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ACTIVATION AND DEGRADATION OF THE PHOSPHOROTHIONATE INSECTICIDES PARATHION AND EPN BY RAT BRAIN*

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Abstract—Cytochrome P-450-dependent monooxygenases are known to activate phosphorothionate insecticides to their oxon (phosphate) analogs by oxidative desulfuration. These activations produced potent anticholinesterases, decreasing the I_{50} values to rat brain acetylcholinesterase almost 1000-fold (from the 10^{-5} M range to the 10^{-8} M range). Since the usual cause of death in mammals from organophosphorus insecticide poisoning is respiratory failure resulting, in part, from a failure of the respiratory control center of the brain, we investigated the ability of rat brain to activate and subsequently degrade two phosphorothionate insecticides, parathion (diethyl 4-nitrophenyl phosphorothioate) and EPN (ethyl 4-nitrophenyl phenylphosphonothioate). Microsomes from specific regions (cerebral cortex, corpus striatum, cerebellum, and medulla/pons) of the brains of male and female rats and from liver were incubated with the phosphorothionate and an NADPH-generating system. Oxon production was quantified indirectly by the amount of inhibition resulting in an exogenous source of acetylcholinesterase added to the incubation mixture as an oxon trap. The microsomal activation specific activity was low for brain when compared to liver [0.23 to 0.44 and 5.1 to 12.0 nmol·min^{-1}·(g tissue)$^{-1}$ respectively]. The mitochondrial fraction of the brain possessed an activation activity for parathion similar to that of microsomes [about 0.35 nmol·min^{-1}·(g tissue)$^{-1}$ for each fraction], but mitochondrial activity was slightly greater than microsomal activity for EPN activation [0.53 to 0.58 and 0.23 to 0.47 nmole·min^{-1}·(g tissue)$^{-1}$]. Whole homogenates were tested for their ability to degrade paraoxon and EPN-oxon (ethyl 4-nitrophenyl phenylphosphonate), quantitated by 4-nitrophenol production. Specific activity for oxon degradation in liver was greater than that in brain [31 to 74 and 1.1 to 10.7 nmole·min^{-1}·(g tissue)$^{-1}$ respectively]. Overall, the brain and liver had about 1.5- to 12-fold higher specific activities for degradation than activation depending on the compound used. These findings demonstrate that the brain possesses both phosphorothionate activation and oxon degradation abilities, both of which may be significant during exposures to organophosphorus insecticides.

The ability of cytochrome P-450-dependent monooxygenases to activate phosphorothionate insecticides to potent anticholinesterases (i.e. the phosphate or oxon forms) has been well known for many years [1–4]. The organophosphorus insecticides parathion (diethyl 4-nitrophenyl phosphorothioate) and EPN (ethyl 4-nitrophenyl phenylphosphonothioate) are activated to their oxon analogs, paraoxon (diethyl 4-nitrophenyl phosphate) and EPN-oxon (ethyl 4-nitrophenyl phenylphosphonate) respectively [5, 6]. This reaction requires reduced NADP and O_2 and is inhibited by CO [7, 8].

The usual cause of death in mammals from acute organophosphate poisoning is respiratory failure. One factor leading to death by respiratory failure is inhibition of the respiratory control center of the brain [9]. It has been generally assumed that the majority of the active metabolite is produced in the liver and is released into the circulation to find its target, acetylcholinesterase (AChE). While the liver is typically the most active organ in the body for xenobiotic activation and detoxication reactions, Neal and coworkers suggested that extrahepatic activation of parathion to paraoxon may be very important to its toxicity [6, 10]. Parathion activation activity by microsomes isolated from rat lung has been documented [6]. While isolated hepatocytes absorbed parathion rapidly but reversibly, perfusion studies indicated that intact parathion exits the liver [11]. This parathion released into the circulation could play a major role in phosphorothionate poisoning if it were activated at a critical target site. Additionally, phosphorothionate absorbed following a dermal exposure, a more likely route for occupational and many accidental poisonings, could circulate directly to extrahepatic tissues, including the target tissues. Sultatos *et al.* [12] found that albumin could serve as a means of transport for parathion in the blood. They reported an esterase-like activity of bovine serum albumin for paraoxon. Further, the oxons can phosphorylate other blood components such as erythrocyte AChE and serum butyrylcholinesterase and B-esterases. Thus, because of the potential degradation of the oxons by the blood proteins and phosphorylation of blood proteins by the oxons, it is unlikely that significant amounts of oxon produced in the liver could reach brain AChE.

Cytochrome P-450-dependent monooxygenase activity has been documented for microsomes isolated from rat brain [6]. More recent work, however, suggests that monooxygenase activity is present in

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brain mitochondrial fractions as well [13, 14]. Even a small rate of phosphorothionate activation activity in brain may be significant in intoxication because of the proximity of the reaction to target AChE.

The major route of detoxication of the active metabolite is hydrolysis. Oxon analogs of organophosphorus insecticides are cleaved by Ca^{2+}-dependent phosphotriesterases. These soluble enzymes are classified as A-esterases and cleave paraoxon [15, 16] and EPN-oxon, releasing 4-nitrophenol.

This study was designed to determine the specific activities in rat brain of enzymes activating phosphorothionate insecticides and of enzymes hydrolyzing the activated oxon metabolites. Studies were conducted on parathion and EPN and their oxon analogs, paraoxon and EPN-oxon, respectively. While activation activity of the phosphorothionates in brain was found to be low when compared to liver, it may be sufficient to contribute to symptoms of organophosphate poisoning. However, subsequent oxon degradation by brain tissue may offer a limited means of target site protection during exposures to organophosphorus insecticides.

METHODS

Chemicals. All reagents and biochemicals were obtained from the Sigma Chem. Co. (St. Louis, MO). Parathion, EPN, paraoxon, and EPN-oxon were synthesized by Howard Chambers; purities of the phosphorothionates were greater than 99% and of the oxons, greater than 95%.

Tissue preparation for monooxygenase assay. Prior to use, adult female and male Sprague–Dawley derived rats [Crl:CD(SD)BR] from an original Charles River stock, 2- to 5-months-old, were housed in a room with controlled temperature (22 ± 2°) and a 12:12 hr light cycle; they had free access to Purina laboratory rodent chow and water. Animals were killed by decapitation, and the liver and brain were rapidly removed and chilled. The tissues were homogenized in 50 mM Tris–HCl buffer + 0.15 M KCl (pH 7.4) with a glass–Teflon motor-driven homogenizer. A mitochondrial fraction was isolated from brain tissue by centrifuging the supernatant fraction of a 5000 *g* (10 min) centrifugation at 17,000 *g* for 15 min. The resulting pellet will be referred to as mitochondria and is considered to be a mitochondrial–synaptosomal preparation. A microsomal fraction was isolated from both brain and liver homogenates by centrifuging a 17,000 *g* (15 min) supernatant fraction at 110,000 *g* for 60 min. All centrifugations were conducted in a Beckman L2–50 ultracentrifuge at 4°. The mitochondrial and microsomal pellets were layered with 1 vol. of 100 mM Tris buffer + 5 mM $MgCl_2$ (pH 7.4) and were frozen until used.

Monooxygenase activation assay. Monooxygenase procedures incorporated methods described by Norman and Neal [6] and Levi and Hodgson [5]. Microsomes from brain and liver and mitochondria from brain were incubated in 100 mM Tris–HCl buffer + 5 mM $MgCl_2$ (pH 7.4) in the presence or absence of an NADPH-generating system consisting of 75 mM glucose-6-phosphate, 7.5 mM NADP, and 1 unit glucose-6-phosphate dehydrogenase. The total incubation volume of 1 ml contained either 0.02 g brain microsomes or mitochondria or 0.004 g liver microsomes (wet weight equivalent). As an exogenous source of AChE, used to trap the oxon as it was formed, 32 mg (wet weight equivalent) of a bovine brain suspension was also added to each reaction mixture. This suspension was a 1000 *g* (15 min) pellet resuspended in 100 mM Tris–HCl buffer (pH 7.4). Reaction mixtures not containing an NADPH-generating system and bubbled for 1 min with CO were used as controls. Reactions containing an NADPH-generating system were bubbled for 30 sec with an $O_2:CO_2$ (95:5) mixture to assure adequate amounts of oxygen. Incubations were carried out in 25-ml Erlenmeyer flasks in a shaking water bath at 37°. Incubation times were 5 min for brain microsomes or mitochondria and 2 min for liver microsomes. Reactions were started by adding 5 μl parathion or EPN solution (in ethanol) to a final concentration of 50 μM. All reactions were stopped by bubbling CO through the mixtures for 1 min, chilling, and diluting with cold 100 mM Tris–HCl buffer + 5 mM $MgCl_2$ (pH 7.4) to 4 ml. These reaction mixtures were immediately assayed for AChE activity as described below.

Acetylcholinesterase assay. Reaction mixtures from the monooxygenase assays were incubated at 37° for 15 min to allow ample time for the inhibition of the exogenous AChE by the oxon produced by the microsomal or mitochondrial monooxygenases. The AChE was isolated from the reaction mixtures by centrifuging for 2 min at 17,500 *g* in an Eppendorf microcentrifuge at room temperature. The pellets were resuspended to 2 mg equivalents/ml in 50 mM Tris–HCl buffer (pH 8.0) in a total volume of 4 ml. All homogenates were then incubated for 15 min at 37° in a shaking water bath to allow for temperature equilibration. The amount of enzyme activity was quantified by a modification of the technique of Ellman *et al.* [17] using 0.75 mM acetylthiocholine iodide as the substrate with a 15-min incubation time. Each reaction was stopped with the addition of 0.5 ml of a mixture of 0.02% 5,5′-dithiobis(nitrobenzoic acid) plus 0.4% sodium dodecyl sulfate (final concentrations). The E_{412} was determined using a Perkin–Elmer Lambda 5 spectrophotometer, and the percent inhibition was determined for each set of reaction conditions compared to a non-inhibited control. The results were compared to standard curves of bovine brain AChE inhibited by each of the oxons to quantify the amount of the oxons. The amount of the oxons produced was calculated by linear regression of the log concentration versus logit inhibition which is mathematically equivalent to the computations used in a Hill plot [18].

The I_{50} values of the two phosphorothionates and their oxon analogs were also determined for the AChE of selected rat brain regions. Previously frozen intact regions were homogenized in 50 mM Tris–HCl buffer (pH 8.0) and were assayed at the following concentrations: cerebral cortex, 1 mg/ml; corpus striatum, 0.2 mg/ml; cerebellum, 1.5 mg/ml; medulla/pons, 1 mg/ml; and combined regions, 1 mg/ml (contained about 12% cerebral cortex, 10% corpus striatum, 42% cerebellum, and 36% medulla/

pons to serve as a representative sample of critical brain regions). After a 15-min temperature equilibration period, the reactions were initiated by the addition of the phosphorothionate or its oxon analog (in 40 μl ethanol) and incubating for 15 min at 37° in a shaking water bath to allow for inhibition of the AChE. The control contained only ethanol. The amount of AChE activity was then assayed as described above and the percent inhibition was calculated compared to the control. The I_{50} for each replication was calculated by linear regression of the log concentration versus logit inhibition.

Oxon degradation assay. Phosphotriesterase activity toward paraoxon and EPN-oxon was determined as described by Chambers and Yarbrough [19] by the spectrophotometric detection of the 4-nitrophenol released. Whole homogenates of 1% liver and 20% brain were prepared in 0.05 M sodium acetate buffer + 1 mM $MgCl_2$ + 1 mM $CaCl_2$ (pH 6.8). To prevent reaction with AChE in the brain samples, 4 μl of the carbamate anticholinesterase eserine was added (in ethanol) to a final concentration of 0.1 mM 15 min prior to the addition of substrate. Reactions were started by adding a final concentration of the oxon (in ethanol) of 1 mM. A buffer blank was also run to correct for nonenzymatic hydrolysis. Samples were incubated at 37° for 45 min for brain homogenates and 20 min for liver homogenates. Reactions were stopped with an equal volume of 10% trichloroacetic acid in 1:1 acetone–water and the precipitated proteins were removed by centrifugation for 3 min at 17,500 *g* at room temperature. The E_{400} of 0.8 ml of the clear supernatant fraction plus 1.6 ml of 8% Tris base was read to determine the amount of 4-nitrophenol released. To correct for turbidity, 3–4 drops of concentrated HCl was added to the mixture and the absorbance was subtracted from the original reading. The amount of 4-nitrophenol produced was calculated using a molar extinction coefficient of $18.8 \times 10^3\ AU^{-1}$ mole-cm^{-1}.

Protein determinations. Protein concentrations were determined by the method of Lowry *et al.* [20] using bovine serum albumin as the standard. Protein concentrations were determined for each tissue sample to ensure that individual samples were prepared consistently.

Statistical analysis. An analysis of variance was run for each data set (oxon production, I_{50}, or 4-nitrophenol production), using the SAS computer program, and the means were separated by the Least Significant Difference method (LSD).

RESULTS

Acetylcholinesterase sensitivity studies. The rat brain AChE I_{50} values of parathion were about 1000-fold greater than those of paraoxon (Table 1). Female brain regions were less sensitive than male brain regions to both parathion and paraoxon, with significant differences occurring between the sexes in the cerebral cortex and the corpus striatum. Small but significant differences were seen among brain parts within females for both parathion and paraoxon, but not within male brain parts. An almost 1000-fold higher I_{50} was also seen for EPN when

Table 1. Sensitivity of acetylcholinesterase in various brain regions to the phosphorothionates parathion and EPN and their oxon analogs, paraoxon and EPN-oxon, respectively, in the rat

	I_{50}^*				
Sex	Combined brain regions	Cerebral cortex	Corpus striatum	Cerebellum	Medulla/pons
Parathion ($\times 10^{-5}$ M)					
Female	4.35 ± 0.13^{aA} (4)	5.26 ± 0.25^{bcA} (3)	5.50 ± 0.19^{bA} (3)	4.67 ± 0.37^{acA} (3)	4.42 ± 0.27^{aA} (3)
Male	4.00 ± 0.53^{aA} (3)	4.52 ± 0.04^{aB} (3)	4.05 ± 0.12^{aB} (3)	4.34 ± 0.07^{aA} (3)	4.24 ± 0.09^{aA} (3)
Paraoxon ($\times 10^{-8}$ M)					
Female	2.62 ± 0.18^{aA} (4)	3.23 ± 0.20^{bA} (3)	3.09 ± 0.28^{abA} (3)	2.64 ± 0.18^{aA} (3)	2.99 ± 0.18^{abA} (3)
Male	2.37 ± 0.10^{aA} (3)	2.61 ± 0.15^{aB} (2)	2.38 ± 0.20^{aB} (3)	2.38 ± 0.05^{aA} (3)	2.44 ± 0.05^{aB} (3)
EPN ($\times 10^{-5}$ M)					
Female	2.73 ± 0.08^{aA} (3)	2.95 ± 0.08^{bA} (3)	2.69 ± 0.64^{aA} (3)	2.72 ± 0.14^{abA} (3)	2.67 ± 0.02^{aA} (3)
Male	2.40 ± 0.06^{aB} (3)	2.46 ± 0.14^{aB} (3)	2.42 ± 0.07^{aB} (3)	2.38 ± 0.05^{aB} (3)	2.73 ± 0.06^{bA} (3)
EPN-oxon ($\times 10^{-8}$ M)					
Female	3.55 ± 0.13^{acA} (3)	3.53 ± 0.05^{acA} (3)	2.97 ± 0.05^{aA} (3)	4.21 ± 0.20^{bA} (3)	3.75 ± 0.12^{bcA} (3)
Male	3.30 ± 0.12^{aA} (3)	3.58 ± 0.12^{aA} (3)	3.46 ± 0.39^{aA} (3)	4.28 ± 0.20^{bA} (3)	3.55 ± 0.08^{aA} (3)

* Values are means ± SEM (N).

[abc] Means within rows not followed by the same letter differ ($P < 0.05$) as determined by LSD.

[AB] Means within columns for each compound not followed by the same letter differ ($P < 0.05$) as determined by LSD.

Table 2. Phosphorothionate activation activity by rat brain regions and liver microsomes

Sex	Specific activity* [nmol · min^{-1} · g tissue^{-1}]				
	Cerebral cortex	Corpus striatum	Cerebellum	Medulla/pons	Liver
Parathion					
Female	0.36 ± 0.05^{aA} (4)	0.33 ± 0.05^{aA} (5)	0.27 ± 0.04^{aA} (5)	0.33 ± 0.04^{aA} (5)	5.09 ± 0.49^{bA} (6)
Male	0.29 ± 0.06^{aA} (4)	0.23 ± 0.07^{aA} (4)	0.37 ± 0.05^{aA} (6)	0.44 ± 0.09^{aA} (6)	11.95 ± 1.94^{bB} (8)
EPN					
Female	0.28 ± 0.02^{aA} (3)	0.30 ± 0.10^{aA} (4)	0.27 ± 0.04^{aA} (4)	0.40 ± 0.03^{aA} (5)	7.12 ± 2.31^{bA} (4)
Male	0.34 ± 0.13^{aA} (5)	0.23 ± 0.05^{aA} (5)	0.44 ± 0.10^{aA} (6)	0.44 ± 0.04^{aA} (5)	10.59 ± 0.46^{bB} (4)

* Values are means ± SEM (N).
^{ab} Means within rows not followed by the same letter differ ($P < 0.05$) as determined by LSD.
^{AB} Means within columns for each compound not followed by the same letter differ ($P < 0.05$) as determined by LSD.

compared to that of EPN-oxon, again reflecting the potency of the oxon metabolite (Table 1). Female brain regions were generally less sensitive to EPN than male brain regions, with significant differences seen between the combined regions, cerebral cortex, corpus striatum, and cerebellum. Overall, however, there was no significant difference in the sensitivity of male and female brain regions to EPN-oxon. Small significant differences were seen among brain parts within female and male regions to both EPN and EPN-oxon.

Phosphorothionate activation. Microsomes from male and female rat brain regions and liver were capable of activating both parathion and EPN to their oxon analogs, paraoxon and EPN-oxon, respectively (Table 2). However, specific activities for brain were low when compared to those of the liver. In the method used, as described above, an exogenous source of AChE was present during the activation reaction to serve as a trap for the oxons as they were formed. In preliminary studies, a substantially lower amount of AChE inhibition was observed if the exogenous AChE was added after termination of the activation reaction (data not shown). It is assumed that either detoxication of the paraoxon was occurring very rapidly after its formation, or that the paraoxon was rapidly phosphorylating microsomal proteins; the latter is likely since the oxons are quite reactive and would not be expected to remain unreacted in a protein-rich environment for any length of time. Therefore, it is assumed that this method, by including a trapping system for the active metabolite within the assay, yielded a more accurate indication of the amount of oxon produced. There were no significant differences in the amount of either oxon formed among brain regions within either sex, nor was there a difference between males and females for the individual brain regions. Microsomes isolated from male rat livers activated a significantly greater amount of both parathion (about 2.3-fold) and EPN (about 1.5-fold) than microsomes isolated from female rat livers. Activation activity was also found in the mitochondrial fraction as well as the microsomal fraction of combined brain regions (Table 3). There was no significant difference for either compound between the activation activity of male and female microsomes or mitochondria in combined brain regions. Parathion activation activities in brain microsomes and mitochondria were similar. However, mitochondrial EPN activation activity was about 1.1- and 2.5-fold higher than microsomal activity for males and females respectively.

Oxon degradation. Paraoxon was degraded by whole homogenates of rat brain and liver (Table 4). When eserine was not present in the reaction mixture, there was approximately 30% greater formation of 4-nitrophenol by both male and female combined brain regions (data not shown), presumably because of the release of 4-nitrophenol from

Table 3. Phosphorothionate activation activity by rat combined brain regions

Sex	Specific activity* [nmol · min^{-1} · g tissue $^{-1}$]	
	Microsomes	Mitochondria
Parathion		
Female	0.39 ± 0.05^{a} (5)	0.40 ± 0.05^{a} (5)
Male	0.30 ± 0.03^{a} (10)	0.34 ± 0.05^{a} (5)
EPN		
Female	0.23 ± 0.04^{a} (4)	0.58 ± 0.05^{a} (4)
Male	0.47 ± 0.10^{a} (4)	0.53 ± 0.09^{a} (4)

* Values are means ± SEM (N).
^{a} Means within columns for each compound were not significantly different ($P > 0.05$) as determined by LSD.

Table 4. Oxon degradation activity by various brain regions and liver

Sex	Specific activity* [nmol 4-nitrophenol · min^{-1} · g tissue^{-1}]					
	Combined brain regions	Cerebral cortex	Corpus striatum	Cerebellum	Medulla/pons	Liver
Paraoxon						
Female	1.26 ± 0.05^{abA} (4)	2.06 ± 0.07^{abA} (4)	2.45 ± 0.12^{bA} (4)	1.34 ± 0.03^{abA} (4)	0.73 ± 0.09^{aA} (4)	63.10 ± 1.79^{cA} (4)
Male	1.07 ± 0.06^{aA} (4)	2.01 ± 0.07^{abA} (4)	2.77 ± 0.26^{bA} (4)	1.25 ± 0.08^{abA} (4)	0.48 ± 0.12^{aA} (4)	73.80 ± 0.89^{cB} (4)
EPN-oxon						
Female	10.66 ± 0.20^{aA} (4)	11.93 ± 0.20^{aA} (3)	12.14 ± 0.38^{aA} (3)	10.05 ± 0.34^{aA} (3)	8.49 ± 0.43^{aA} (3)	30.72 ± 6.95^{bA} (4)
Male	10.45 ± 0.20^{aA} (4)	12.00 ± 0.30^{aA} (4)	12.31 ± 0.39^{aA} (4)	9.83 ± 0.39^{aA} (4)	8.67 ± 0.28^{aA} (4)	37.43 ± 2.84^{bB} (4)

* Values are means ± SEM (N).
^{ab} Means within rows not followed by the same letter differ ($P < 0.05$) as determined by LSD.
^{AB} Means within columns for each compound not followed by the same letter differ ($P < 0.05$) as determined by LSD.

paraoxon when AChE is phosphorylated. The 4-nitrophenol reported here would also not have been the result of monooxygenase-mediated oxidative dearylation since this reaction occurs with the phosphorothionates but not the oxons. Thus, the 4-nitrophenol released here in the eserine-inhibited samples would have been only from phosphotriesterase action. Significant differences in oxon degradation were found among brain regions within each sex, with the medulla/pons region having the lowest activity in each case. There was no difference between males and females for each of the brain regions tested, but male liver degraded a significantly greater amount of paraoxon (1.2-fold) than did female liver. Brain and liver homogenates also degraded EPN-oxon (Table 4). However, brain degradation activity was 9.1-fold greater for EPN-oxon than for paraoxon. There were no significant differences among brain regions for EPN-oxon degradation, but the medulla/pons region again showed lower activity than the other brain regions. Male liver homogenates degraded a significantly greater amount of EPN-oxon (1.2-fold) than female liver homogenates.

DISCUSSION

Xenobiotics that can cross the blood–brain barrier are potential substrates for the cytochrome P-450-dependent monooxygenase system of the brain. A greater amount of brain cytochrome P-450 has been reported in the mitochondrial fraction than in the microsomal fraction [13]. The results presented here indicate that rat brain is capable of activating the phosphorothionate insecticides parathion and EPN to the potent anticholinesterases, paraoxon and EPN-oxon, respectively, by an NADPH-dependent enzyme system, presumably cytochrome P-450-dependent monooxygenases. This activation specific activity is located in the microsomal as well as the mitochondrial fractions of the brain. The microsomal activation of parathion by male rat brain microsomes was reported by Norman and Neal [6]. As expected, brain activity was low when compared to liver. Mitochondrial activation specific activity has also been found in rat lung [6].

EPN-oxon degradation specific activity was much higher than EPN activation specific activity in both sexes in brain and liver (Table 5); the difference was particularly pronounced in brain. The same was true for paraoxon by livers of both sexes. However, paraoxon degradation specific activity by brain regions from males and females was only slightly greater than parathion activation specific activity. A several-fold greater oxon degradation activity compared to activation activity implies that the toxic metabolite may not accumulate to concentrations sufficient to cause symptoms of organophosphate poisoning from AChE inhibition. However, a kinetic analysis of parathion activation and paraoxon hydrolysis revealed that the K_m for paraoxon hydrolysis is significantly greater than the K_m for parathion activation [15, 21, 22]. This difference is reflected in the substrate concentrations used for the activation and degradation reactions, 50 μM and 1 mM respectively. Thus, a much higher con-

Table 5. Ratio of specific activities of degradation to total activation (microsomal + mitochondrial) of organophosphates by rat brain (combined regions) and liver

	Paraoxon degradation: Parathion activation		EPN-oxon degradation: EPN activation	
Tissue	Female	Male	Female	Male
Brain	1.59	1.67	13.16	10.45
Liver	12.40	6.17	4.31	3.53

centration of oxon is needed to reach maximum phosphotriesterase activity than phosphorothionate required to achieve maximum activation activity. Consequently, at a comparatively low rate of activation, as would be occurring in most tissues including the brain, the oxon would be hydrolyzed with a very low velocity since the substrate concentration would be expected to be considerably lower than the K_m.

As suggested by Wallace and Dargan [22], appreciable concentrations of paraoxon or EPN-oxon probably accumulate in the liver before being further metabolized and, therefore, exit from the liver. Once in the circulation, however, serum albumin has been shown to nonspecifically hydrolyze paraoxon while at the same time acting as a transport protein for parathion [12]. Additionally, other blood proteins, such as B-esterases and butyrylcholinesterase, are phosphorylated by a variety of oxons at very low concentrations*, leading to further oxon degradation. Thus, very little, if any, intact oxon would reach target sites in the brain, suggesting the importance of phosphorothionate activation activity in the brain. Also especially important is the fact that, even though brain activation specific activity was low, the rate of phosphorylation of rat AChE has been found to be rapid [23], indicating that the target enzyme will be quickly affected.

The well known sex-related differences in drug metabolism were seen in liver but not in brain. The lack of differences observed here between brain regions of males and females in either activation specific activity or phosphotriesterase oxon degradation does not explain why males are more resistant to parathion and EPN *in vivo* than females [24]. However, the *in vivo* toxicity data were reported from Sherman rats, a different strain than those used here. Greater LD_{50} values for males than females with oral doses of EPN and parathion [24] do not correlate with the I_{50} data presented here where female brain AChE was generally less sensitive than male to parathion, paraoxon, and EPN. However, other parameters which could influence sex differences in toxicity, such as other metabolic pathways (e.g. oxidative dearylation) or levels and sensitivities of protective proteins and enzymes, are unknown and are presently under investigation in our laboratories.

* Personal communication, Howard Chambers, Department of Entomology, Mississippi State University; cited with permission.

Both male and female rats are reported to be more sensitive to parathion than to EPN [24]. While no large difference between activation activity of parathion and EPN by brain was observed here, degradation by rat brain of EPN-oxon was much greater than that of paraoxon and, therefore, may be a means of protection against poisoning by EPN.

In summary, because of the high capacity of liver and blood proteins to be phosphorylated by the oxons and to hydrolyze them (rapidly in the case of the A-esterases and slowly in the case of the B-esterases), extrahepatic activation of phosphorothionate insecticides may be extremely important to their toxicity, as suggested earlier by Norman and Neal [6]. The results here indicate that the brain does have measurable levels of phosphorothionate activation activity, so it would be capable of producing some active metabolites. Although the brain has higher specific activities of the phosphotriesterases than the phosphorothionate activation enzymes, the former probably do not function very efficiently at low substrate concentrations, so oxon accumulation would occur. The slightly lower phosphotriesterase activities observed in the medulla/pons than the other brain parts may well make accumulation even more likely near the target respiratory control centre. Studies are currently underway to characterize more fully the rat brain phosphorothionate activation activity.

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