	4 ± 1
2 *n*-$C_5H_{11}O$	*n*-$C_5H_{11}O$		62 ± 13	2,800 ± 1,400	7,900 ± 170	17,000 ± 1,400	400 ± 90	0.65 ± 0.11	130 ± 67
3 *i*-C_3H_7O	*i*-C_3H_7O		75 ± 4		>100,000	>100,000	12,000 ± 600	70 ± 2	20
4 *n*-$C_8H_{17}O$	*n*-$C_8H_{17}O$		95 ± 15	7,100 ± 1,400	150 ± 26	7.6 ± 1.2	210 ± 38	1.7 ± 0.1	>1,000
5 C_2H_5O	C_2H_5O	paraoxon	170 ± 15	13,000 ± 2,000	>100,000	6,200 ± 800	2,300 ± 1,100	34 ± 0.8	14 ± 3
6 CH_3	*n*-$C_{12}H_{25}$		220 ± 21			>100,000	0.60 ± 0.03	5.7 ± 0.1	3 ± 1
7 *n*-$C_{12}H_{25}O$	$C_6H_{11}O$		2,000 ± 200			>100,000	100 ± 3	21 ± 2	230 ± 38
8 *n*-$C_{12}H_{25}O$	*n*-$C_{12}H_{25}O$		26,000 ± 5,000	4,500 ± 2,000	4,700 ± 1,100	19,000 ± 3,000	320 ± 30	460 ± 50	>1,000
R_1R_2 P(O)OC_6H_3-3-CH_3-4-NO_2									
9 C_2H_5O	C_2H_5O	fenitrothion-ethyl oxon	63 ± 8	3,100 ± 1,000	>100,000	>100,000	5,900 ± 900	43 ± 9	230 ± 38
10 *n*-$C_5H_{11}O$	*n*-$C_5H_{11}O$	fenitrothion-pentyl oxon	76 ± 4	1,500 ± 490	27,000 ± 15,000	34,000 ± 5,000	330 ± 120	1.5 ± 0	
11 *i*-C_3H_7O	*i*-C_3H_7O	fenitrothion-isopropyl oxon	57 ± 5			>100,000	3.6 ± 0.2	32 ± 4	110 ± 2
$R_1R_2P(O)OCH = CCl_2$									
12 *n*-$C_5H_{11}O$	*n*-$C_5H_{11}O$	dichlorvos-pentyl	63 ± 7	59,000 ± 17,000	>100,000	>100,000	3,300 ± 2,000	1.9 ± 0.2	
13 CH_3O	CH_3O	dichlorvos	140 ± 10	>100,000	>100,000	>100,000	13,000 ± 600	6,800 ± 800	18,000 ± 750
$R_1R_2P(O)O$-pyridinyl-3,5,6-Cl_3									
14 *n*-$C_5H_{11}O$	*n*-$C_5H_{11}O$	CPO-pentyl	78 ± 5	1,200 ± 800	140 ± 80	>100,000	43 ± 5	1.5 ± 0.1	31 ± 1
15 *n*-C_4H_9O	*n*-C_4H_9O	CPO-butyl		4,400 ± 1,900	2,500 ± 250	>100,000	21 ± 2	1.3 ± 0.1	11 ± 0
16 *i*-C_3H_7O	*i*-C_3H_7O	CPO-isopropyl	94 ± 7		>100,000	>100,000	2,200 ± 200	2.6 ± 0.1	14 ± 2
17 C_2H_5O	C_2H_5O	CPO	80 ± 13	7,100 ± 1,400	2,200 ± 1,000	20,000 ± 4,600	34 ± 12	5.0 ± 1.7	8 ± 2
$R_1R_2P(O)F$									
18 $C_{12}H_{25}$	*i*-C_3H_7O	IDFP	97 ± 12	20 ± 3.8	>100,000	>100,000	0.76 ± 0.33	3.1 ± 0.3	270 ± 37
19 C_8H_{17}	C_2H_5O	EOPF	160 ± 8	7.3 ± 2.9	3,500 ± 640	33,000 ± 6,200	3.0 ± 0.7	3.0 ± 0.6	52 ± 9
20 *i*-C_3H_7O	*i*-C_3H_7O	DFP	170 ± 5	>100,000	*ca.* 100,000	66,000 ± 8,000	45,000 ± 4,000	2,700 ± 400	17,000 ± 5,000
21 *n*-$C_5H_{11}O$	*n*-$C_5H_{11}O$		180 ± 36		>100,000	>100,000	550 ± 170	30 ± 3	
22 C_6H_5	C_6H_5		550 ± 29		>100,000	>100,000	>100,000	7,900 ± 300	
23 CH_3	*n*-$C_{14}H_{29}O$			18 ± 1.5	160 ± 50	470 ± 17	560 ± 50	38 ± 3	
24 CH_3	*n*-$C_{13}H_{27}O$		670 ± 91	51 ± 8.6	170 ± 20	650 ± 220	1,000 ± 20	25 ± 1	11 ± 1
25 CH_3	*n*-$C_{12}H_{25}O$			9.3 ± 0.6	170 ± 75	210 ± 17	81 ± 5	6.3 ± 0.6	8 ± 0
26 CH_3	*n*-$C_{11}H_{23}O$			64 ± 14	590 ± 81	290 ± 83	120 ± 4	4.1 ± 0.4	
27 CH_3	*n*-$C_8H_{17}O$		4,200 ± 700	290 ± 28	5,700 ± 1,500	10,000 ± 1,600	440 ± 49	120 ± 10	24 ± 6
28 C_2H_5	*n*-$C_5H_{11}O$		11,000 ± 2,000		>100,000	>100,000	11,000 ± 1,000	2,400 ± 300	
									
29 *n*-$C_5H_{11}S$		*S*-pentyl BDPO	93 ± 20	420 ± 14	2,400 ± 1,200	>100,000	3.1 ± 0.2	7.9 ± 0.6	
30 *n*-$C_8H_{17}O$		*O*-octyl BDPO	67 ± 13	15 ± 3.4	3,600 ± 520	25,000 ± 13,000	150 ± 5	2.8 ± 0.3	
31 *n*-C_7H_{15}		heptyl BDPO	120 ± 12	15 ± 2.1	5,800 ± 1,900	6,300 ± 310	45 ± 3	8.8 ± 0.3	
$R_1R_2P(O)O$-pyrimidinyl-2-*i*-C_3H_7-6-CH_3									
32 C_2H_5O	C_2H_5O	diazoxon	200 ± 29	8,900 ± 1,000	>100,000	>100,000	14,000 ± 1,000	52 ± 11	380 ± 140

[a]The sources were as follows: **1–4, 6–11,** and **14–16** synthesized by standard methods in this laboratory for these studies; **5, 13,** and **17** from Chem Service (West Chester, PA); **12** and **22** (Wu and Casida, 1996); **18** (Segall *et al.*, 2003); **19**, **21,** and **28** (Wu and Casida, 1995); **20** (Sigma); **32** (Quistad *et al.*, 2001), **23–27** (Quistad *et al.*, 2005a); and **29–31** (Wu and Casida, 1992, 1994).
[b]Mean ± SD, $n = 3–6$.

MAG lipase OP SAR (Table 2). Twenty-two new OPs were tested for mouse brain MAG lipase inhibition and compared to 10 others from our previous studies (Quistad *et al.*, 2006). 4-Nitrophenyl methyldodecylphosphinate (**6**) is the most potent OP yet tested (IC_{50} 0.6 nM). Fenitrothion isopropyl oxon (**11**) and S-pentyl BDPO (**29**) of the new series are also very potent (IC_{50} 3–4 nM).

Cholesterol esterase OP SAR (Table 2). Cholesterol esterase (bovine pancreas) is very sensitive to all of the classes of OPs tested with a great variety of alkyl chain lengths ($IC_{50} < 10$ nM for 17 compounds). Seven OPs (**1, 2, 4, 10, 12, 14, 15**) are particularly active (IC_{50} 0.7–1.9 nM).

KIAA1363 OP SAR (Table 2). ^{3}H-CPO binding to KIAA1363 in mouse brain is also sensitive to many classes of OPs, i.e., 4-nitrophenyl, 3-methyl-4-nitrophenyl, trichloropyridinyl, and fluorophosphorus compounds. Of the fourteen new compounds tested, many have potency (IC_{50} 8–14 nM) similar to our previous investigation (Nomura *et al.*, 2005), but improved activity occurs for phosphate **1** and phosphinate **6** (IC_{50} 3–4 nM).

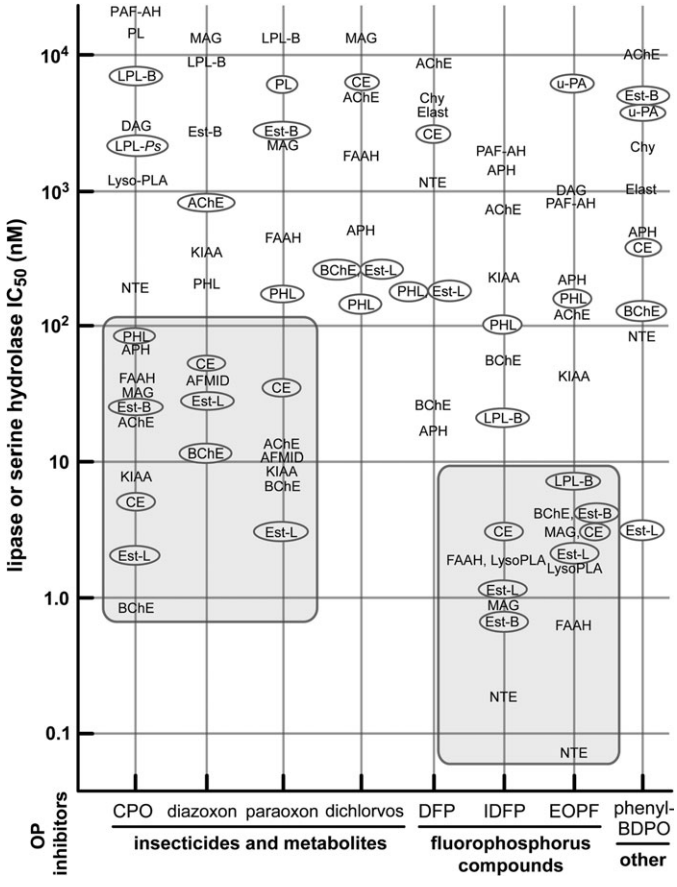


FIG. 1. Relative sensitivities of serine hydrolases to inhibition by eight OP compounds. Exact IC_{50} values are in Supplementary Table 2. Additional references for these data not included in the text are: Quistad and Casida, 2004; Quistad *et al.*, 2002, 2003. Assays involved the same conditions (15 min inhibition, 25°C). Circled hydrolases represent data from this investigation. The boxes show a generally higher sensitivity to fluorophosphorus compounds compared to insecticides and metabolites. Abbreviations are as follows: AChE, acetylcholinesterase (mouse brain); AFMID, arylformamidase (mouse liver); APH, acylpeptide hydrolase (mouse brain); BChE, butyrylcholinesterase (mouse plasma); CE, cholesterol esterase (bovine pancreas); Chy, chymotrypsin (bovine pancreas); DAG, diacylglycerol lipase (mouse brain); Elast, elastase (porcine pancreas); Est-B, brain esterase (mouse); Est-L, liver esterase (porcine); FAAH, fatty acid amide hydrolase (mouse brain); KIAA, KIAA1363 (mouse brain); LPL-B, lipoprotein lipase (bovine milk); LPL-*Ps*, lipoprotein lipase (*Pseudomonas*); Lyso-PLA, lysophospholipase (mouse brain); MAG, monoacylglycerol lipase (mouse brain); NTE, neuropathy target esterase-lysophospholipase (mouse brain); PAF-AH, platelet-activating factor (mouse brain); PHL, postheparin lipoprotein lipase (rat serum); PL, lipase (porcine pancreas); u-PA, urokinase-type plasminogen activator (human urine).

DISCUSSION

Each Lipase has a Unique OP Sensitivity Profile

Lipases show a wide range of sensitivities to OPs (Table 2). Some of the differences may be related to varying species and degrees of enzyme purification, leading to an emphasis on sensitivity profiles more than individual potencies. The IC_{50} values for each of the lipases (postheparin serum, bovine milk, *Pseudomonas*, pancreatic, MAG), cholesterol esterase, and KIAA1363 binding were plotted (log-log scale) against each other individually. The best correlation of these sensitivity profiles for lipases occurred for *Pseudomonas* versus pancreatic assayed with *O,O,S*-TB ($r^2 = 0.74$, $n = 15$), and the next best for inhibition of cholesterol esterase hydrolytic activity versus KIAA1363 binding of OPs ($r^2 = 0.52$, $n = 20$). In general, activity correlates poorly, and each lipase has a unique sensitivity profile for OP inhibitors.

Potency of OP Pesticides Compared with Designer Compounds

Of the OPs studied, CPO is generally the most potent pesticide-related inhibitor of serine hydrolases (Fig. 1), justifying the focus on chlorpyrifos in studies of secondary targets in OP safety (Casida and Quistad, 2004). Paraoxon, diazoxon, and dichlorvos are less active overall, inhibiting serine hydrolases in decreasing order. Even though DFP is the prototype serine hydrolase inhibitor, it generally is only moderately potent [except for BChE and acylpeptide hydrolase (APH)]. DFP is only a moderate inhibitor of brain AChE *in vitro*, which is surprising considering its high toxicity to mice (ip and as vapor) (Quistad *et al.*, 2005b). The designer compounds IDFP and EOPF are the best general serine hydrolase inhibitors with multiple high-sensitivity targets. Their high potency is probably derived from a similarity to lipase substrates (C_8 and C_{12} chain) without the long chain (C_{16}–C_{20}) which compromises solubility.

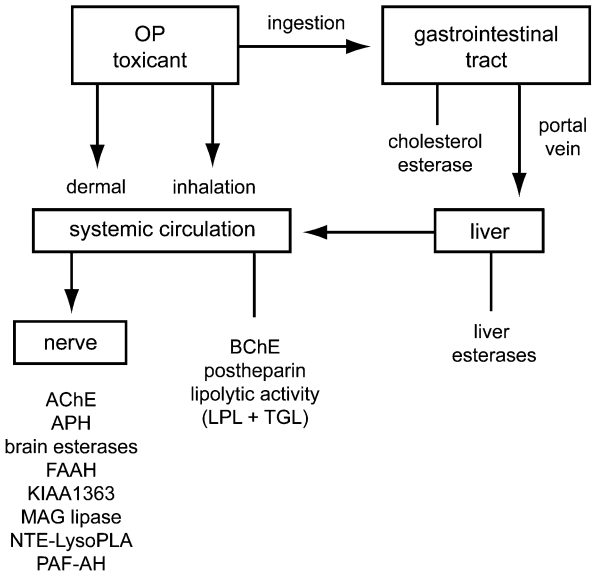


FIG. 2. Serine hydrolases in various tissues most sensitive to OPs following ingestion, inhalation, and dermal exposure. Secondary targets in the gastrointestinal tract, blood, liver, and brain reduce the level of OP available for AChE inhibition.

Sensitivity of Lipases Compared with Other Serine Hydrolases

Eight OPs representative of pesticides (CPO, diazoxon, paraoxon, dichlorvos), fluorophosphorus acute (DFP) and delayed toxicants (EOPF and IDFP) and a cyclic phosphonate (phenyl-BDPO) were compared for potency using the lipase data from this investigation, new data from two serine hydrolases (long-chain acyl-CoA hydrolase and urokinase-type plasminogen activator), and the literature for numerous past studies from this laboratory (Fig. 1 and Supplementary Table 2). Interestingly, for none of the OPs examined was AChE the most sensitive target. The most sensitive enzymes for the active oxons from the pesticides are BChE and porcine liver esterase, generally followed by cholesterol esterase and KIAA1363. For the delayed toxicants (IDFP and EOPF), NTE-LysoPLA is most sensitive, with three orders of magnitude lower potency for AChE. Although PAF-AH is not very sensitive to the OPs tested here, potent methylphosphonates are known (Quistad *et al.*, 2005a). Digestive peptidases (except DFP with APH), blood clotting factors, and plasminogen activators (tissue-type and urokinase) are weakly inhibited by the OPs. Long-chain acyl CoA hydrolase is not inhibited by CPO, IDFP, EOPF, or phenyl-BDPO (IC_{50} > 100 μM). Urokinase is inhibited moderately to weakly by DFP, IDFP, EOPF, and phenyl-BDPO (IC_{50} 4–30 μM) but not by the pesticides or oxon metabolites (IC_{50} > 100 μM).

Toxicological Implications of OP-Sensitive Lipase Targets

Some OP pesticides cause changes in lipid metabolism. Degradation of 2-arachidonoylglycerol in the cannabinoid system is disrupted by chlorpyrifos (30 mg/kg) inhibition of MAG lipase, leading to hypomotility, in part from interference with this signal transduction pathway (Quistad *et al.*, 2006). Fenitrothion at 50 mg/kg increases triacylglycerols and certain phospholipids in liver, kidney, and brain of rats (Roy *et al.*, 2004). A 50% LD_{50} dose of dichlorvos significantly inhibits postheparin lipolytic activity in rat plasma and increases triacylglycerols (Kozlowska *et al.*, 1988; Lucić *et al.*, 2002). Thus, at high doses these OP pesticides elevate blood triacylglycerols, which may have undesirable consequences to health, but the overall impact of lipase inhibition is probably minimal compared to the ramifications of AChE or NTE-lysoPLA inhibition, causing acute and delayed neurotoxicity, respectively. The effects on lipases from chronic exposure to OP pesticides are unknown.

There are many tissue and enzyme targets or detoxification sites for OPs following ingestion, inhalation, or dermal exposure (Fig. 2) (Casida and Quistad, 2004; Chambers *et al.*, 2001). Digestive proteases and pancreatic lipases are fairly insensitive, whereas cholesterol esterase is strongly inhibited. Liver esterase has a high affinity for OPs. As OPs enter systemic circulation, BChE is a major serine hydrolase protectant, although postheparin serum lipase is moderately reactive. Reaction with brain APH, esterases, FAAH, KIAA1363, MAG lipase, and PAF-AH may neutralize OP toxicants and thereby reduce AChE and NTE-LysoPLA inhibition. Thus, several serine hydrolases with high OP reactivity may protect sensitive nerve targets from toxicity.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/. Heparin-induced serum lipolytic activity is differentiated by ethephon inhibition for BChE activity (Supplementary Table 1). The sensitivity of 28 serine hydrolases to eight organophosphorus compounds is given as IC_{50} values (Supplementary Table 2).

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