Journal of Toxicology and Environmental Health, Part B: Critical Reviews
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uteb20

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Version of record first published: 28 Nov 2007.

To cite this article: Floria Pancetti, Cristina Olmos, Alexies Dagnino-Subiabre, Carlos Rozas & Bernardo Morales (2007): Noncholinesterase Effects Induced by Organophosphate Pesticides and their Relationship to Cognitive Processes: Implication for the Action of Acylpeptide Hydrolase, Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 10:8, 623-630

To link to this article: http://dx.doi.org/10.1080/10937400701436445

Journal of Toxicology and Environmental Health, Part B, 10:623–630, 2007

ISSN: 1093-7404 print / 1521-6950 online
DOI: 10.1080/10937400701436445

Taylor & Francis
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NONCHOLINESTERASE EFFECTS INDUCED BY ORGANOPHOSPHATE PESTICIDES AND THEIR RELATIONSHIP TO COGNITIVE PROCESSES: IMPLICATION FOR THE ACTION OF ACYLPEPTIDE HYDROLASE

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Organophosphate pesticides have been classically described as inhibitors of acetylcholinesterase (AChE) activity in insects and invertebrates. However, there is now more evidence supporting the hypothesis that these compounds also act through noncholinergic pathways, especially those related to cognitive processes. The enzyme acylpeptide hydrolase was identified as a new target for organophosphate pesticides. This enzyme is more sensitive than AChE to some organophosphates (OP), including dichlorvos, which is the parent compound for metrifonate, a therapeutic agent used in the treatment of cognitive impairment associated to Alzheimer´s disease. Therefore, there is some doubt as to whether the mechanism of action of this drug is mediated by a potentiation of cholinergic transmission. However, the direct action of acylpeptide hydrolase in cognitive processes and the physiological and molecular mechanisms underlying subacute exposure to OP have yet to be demonstrated. This review deals with evidence demonstrating the existence of mechanisms of actions of OP, which are independent of cholinergic pathway potentiation and which have an effect on cognitive processes. In addition, the possible participation of the enzyme acylpeptide hydrolase in these processes is also discussed. Finally, the possibility of using this enzyme activity as a new biomarker for exposure to OP is considered.

Organophosphate pesticides are widely used as domestic insecticides and also in agriculture for plague control. The use of these pesticides constitutes a main problem for public health since a high percentage of cases related to accidental intoxication seen in emergency rooms are produced by domestically used insecticides (Cole et al., 1998). Furthermore, chronic exposure is caused by the consumption of food contaminated with residues of these pesticides, and in rural areas by respiration of aerial fumigation residues. Despite the fact that strict measures have been taken concerning the commercialization and use of these pesticides, their sale has increased in recent years (Carlock et al., 1999). According to the World Health Organization (WHO), it is estimated that there are about 3 million cases of acute intoxication and 220,000 deaths each year. The majority of these acute cases occur in underdeveloped countries (He, 2000; Clegg & van Gemert, 1999), particularly in Africa, Asia, Central America, and South America. For example, between the years 1992 and 2000 the use of pesticides in Central America notably increased and the incidence of acute intoxications rose from 6.3 per 100,000 habitants to 19.5 per 100,000 habitants, while the mortality rate increased from 0.3 per 100,000 habitants to 2.1 per 100,000 habitants (Henao & Arbelaez, 2002).

In Chile, according to the National Institute of Statistics, the importation of insecticides for agricultural use increased approximately 20% between the years 1999 and 2003 (Rojas, 2004). In addition, data from the Ministry of Health indicate that the number of nonoccupational intoxications has increased from 20 cases in the year 1999 to 42 cases in the year 2003 (Vallebuona, 2004), indicating a greater risk of exposure to these substances within the general population.

This work was supported by the General Direction of Investigation and Postgraduate of the Universidad Católica del Norte for F. Pancetti, Bicentennial Program in Science and Technology PSD-11 for F. Pancetti and A. Dagnino-Subiabre, and Fondecyt 1030220 for B. Morales and F. Pancetti.

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HEALTH EFFECTS FROM EXPOSURE TO ORGANOPHOSPHATES

The effects induced by exposure to organophosphates in both humans and animals have been clinically and toxicologically described and depend basically on three factors: the type of organophosphate, the doses, and the duration of exposure (Pope, 1999). For example, exposure to high concentrations of organophosphates for short periods leads to the classic cholinergic syndrome, which is produced by inhibition of acetylcholinesterase activity (Ivens et al., 1998; Clegg & van Gemert, 1999) resulting in over stimulation of nicotinic and muscarinic receptors. This produces the typical signs and symptoms of acute intoxication such as hypersecretion in respiratory pathways, ataxia, and respiratory paralysis, among others (Abou-Donia, 1992). Exposure to high doses of some organophosphates for short periods of time induces a syndrome called delayed peripheral neuropathy (OPIDN, organophosphorus-induced delayed neuropathy), whose signs and symptoms appear days after acute exposure (Abou-Donia & Lapadula, 1990; Pope et al., 1993) and coincide with the inhibition of an enzyme called neurotoxic esterase (NTE) (Johnson, 1993; Glynn, 1999). The appearance of a neuromuscular transmission syndrome known as intermediate syndrome also occurs after an episode of acute intoxication; however, its onset is earlier than that of OPIDN and its etiology is unknown (Senanayake & Karalliedde, 1987; Good et al., 1993; Karalliedde et al., 2006). Each of the three syndromes just mentioned occurs after an episode of acute intoxication characterized by an inhibition in acetylcholinesterase (AChE) activity. However, the participation of other targets cannot be ruled out. For instance, the enzyme NTE is involved in OPIDN, and there may be other targets not yet described that participate in the intermediate syndrome (Pope, 1999; Jamal et al., 2002; Karalliedde et al., 2006).

EXPOSURE TO ORGANOPHOSPHATES AND COGNITIVE SYNDROME

Aside from the three syndromes already described, several epidemiology studies and other studies done in animals demonstrated the existence of a syndrome related to cognitive dysfunction in which memory and learning processes deteriorate (McDonald et al., 1988; London et al., 1997; Prendergast et al., 1997; Ivens et al., 1998; Ray, 1998). However, the relationship between the cognitive dysfunction and inhibition of AChE activity has not been established in these studies (Nio & Breton, 1994; Richards et al., 2000; Bomser and Casida, 2001; Ray & Richards, 2001). This was clearly demonstrated in epidemiological studies where exposure to low doses of organophosphates was unable to evoke any cholinergic symptoms or inhibition of plasma cholinesterase activity (Ray & Richards, 2001). It is also important to mention that studies aimed at elucidating the effects of exposure to low doses of organophosphates in cognitive processes produced contradictory results where it was shown that duration of exposure is decisive in determining the type of effect observed (Ray & Richards, 2001).

EFFECTS OF LONG-TERM EXPOSURE TO ORGANOPHOSPHATES ON COGNITIVE PROCESSES

Review of the literature demonstrates that the neurotoxicity, that is, the deterioration of cognitive or neurological processes, is directly related to duration of exposure to organophosphates (Jamal et al., 2002). In humans, several epidemiological studies employed a battery of neurological and cognitive tests (Metcalf & Holmes, 1969; Rosenstock et al., 1991; Steenland et al., 1994; London et al., 1997; Roldan-Tapia et al., 2005, 2006) that established that long-term exposure to organophosphates produces a neurotoxicity described as a "neuropsychiatric disorder induced by chronic exposure to organophosphates" (Jamal et al., 2002). Furthermore, it was shown that the appearance of this disorder did not always correlate with a decreased cholinesterase activity, neither before nor during the clinical period, suggesting that other proteins may be susceptible to the action of organophosphates (Jamal et al., 2002; Salvi et al., 2003). Similar results were found in lab animals chronically exposed to organophosphates that were tested using the Morris I maze for referential memory, Morris II for working memory, and tests for active and passive avoidance (Kelly

et al., 1994, 1997; Prendergast et al., 1998). However, there is no agreement concerning to this issue, due to some authors considering that there is not sufficient evidence in order to conclude that chronic, low-level exposure to organophosphates could be related to neuropsychological impairment (Clegg & van Gemert, 1999). Costa (2006) clearly states the difference in "long-term adverse central nervous system effects" with and without initial cholinergic signs of toxicity. At least in those cases of initial acute exposure to organophosphates related to AChE, inhibition seems to be in consensus with the association between chronic exposure to organophosphates and long-term neurobehavioral adverse effects (Costa, 2006).

EFFECTS OF SUBACUTE EXPOSURE TO ORGANOPHOSPHATES ON COGNITIVE PROCESSES

Some studies reported that exposure to low doses of organophosphates for short periods of time, instead of producing neurotoxicity, exerted just the opposite effect, with potentiation of cognitive functions. For example, a study that only applied the Morris I maze test to rats exposed for 13 wk to low doses of the insecticide parathion showed that these rats displayed an increase in behavior related to memory and learning only during the first week, with the effect disappearing during the following weeks (Ivens et al., 1998). Interestingly, prolonged exposure to low doses of parathion in this study did not produce effects that could be considered neurotoxic and at no time was a decrease in AChE activity detected. The authors of this work concluded that neurotoxicity is only related to a higher inhibition of AChE. This study contradicts the conclusion of Jamal et al. (2002), who stated that neurotoxicity is related to the time of exposure, independent of the type of target protein.

Other investigations also detected an increase in cognitive performance unrelated to inhibition of AChE. For example, one study administered metrifonate, dichlorvos, diisopropylfluorophosphate (DFP), and paraoxon to a group of rats 30 min before undergoing a Morris water maze test (van der Staay et al., 1996b). Metrifonate is a pro-drug that produces dichlorvos and has been used in therapy for Alzheimer´s disease patients to increase their cognitive performance (Knopman, 1998). After administration, metrifonate is converted to the active agent dichlorvos by a nonenzymatic reaction (Hinz et al., 1996). To date, this drug has been withdrawn by Bayer due to reported cases of patients suffering respiration paralysis and problems with muscular neurotransmission after taking this medicine (Lopez-Arrieta & Schneider, 2006). Interestingly, it was reported that paraoxon, a potent inhibitor of AChE, exerted no effect on the cognitive parameters measured (van der Staay et al., 1996b). In another study carried out by the same group, it was found that the potentiation of cognitive processes only occurred at low doses of metrifonate at which there was no inhibition of AChE (van Der Staay et al., 1996a). Proposed therapies to treat Alzheimer´s disease with drugs derived from organophosphates are based on potentiating cholinergic transmission. However, this study showed that such an effect was brought about by other pathways, indicating that metrifonate and/or its degradation product dichlorvos may act on target proteins other than AChE. These results reinforce the hypothesis that exposure to low doses of organophosphates for short periods of time (subacute exposure) potentiates cognitive processes through pathways independent of cholinergic transmission.

ACYLPEPTIDE HYDROLASE AND COGNITIVE ACTIONS

A new target of action may be implicated in the participation of organophosphates on cognitive processes through noncholinergic pathways: the enzyme acylpeptide hydrolase. In studies using homogenized pig brain, this enzyme was identified as a direct target for some organophosphate compounds (Richards et al., 2000). Despite the fact that this enzyme was described more than 30 years ago (Witheiler & Wilson, 1972), its exact biological function has not been determined. Acylpeptide hydrolase belongs to a family of prolyl-oligopeptidases and catalyzes the hydrolysis of short peptides of the type N^{α}-acyl to form an acyl amino acid and a peptide with a free N-terminus. Richards et al. (2000) claimed that the discovery of this new target of action has pharmacological

relevance since dichlorvos exhibits a high affinity for acylpeptide hydrolase. Given the proposal that the mechanism of action of dichlorvos is through inhibition of AChE, it is surprising that it binds with such high affinity to acylpeptide hydrolase. Indeed, dichlorvos and DFP react 6.6 and 10.6 times more quickly, respectively, with acylpeptide hydrolase than with AChE (Richards et al., 2000). In addition, as previously mentioned, these organophosphate compounds improve memory and learning processes in rats at low doses that are unable to inhibit AChE (van der Staay et al., 1996b). Taken together, these facts support the hypothesis that inhibition of acylpeptide hydrolase activity is related to cognitive improvement.

There is evidence that implicates other members of the prolyl-oligopeptidase family with processes associated with the nervous system. For example, prolyl-oligopeptidase (EC 3.4.21.26) exhibits the highest concentration of all other brain peptidases and is involved in various disorders of the central nervous system such as depression and anorexia (Maes et al., 1994, 2001). Interesting evidence that helps to reaffirm the role of prolyl-oligopeptidase in cognitive processes comes from a study showing that increased activity levels of this enzyme may contribute to symptoms such as memory loss in Alzheimer´s disease patients (Polgar, 2002). In addition, some inhibitors of prolyl-oligopeptidase have been designed to function as potentiators for cognitive processes (Morain et al., 2002).

POSSIBLE PARTICIPATION OF ACYLPEPTIDE HYDROLASE IN PROCESSES OF SYNAPTIC PLASTICITY

The area of the brain responsible for the adquisition of new memories is the hippocampus (Fries et al., 2003); therefore, different models of synaptic plasticity can be studied in this section. Synaptic plasticity is fundamental in the regulation of efficient synaptic transmission between two or more neurons and is crucial for cognitive processes such as memory and learning.

Plasticity is defined as the capacity to produce permanent changes in the characteristics of a system (anatomical or functional) based on experience. On a cellular level, neurons depend on synaptic transmission to initiate changes that lead to plasticity processes. Long-term potentiation (LTP) is a model of synaptic plasticity that consists of an increase in the size of the postsynaptic response during a long period of time, after the application of presynaptic tetanic stimuli at 100 Hz. On the other hand, application of a low-frequency stimulus (1 Hz for 15 min) induces a decrease in the postsynaptic response known as long-term depression (LTD). Extensive studies related to models of synaptic plasticity were carried out in the hippocampus due to its defined anatomic structure and its important role in the formation of new memories (Bliss & Lomo, 1973; Soderling & Derkach, 2000). Transversal slices clearly demonstrate a layer of pyramidal neurons located in the periphery of the hippocampus denominated as CA1, CA2, CA3, and CA4 according to their position in the tissue. Usually, LTP is induced by stimulation of the area comprising CA3 pyramidal neurons and registered in CA1 neurons. This protocol is known as stimulation in Schaffer´s collaterals, which corresponds to CA3 neuronal axons that project towards the dendrites of CA1 neurons (Malenka & Nicoll, 1999; Malenka & Bear, 2004). Indeed, the biophysical basis for this type of synaptic plasticity is well known due to studies done in CA1 pyramidal neurons (Malenka & Nicoll, 1999; Malenka & Bear, 2004). Briefly, the more accepted LTP model suggests that the release of glutamate from the presynaptic terminal activates an AMPA-type glutamate receptor. This allows the entrance of Na^+, leading to a depolarization in the postsynaptic membrane that removes Mg^{2+} blocking the NMDA-type glutamate receptor permitting the entrance of Ca^{2+} into the dendritic button. Finally, Ca^{2+} activates protein kinases such as calcium- and calmodulin-dependent protein kinase II (CaMKII) and camp-dependent protein kinases (PKA). Activation of these kinases enables the insertion of new AMPA-type receptors in the membrane, generating a second response of greater magnitude than that seen with the first stimulus (Sanes & Lichtman, 1999).

Results of LTP experiments done in our lab and recently published in abstract form demonstrated that inhibition of acylpeptide hydrolase by doses of dichlorvos that do not affect AChE increased significantly (approximately 100%) the magnitude of the LTP in glutamatergic synapses within the CA3→CA1 pathway of the hippocampus. It was also shown that α_7 nicotinic receptors

participated, since the effect was reversed when these receptors were specifically blocked with methyllicaconitine (MLA) (Ji et al., 2001).

Nicotinic receptors constitute a diverse family of ion channels possessing nine α subunits (α_2–α_{10}) and three different β subunits (β_2–β_4). Acetylcholine nicotinic receptors having the α_7 subunit are the most abundant in the brain and participate in important processes such as modulation of neurotransmitter release, induction of postsynaptic excitatory responses, regulation of synaptic plasticity processes in the hippocampus like LTP, and regulation of cognitive functions (Kawai et al., 2002; Levin, 2002). The α_7 nicotinic receptors were detected immunohistochemically in both presynaptic and postsynaptic elements (Fabian-Fine et al., 2001). In rat hippocampus, presynaptic α_7 nicotinic receptors modulate the release of neurotransmitters such as glutamate and GABA (Alkondon et al., 1997; Maggi et al., 2001). Postsynaptically, α_7 nicotinic receptors are distributed in dendritic spines and other membrane structures, suggesting that these receptors may undergo dynamic regulation of insertion and internalization (Fabian-Fine et al., 2001).

It was confirmed in the literature that α_7 nicotinic receptors are susceptible to allosteric modulation by peptides such as the β–amyloid 1–42 fragment (Wang et al., 2000a; 2000b; Pettit et al., 2001; Dineley et al., 2002; Espinoza-Fonseca, 2004), peptides derived from apoliprotein E (Klein & Yakel, 2004), or a peptide fragment originating from the C-terminus of AChE (Greenfield et al., 2004; Zbarsky et al., 2004). Nicotinic receptors are also be affected by direct action of anticholinesterase agents that result in either a blockade or potentiation of these receptors (Maskell et al., 2003; Smulders et al., 2004, 2005). However, such a significant effect induced by dichlorvos is excluded since this receptor desensitizes quickly, thereby ruling out any possible direct effect of this organophosphate (Dr. Henk Vijverberg, personal communication).

Results obtained in our laboratory lead us to believe that inhibition of acylpeptide hydrolase by dichlorvos possibly blocks its activity toward *N*-acylpeptide, which is its endogenous substrate. Since *N*-acylation of peptides is, in general terms, a protective mechanism from proteolysis, inhibition of acylpeptide hydrolase would enable either acylpeptide hydrolase or its substrates to remain in the tissue inducing a type of allosteric affect on α_7 nicotinic receptors. If the effect is produced on postsynaptic α_7 nicotinic receptors that are homomeric channels permeable to Ca^{+2} (Utkin Yu et al., 2000), the increase in postsynaptic Ca^{+2} may be a triggering factor for LTP.

In summary, evidence suggests that acylpeptide hydrolase plays a predominant role in the control of presynaptic biopeptide concentrations that are contained in synaptic vesicles and released to the synaptic space exerting a neuromodulator effect at a postsynaptic level. One issue still remaining is to determine the endogenous substrate for acylpeptide hydrolase in these hippocampal synapses.

ACYLPEPTIDE HYDROLASE: A NEW BIOMARKER?

A biomarker for molecular exposure is a molecule of biological origin that is found in blood, urine or any body fluid that can be collected. A good biomarker should undergo alteration in function and/or expression (presence in the fluid) when exposed to a contaminating agent. In addition, the alteration should be specific for only the biomarker and no other molecules present in the sample (Kennedy, 2002; Scaros & Fisler, 2005). A classic biomarker for determining exposure to organophosphates is cholinesterase activity (acetylcholinesterase or butyrilcholinesterase) in plasma (Quistad et al., 2005). However, the inhibition of this enzymatic activity is almost always accompanied by signs of intoxication already mentioned; therefore, its usefulness as an exposure indicator to low doses of organophosphates is limited and does not serve as a means to predict exposure risk.

There exist different strategies to find and identify new and better biomarkers of exposure. One of them is to first determine the physiological functions that are affected by the action of low doses of a compound using in vitro experimental models. Once the effect has been identified, the molecular mechanisms responsible for this effect can be elucidated. In this way, the biological molecules (generally proteins with enzymatic activity) that are direct targets of action for a compound in a tissue of interest can be pinpointed. In the case of searching for biomarker molecules in neurotoxicological processes, it is necessary to look for proteins equivalent to those found in the

nervous system but that are present in a body fluid that can be collected. The enzyme acylpeptide hydrolase possesses sufficient characteristics to be considered as a potential biomarker for exposure to organophosphates since it has a high affinity for some compounds, and besides being present in the brain, it is also found in erythrocytes (Jones & Manning, 1985). Furthermore, published studies as well as our preliminary results point to acylpeptide hydrolase as having an important role in transmission and synaptic plasticity processes at a molecular level in cognitive phenomena. This finding, together with the increased sensitivity of this enzyme to inhibition by some organophosphates, indicates that acylpeptide hydrolase is a highly sensitive biomarker for organophosphate exposure in the screening for neurotoxicity. The inhibition of acylpeptide hydrolase by the organophosphate DFP in erythrocytes was previously reported (Scaloni et al., 1992). Recently, Quistad et al. (2005) evaluated the usefulness of erythrocyte acylpeptide hydrolase as a biomarker enzyme for exposure to organophosphates. They concluded that acylpeptide hydrolase is a reliable biomarker for some organophosphates that showed a higher specificity for this enzyme than for cholinesterases found in the blood and brain. Specifically, their work demonstrated that dichlorvos is one of the organophosphates that showed selectivity for acylpeptide hydrolase inhibition in vivo.

CONCLUSION

In summary, evidence shows that organophosphate pesticides exert a wide range of effects that vary from neurotoxicity to an increase in cognitive capacity, depending on the type of organophosphate, the dose, and the duration of exposure. The mechanisms underlying the neurotoxic effects produced by organophosphates have been widely studied, but that has not been the case concerning mechanisms responsible for the increase in cognitive performance observed at subacute doses. With regard to some organophosphates, cholinergic transmission does not seem to be implicated. Based on the presented evidence, it is possible to conclude that: (1) The existence of other targets than AChE for organophosphate actions is gaining acceptance; (2) there is a direct relationship between the appearance of neurotoxicity and duration of exposure, independent of whether the effect is mediated by AChE or other target proteins; (3) at high doses of organophosphates, neurotoxicity is related to AChE inhibition; (4) exposure to low doses of organophosphates for short time periods (subacute) favors cognitive processes through cholinergic-independent pathways, with these mechanisms being the object of investigation; (5) the enzyme acylpeptide hydrolase has been identified as a new target of action for organophosphate compounds—this enzyme may be implicated in synaptic plasticity processes responsible for the cognitive effects induced by organophosphates; and finally, (6) the evaluation of acylpeptide hydrolase is a possible biomarker for exposure to organophosphates, which from an epidemiological point of view is an interesting aspect to develop.

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