Pharmacology Biochemistry & Behavior, Vol. 40, pp. 929–936. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Acute Effects of the Organophosphate Paraoxon on Schedule-Controlled Behavior and Esterase Activity in Rats: Dose-Response Relationships[1]

RUSSELL L. CARR[2] AND JANICE E. CHAMBERS

College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762

Received 3 June 1991

CARR, R. L. AND J. E. CHAMBERS. *Acute effects of the organophosphate paraoxon on schedule-controlled behavior and esterase activity in rats: Dose-response relationships.* PHARMACOL BIOCHEM BEHAV **40**(4) 929–936, 1991.—The effects of acute intraperitoneal administration of paraoxon on behavioral and biochemical parameters were studied in male rats. Rats were trained to press a lever under an FR10 schedule of reinforcement. Rats were injected with 3 sublethal doses of paraoxon (0.5, 0.75, and 1.0 mg/kg) and performance was monitored for four days after exposure. Response rates were depressed significantly for days 1 and 2 with 0.75 and 1.0 mg/kg, but not 0.5 mg/kg, even though there was inhibition of brain and plasma cholinesterases at all doses. Performance recovered prior to brain AChE recovery. There was no clear-cut threshold of brain AChE inhibition required to yield performance deficits, nor was there a direct correlation between significant inhibition in peripheral enzymes which could serve as markers (plasma aliesterases, butyrylcholinesterase, non-iso-OMPA-sensitive cholinesterase, and hepatic aliesterases) and performance deficits, suggesting that other noncholinergic targets may play a role in OP-induced behavioral deficits.

Acetylcholinesterase Aliesterase Dose-response Paraoxon Schedule-controlled behavior

PRESENTLY, low levels of organophosphorus (OP) insecticides are frequently applied in agricultural and household situations. The risk of acute exposure to these compounds is a constant threat, and they are responsible for numerous poisonings annually (1). These insecticides or their metabolites are inhibitors of acetylcholinesterase (AChE) and exposure can lead to behavioral deficits (22, 30, 34).

The brain cholinergic system is implicated in learning and memory (1, 2, 35). According to the cholinergic theory of memory (8), remembering and forgetting are the results of time-dependent changes in the cholinergic synapse. It is thought that the OP's play a role in inhibition of memory by producing a cholinergic dysfunction at the level of the synapse. However, the main focus of past work dealing with the effects of OP's on behavior has been investigation of the more potent nerve gases (i.e., soman and sarin) and diisopropylfluorophosphate (DFP) (4, 13, 14, 19, 21). These compounds have somewhat different physiological effects than the insecticides. However, little behavioral work has been done with the less potent OP insecticides and that done has focused primarily on either higher doses (6), repeated exposures (22) or the development of tolerance (15). The adverse behavioral effects of acute low level insecticidal exposures are commonly overlooked in the investigation of OP's. Therefore, the evaluation of the effects of acute low levels of organophosphate insecticides and their metabolites on complex behavioral tasks is critical in determining the ability of exposed organisms to meet the demands of their environment.

Parathion (diethyl *p*-nitrophenyl phosphorothioate) inhibits learned behavior in mice during a simple one-trial passive avoidance test (30) and in monkeys during a visual discrimination test (31). Parathion, in its original form, has extremely low acute toxicity (12). It is activated to its oxygen analog, paraoxon (diethyl *p*-nitrophenyl phosphate) through the cytochrome P450-dependent oxidative desulfuration of parathion (20, 27, 36).

In previous work, it has been found that acute exposure to lethal levels of paraoxon when administered with an antidote (to mimic an acute accidental poisoning and treatment) produces deficits in schedule-controlled behavior. In this work, a high sublethal dose of paraoxon, 1.3 mg/kg, resulted in 52% and 34% depressions in schedule-controlled performance at days 1 and 2, respectively, following exposure, while brain AChE levels remained below controls (6). However, no work has been done on the effects of lower levels of paraoxon on this type of positively reinforced behavior and its relation to the cholinergic system in the brain. Thus it is not presently known how much AChE inhibition can occur without eliciting adverse effects. It has also been proposed that OP-induced behavioral deficits result from peripheral effects as well as central effects (4), though this is not a widely accepted theory. Therefore, analysis of peripheral plasma cholinesterase (ChE) inhibition was also included. Also, other serine esterases which could serve as alternate phosphorylation sites for OP's, such as aliesterases (carboxylesterases),

[1]Work performed in the Department of Biological Sciences, Mississippi State University.

[2]Requests for reprints should be addressed to Russell L. Carr, P.O. Drawer V, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762.

TABLE 1

ACETYLCHOLINESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE CEREBRAL CORTEX OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

Time	Dosages 0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	17.36 ± 4.28*A 58.13% (5)	6.71 ± 1.11*A 83.82% (5)	3.58 ± 0.98*A 91.37% (5)
1 Day	20.07 ± 1.15*A 51.60% (5)	15.15 ± 1.25*B 63.46% (5)	13.63 ± 0.81*B 67.13% (5)
2 Day	25.96 ± 1.00*B 37.39% (5)	21.71 ± 1.35*C 47.64% (5)	21.62 ± 1.04*C 47.86% (5)
3 Day	30.11 ± 1.38*BC 27.39% (5)	26.43 ± 1.37*CD 36.26% (5)	22.10 ± 0.99*CD 46.70% (5)
4 Day	33.07 ± 3.73*C 20.25% (4)	29.55 ± 0.81*D 28.74% (4)	26.73 ± 0.58*D 35.54% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 41.46 ± 0.70 (24).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

have been postulated to be a protective mechanism against OP-induced toxicity. Therefore, the initial inhibition and recovery of liver and plasma aliesterases were quantified to study their possible protective role in low-level paraoxon exposures. A correlation between inhibition of these peripheral enzymes and inhibition of performance would suggest that the enzymes could serve as a biomarker of OP-induced neurobehavioral deficits.

Currently, the literature contains many behavioral studies in which only a single dose of individual neurotoxic compounds were tested (32,37). Other studies present behavioral results but no accompanying biochemical analysis for comparison (26,29). By establishing a behavioral dose-response curve with accompanying biochemical analysis, inferences can be made about the biochemical causes of the behavioral effects. These experiments were designed to study the effects of acute low-level exposures to paraoxon on fixed ratio performance. Three dosages of paraoxon (0.5, 0.75, and 1.0 mg/kg) were administered. The initial inhibition and recovery of brain AChE, two plasma cholinesterases (butyrylcholinesterase (BChE) and plasma non-iso-OMPA-sensitive ChE), and liver and plasma aliesterases were also studied to determine any correlations between central and/or peripheral biochemical parameters and performance deficits.

METHOD

Animals

Male experimentally naive Sprague-Dawley [Crl:CD(SD)BR] rats (originally from Charles River) weighing 250–300 g were used. The rats used in the behavior studies were housed separately in wire-bottom cages (28 × 21 × 23 cm) with tap water freely available except during behavioral testing. Food (Purina rat chow) was freely available prior to experimentation. The rats used for biochemical studies were housed in the colony until designated for the study, at which time they were injected and housed in groups of four; food and water were available ad lib throughout testing. The room was maintained at a temperature of 22 ± 2°C on a 12:12 LD cycle. Eight animals were used for each behavioral treatment group and 4–5 for the biochemical experiments.

Chemicals

The paraoxon was a gift from Howard W. Chambers, Department of Entomology, Mississippi State University, and was synthesized according to described procedures (7). The same batch of paraoxon was used throughout the study and was greater than 99% pure. Corn oil was used as the vehicle. All biochemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Behavioral Protocol

In preparation of the animals for training in the schedule-controlled behavioral study, food was deprived for 48 h with water freely available. The rats were housed in pairs in order to reduce the stress of food deprivation. The rats were separated into individual cages following the 48 h. The rats were placed in an experimental chamber and trained by successive approximations to press a lever for 48 mg food pellets (P. J. Noyes Company, Inc.). Training took place for 15 min/day until the rats were responding independently. The subjects were then placed in a microcomputer-monitored experimental chamber. All experimental chambers were BRS-LVE (Model #143-03) and were equipped with a houselight, a sound-masking ventilation fan, a single response lever, and a magazine for delivery of food pellets. The behavioral testing was performed at the same time daily and no water was available during the tests. To maintain motivation, the rats were kept at about 80% of their free-feeding weight through a restricted feeding schedule which involved providing an additional 10–12 g of Purina Lab Chow to each rat after a test. Each test session ended after 50 min or after the delivery of 50 food pellets, whichever occurred first.

The rats began on a Fixed Ratio of One (FR1) schedule on the first day in the microcomputer-monitored box. The rats progressed to and were maintained under an FR10 schedule. The

TABLE 2

ACETYLCHOLINESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE MEDULLA OBLONGATA OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

	Dosages		
Time	0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	35.72 ± 8.37*A 54.67% (5)	12.93 ± 1.91*A 83.60% (5)	8.05 ± 1.57*A 89.78% (5)
1 Day	51.57 ± 5.01*B 34.56% (5)	39.19 ± 1.54*B 50.27% (5)	37.70 ± 2.29*B 52.16% (5)
2 Day	58.09 ± 5.09*BC 26.28% (5)	49.63 ± 4.80*B 37.02% (5)	47.22 ± 4.60*B 40.08% (5)
3 Day	54.30 ± 1.99*BC 28.09% (5)	48.27 ± 2.38*B 38.74% (5)	48.74 ± 2.78*BC 38.15% (5)
4 Day	67.33 ± 5.98*C 14.56% (4)	64.24 ± 4.22*C 18.48% (4)	62.41 ± 2.77*C 20.80% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 78.80 ± 1.64 (24).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

daily response rate (responses/s) was monitored by the microcomputer. After about 3–5 weeks, the animals normally developed a stable response rate that varied less than 10% over 3 days. After reaching this criterion of stability, the rats were treated. Each dose was injected intraperitoneally (IP) at 1 ml/kg about 22 h prior to the next test session. Controls were injected with an equivalent amount of corn oil. This time period eliminated from the behavioral tests the immediate acute effects of the paraoxon. The rats, 8 per dose, were tested for 4 days following exposure to the paraoxon and the response rate was recorded. The response rates for the 3 days prior to injection were averaged and considered the pretreatment average ("0" day).

Biochemical Studies

On the fourth day, the behavioral animals were euthanized by decapitation and the cerebral cortex and medulla oblongata were removed for AChE analysis. Separate untrained rats were injected with the same dosages and sacrificed after 2 h and 1, 2, 3, and 4 days. From these animals, the brain was removed and chilled to obtain the cerebral cortex and medulla oblongata for AChE analysis. Also, 0.05 g of liver was removed for aliesterase determination. Blood was collected using EDTA to prevent clotting and the plasma was obtained by centrifuging at 17,000 × g for 5 min for subsequent determination of aliesterase, BChE, and

TABLE 3

ALIESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE LIVER OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

	Dosages		
Time	0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	372.9 ± 54.0*A 65.72% (5)	259.9 ± 37.2*A 76.11% (5)	225.6 ± 62.8*A 79.26% (5)
1 Day	765.7 ± 22.2*B 29.61% (5)	535.2 ± 76.1*B 50.80% (5)	499.1 ± 56.0*B 54.12% (5)
2 Day	797.7 ± 42.9*B 26.68% (5)	723.3 ± 70.0*BC 33.51% (5)	656.5 ± 79.4*B 39.65% (5)
3 Day	814.5 ± 72.8*B 25.13% (5)	728.2 ± 82.5*BC 33.06% (5)	686.5 ± 73.8*B 36.89% (5)
4 Day	961.1 ± 68.8 B 11.65% (4)	804.2 ± 65.8*C 26.08% (4)	757.2 ± 70.6*B 30.37% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 1087.9 ± 35.4 (24).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

TABLE 4

ALIESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE PLASMA OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

Time	Dosages 0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	6.9 ± 2.5*A	5.5 ± 1.5*A	3.9 ± 1.3*A
	96.28% (5)	97.06% (5)	97.91% (5)
1 Day	108.0 ± 20.0*B	99.3 ± 8.1*B	76.1 ± 7.3*B
	42.61% (5)	47.22% (5)	59.54% (5)
2 Day	127.9 ± 10.2*BC	115.8 ± 5.1*B	100.9 ± 8.4*BC
	32.04% (5)	38.47% (5)	46.39% (5)
3 Day	141.6 ± 7.3 BC	125.2 ± 8.6*B	121.0 ± 5.3*C
	24.77% (4)	33.48% (4)	35.71% (5)
4 Day	156.5 ± 9.6 C	135.8 ± 17.2*B	125.1 ± 7.0*C
	16.71% (4)	27.84% (4)	33.53% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 188.2 ± 4.9 (24).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

non-iso-OMPA-sensitive ChE activity.

AChE activity in the brain was measured spectrophotometrically using a modification (5) of Ellman et al. (9) using acetylthiocholine as the substrate and 5,5′-dithiobis(2-nitrobenzoate) as the chromogen. Duplicate subsamples were run for all samples at a final concentration of 1 mg/ml.

Liver was homogenized at 5 mg/ml in 0.05 M Tris-HCl buffer (pH 7.4 at 25°C). For determination of aliesterase activity, liver (0.0283 mg/ml) and plasma (0.625 μl/ml) were incubated in 4 ml of the same buffer and paraoxon was used in the enzyme blank at a final concentration of 0.01 mM. Following a 15 min preincubation, the substrate, 4-nitrophenyl valerate (final concentration of 0.5 mM), was added. The reaction was terminated by the addition of 250 μl/ml of a mixture containing 2% sodium dodecyl sulfate and 2% Tris base. Absorbance was measured at 400 nm in a Perkin-Elmer Lambda 5 spectrophotometer.

Plasma non-iso-OMPA-sensitive ChE activity was measured spectrophotometrically using a modification of Ellman et al. (9) similar to Chambers et al. (5). Plasma (50 μl/ml) was preincubated with a final concentration of 0.01 mM iso-OMPA (tetrasopropyl pyrophosphoramide), an inhibitor specific for BChE, to eliminate BChE activity and eserine sulfate (final concentration of 0.01 mM) was used to correct for non-ChE hydrolysis. Acetylthiocholine was the substrate and 5,5′-dithiobis(2-nitrobenzoate) was the chromogen.

TABLE 5

NON-ISO-OMPA-SENSITIVE CHOLINESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE PLASMA OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

Time	Dosages 0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	0.567 ± 0.03*A	0.525 ± 0.03*A	0.447 ± 0.02*A
	74.23% (5)	76.14% (5)	79.68% (5)
1 Day	1.706 ± 0.02*B	1.610 ± 0.04*B	1.613 ± 0.03*B
	22.45% (5)	26.82% (5)	26.68% (5)
2 Day	1.866 ± 0.05*B	1.845 ± 0.04*C	1.777 ± 0.04*BC
	15.18% (5)	16.14% (5)	19.23% (5)
3 Day	2.111 ± 0.04 C	2.036 ± 0.02 CD	1.909 ± 0.07*C
	4.05% (4)	7.45% (4)	13.23% (4)
4 Day	2.136 ± 0.12 C	2.155 ± 0.08 D	1.924 ± 0.04*C
	2.91% (4)	2.05% (4)	12.55% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 2.20 ± 0.03 (23).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

TABLE 6

BUTYRYLCHOLINESTERASE SPECIFIC ACTIVITY AND PERCENT INHIBITION IN THE PLASMA OF RATS FOLLOWING EXPOSURE TO THREE SUBLETHAL DOSAGES OF PARAOXON

Time	Dosages 0.5 mg/kg	0.75 mg/kg	1.0 mg/kg
2 h	0.444 ± 0.06*A 50.94% (5)	0.411 ± 0.05*A 54.59% (5)	0.307 ± 0.03*A 66.07% (5)
1 Day	0.729 ± 0.04AB 19.45% (5)	0.639 ± 0.13*AB 29.39% (5)	0.511 ± 0.03*AB 43.54% (5)
2 Day	0.811 ± 0.10 B 10.39% (5)	0.798 ± 0.13 B 11.82% (5)	0.653 ± 0.07*BC 27.85% (5)
3 Day	0.918 ± 0.08 B 0% (4)	0.824 ± 0.10 B 8.95% (4)	0.766 ± 0.08 BC 15.36% (4)
4 Day	1.051 ± 0.09 B 0% (4)	0.975 ± 0.08 B 0% (4)	0.915 ± 0.15 C 0% (4)

Specific activity is expressed as nmoles substrate hydrolyzed per min per mg protein ± S.E.M. Number of replications in parentheses. Control specific activity is 0.905 ± 0.04 (23).

*Indicates significantly different ($p<0.01$) from control. Means within a column not followed by the same letter are significantly different ($p<0.01$).

Plasma BChE activity was measured spectrophotometrically using a modification of Ellman et al. (9) similar to Chambers et al. (5). Butyrylthiocholine was the substrate and 5,5'-dithiobis(2-nitrobenzoate) was the chromogen. Iso-OMPA was used to correct for non-BChE hydrolysis.

For all tissues, proteins were quantified by the method of Lowry et al. (23), using bovine serum albumin as a standard.

Statistical Analysis

For all acute behavioral studies, the data were analyzed as a repeated measurements (split plot) design using general linear model (GLM) with one grouping variable (treatment) and one within-treatment variable (day). For the analyses the "0" day values were the average of the last 3 pretreatment tests (the day of treatment and the previous two days). Appropriate least significant means (LSM) comparisons were made to separate days within treatment.

For the biochemical assays, the differences in specific activities were determined between control and treated groups using GLM followed by LSM separation of days within treatment.

RESULTS

Signs of Poisoning

Within 30 minutes following exposure, the treated animals showed dose-dependent signs of paraoxon intoxication. The two higher doses caused more typical signs of OP poisoning such as tremors, salivation, and lacrimation with the 1.0 mg/kg dosage being more severe. Although the lowest dosage did not induce these typical signs of poisoning, there was a lack of normal activity. Animals treated with 0.5 mg/kg paraoxon did not seem to be interested when approached and remained stationary in the cage. However, all outward signs of paraoxon toxicity had subsided by one day following all treatments.

Behavior

Behavioral performance was initially decreased on days 1 and 2 following treatment in a dose-dependent fashion with all paraoxon treatments (Fig. 1). The control group maintained a stable response rate throughout the test period. There were no significant differences between the control groups and the pretreatment response rates of any treatment group. Therefore, the posttreatment response rates were statistically compared to the pretreatment response rates of that group.

There was no significant difference between pretreatment response rates and posttreatment response rates with 0.5 mg/kg paraoxon though response rates did decrease. The response rates of rats given 0.75 mg/kg were significantly lower than pretreatment rates by 25% and 18% on days 1 ($p<0.0004$) and 2 ($p<0.008$), respectively, while 1.0 mg/kg yielded performance deficits of 56% and 32% on days 1 ($p<0.0001$) and 2 ($p<0.0001$), respectively.

Biochemical

AChE activity in the cerebral cortex and medulla oblongata

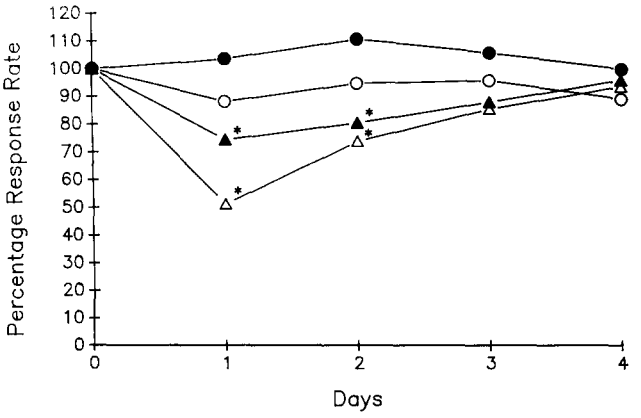

FIG. 1. Percentage depression of FR-10 performance in rats exposed to three sublethal doses of paraoxon. Control = ●; 0.5 mg/kg = ○; 0.75 mg/kg = ▲; 1.0 mg/kg = △. Day 0 indicates three day pretreatment average as 100%. *Indicates a significant difference from pretreatment levels ($p<0.01$).

was significantly inhibited in a dose-dependent manner throughout the study for all paraoxon treatments (Tables 1 and 2). Recovery occurred faster in the medulla oblongata than in the cerebral cortex. Though not presented, percentage brain AChE inhibition on day 4 in the behavioral subjects was comparable to the day 4 percentage inhibition of the nonbehavioral subjects with less than 7% differences in the two lower doses and less than 11% differences in the highest dose. Although rats used for biochemical measures were not food deprived as were the behavioral animals, the similar inhibition of AChE on day 4 in both groups suggests that the biochemical parameters are comparable.

Liver aliesterase activity was significantly inhibited for 3 days for all paraoxon treatments, but the 0.5 mg/kg group recovered by day 4 (Table 3). Initial inhibition of liver aliesterase activity appeared to occur in a dose-dependent manner. Plasma aliesterase activity was significantly inhibited with the two higher doses throughout the study, but activity had recovered by day 3 in rats exposed to 0.5 mg/kg (Table 4). Initial inhibition of plasma aliesterases was nearly 100% with all doses of paraoxon at 2 h.

Plasma non-iso-OMPA-sensitive ChE activity was inhibited for 2 days in all paraoxon-treated groups. Recovery of activity did not occur in the highest dose but the 0.5 mg/kg and 0.75 mg/kg groups recovered by day 3 (Table 5).

Plasma BChE activity was initially inhibited on day 1 with all paraoxon treatments and day 2 with 1.0 mg/kg (Table 6). Activity recovered much faster than plasma aliesterases and non-iso-OMPA-sensitive ChE, and reached 100% of control by day 4 with all dosages.

DISCUSSION

In the present study, acute exposure of rats to different levels of paraoxon produced initial dose-dependent deficits in schedule-controlled behavior. All biochemical measures were also initially inhibited in a dose-dependent manner with the exception of plasma aliesterases whose activity was virtually eliminated.

Aliesterases in the plasma and liver seem to provide a protective mechanism against OP toxicity (8,20). Greater percentage inhibition was observed with plasma than with liver aliesterase activities. This rapid inhibition was expected because the chemical used, paraoxon, was an active antiesterase and did not require hepatic activation as the parent insecticide would. If these phosphorylation sites for paraoxon were not present, the amount of brain AChE inhibition would probably have been greater.

The inhibition of AChE by these lower doses of paraoxon was substantial and persistent, with no significant recovery observed. The slow rate of recovery could have been the result of the aging of the phosphorylated AChE (7). During the latter part of the study when there were no significant deficits in behavior, cholinergic hyperactivity could still have been present in the brain. On the other hand, cholinergic hyperactivity may only result after a certain threshold of AChE inhibition has been achieved.

Behavioral deficits have been shown to recover prior to brain AChE recovery following exposure to several anticholinesterase insecticides (33) including parathion, the parent insecticide of paraoxon (30). Similar results were indicated here with paraoxon. It appears that brain AChE activity is more sensitive than performance to inhibition, suggesting that there is a fraction of brain AChE activity which is not necessary for normal performance of this task.

It has been hypothesized that the obvious signs of OP intoxication are associated with the performance deficits in schedule-controlled behavior (25). Since noticeable signs of intoxication frequently occur during the time of peak AChE inhibition, the correlation of peak inhibition and peak performance deficits could be partially attributed to incapacitation of the animal following exposure to an OP. With these lower doses of paraoxon, the obvious signs of intoxication had disappeared by day 1. Thus deficits in schedule-controlled behavior can occur without concurrent overt signs of toxicity.

These data do not directly agree with studies with DFP in which brain AChE was lowered to 46% of normal and no consequent operant behavioral deficits occurred (28). In the present study, behavioral deficits occurred with brain AChE levels at 50% of control. Thus a difference exists in the sensitivity of brain AChE and performance when exposed to different OP's. In previous studies, following intrastriatal injection of three OP ChE inhibitors, DFP, soman and sarin, only DFP produced gross motor deficits while all three produced similar brain AChE inhibition (24). Also, it has been demonstrated that differences in time course recovery of AChE differs with OP's such as DFP and paraoxon (3), and even different brain areas have different recovery rates after peripheral exposure to OP's (7,19). These rate differences have been observed in this study with the medulla oblongata recovering faster than the cerebral cortex.

It has been proposed that to obtain a deficit in behavior, brain AChE must be lowered to below a threshold at 45% of normal (16). However, no threshold was observed here. The amount of inhibition of AChE in the cerebral cortex at the same time as a behavioral deficit varied among treatment groups. AChE inhibition was greater than 60% on day 1 when behavioral deficits occurred in the 0.75 mg/kg and 1.0 mg/kg paraoxon groups, while no behavioral deficits were present with 0.5 mg/kg paraoxon even though AChE inhibition was 51% (Tables 1 and 2). In contrast, the inhibition of AChE was below 50% on day 2, but behavioral deficits were still present in the two higher groups.

All doses of paraoxon significantly inhibited plasma BChE and non-iso-OMPA-sensitive ChE at 2 h. Non-iso-OMPA-sensitive ChE was initially inhibited to levels comparable to brain AChE inhibition. However, recovery of plasma non-iso-OMPA-sensitive ChE was faster than that of brain AChE. Since the function of non-iso-OMPA-sensitive ChE is not known, it is difficult to make inferences about its toxicological significance. Its lack of sensitivity to iso-OMPA and its sensitivity to eserine suggests that this may be AChE. Plasma BChE seemed to be less sensitive to paraoxon inhibition than non-iso-OMPA-sensitive ChE (Tables 6 and 7). The recovery of BChE activity was much faster than the recovery of both brain AChE and plasma non-iso-OMPA-sensitive ChE. There was no direct correlation between the inhibition of either of the plasma ChE's and behavioral deficits. Unfortunately, the plasma parameters measured here do not seem to be accurate biomarkers for either brain AChE inhibition or behavioral deficits.

The lack of correlation between behavior and central or peripheral ChE activity indicates that AChE is not the sole determinant of this behavior or that the critical brain region was not monitored. There is a difference in the target areas of different OP's (37). For example, studies have been conducted with paraoxon in which paraoxon in vitro was found to act directly as an agonist on certain subtypes of central muscarinic receptors (17). This type of noncholinesterase action could affect behavioral performance.

In schedule-controlled behavior, obtaining the food reward is thought to depend upon appetite and motivation; therefore, these could negate the role of memory in these types of behavioral results (6). However, alterations in appetite and motivation were probably not responsible for the performance deficits observed in this study. Following completion of behavioral testing prior to exposure to paraoxon, about half of the animals were accustomed to obtain their daily ration of food from the author's hand

above the edge of the cage. Following exposure to paraoxon, no animal failed to obtain food in this manner. This behavior suggests that appetite and motivation were still present in these animals. Certain behaviors may be more susceptible to inhibition by OP's than others. It has been proposed that types of behavior that involve higher CNS function and require motor activity, such as schedule-controlled behavior, are more sensitive to ChE inhibitors (37).

It can be hypothesized that exposure to OP's disrupts the ability of an organism to perform a learned task via inhibition of AChE in the brain. The data presented here indicates that brain AChE inhibition alone does not explain the deficits in the performance observed. While the involvement of the brain cholinergic system in learning has been well documented (1, 2, 35), it is possible that the disruption of FR performance caused by paraoxon can be attributed to both central and peripheral effects. Behavioral deficits have been demonstrated with other anticholinesterases such as sarin (21), which has a predominantly peripheral mode of action (37). Following treatment with parathion and DFP, whole brain AChE did not correlate with the saccharin preference ratio while plasma ChE did, suggesting that a peripheral site might mediate OP-induced conditioned taste aversion (32). In comparison, if the peripheral data present in the form of plasma cholinesterases are an indication of the peripheral system, then a much faster recovery occurs in the peripheral than in the central nervous system, and could indicate that both the peripheral and central cholinergic system may be involved in behavioral deficits. There is no doubt that the central cholinergic system plays an important role in behavioral performance but the effects of anticholinesterases may not totally depend upon their action at cholinergic synapses but may also result as a secondary effect upon noncholinergic synapses (18) such as abnormal increases in choline (11) or alterations in postsynaptic receptor mechanisms (10). These types of effects are usually not correlated with OP-induced behavioral deficits.

In conclusion, although there were obvious dose-response relationships present in both biochemistry and behavior, there was no clear-cut threshold of brain AChE inhibition which was required to yield performance deficits, nor was there a direct correlation between significant inhibition in peripheral enzymes and performance deficits. These experiments did not elucidate a peripheral enzyme which could serve as a biomarker to predict OP-induced deficits in schedule-controlled behavior. Therefore, further investigation is needed to understand the OP-induced biochemical and physiological effects that cause alterations in behavior.

ACKNOWLEDGEMENTS

This research was supported by EPA grant R-811295 and by NIH Research Career Development Award ES00190 to J.E.C. The authors wish to express appreciation to Amanda Holland and Jeff Stokes for the animal care rendered.

REFERENCES

1. Beninger, R. J.; Wirsching, B. A.; Jhamandas, K.; Boegman, R. J.; El-Defrawy, S. R. Effects of altered cholinergic function on working and reference memory in the rat. Can. J. Physiol. Pharmacol. 64:376–382; 1986.
2. Bermudez-Rattoni, F.; Mujica-Gonzalez, M.; Prado-Alcala, R. A. Is cholinergic activity of the striatum involved in the acquisition of positively-motivated behaviors? Pharmacol. Biochem. Behav. 24: 715–719; 1986.
3. Bisso, G. M.; Meneguz, A.; Michalek, H. Developmental factors affecting brain acetylcholinesterase inhibition and recovery in DFP-treated rats. Dev. Neurosci. 5:508–519; 1982.
4. Brezenoff, H. E.; McGee, J.; Hymowitz, N. Effect of soman on schedule-controlled behavior and brain cholinesterase in rats. Life Sci. 37:2421–2430; 1985.
5. Chambers, J. E.; Wiygul, S. H.; Harkness, J. E.; Chambers, H. W. Effects of acute paraoxon and atropine exposures on retention of shuttle avoidance behavior in rats. Neurosci. Res. Commun. 3:85–92; 1988.
6. Chambers, J. E.; Chambers, H. W. Short-term effects of paraoxon and atropine on schedule-controlled behavior in rats. Neurotoxicol. Teratol. 11:427–432; 1989.
7. Chambers, H. W.; Chambers, J. E. An investigation of acetylcholinesterase inhibition and aging and choline acetyltransferase activity following a high level acute exposure to paraoxon. Pest. Biochem. Physiol. 33:125–128; 1989.
8. Deutsch, J. A. The cholinergic synapse and the site of memory. Science 174:788–794; 1971.
9. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88–85; 1961.
10. Fisher, S. K.; Figueiredo, S. C.; Bartus, R. T. Differential stimulation of inositol phospholipid turnover in brain analogs of oxotremorine. J. Neurochem. 43:1171–1179; 1984.
11. Flynn, C. J.; Wecker, L. Elevated choline level in brain: A noncholinergic component of organophosphate toxicity. Biochem. Pharmacol. 35:2815–2821; 1986.
12. Gage J. C. A cholinesterase inhibitor derived from *OO*-diethyl *O*-*p*-nitrophenyl thiophosphate in vivo. Biochem. J. 54:426–430; 1953.
13. Gardner, R.; Ray, R.; Frankenheim, J.; Wallace, K.; Loss, M.; Robichaud, R. A possible mechanism for diisopropylfluorophosphate-induced memory loss in rats. Pharmacol. Biochem. Behav. 21:43–46; 1984.
14. Geller, I.; Hartmann, R. J.; Leal, B. Z.; Haines, R. J.; Gause, E. M. Effects of the irreversible acetylcholinesterase inhibitor soman on match-to-sample behavior of the juvenile baboon. Proc. West. Pharmacol. Soc. 27:217–221; 1984.
15. Giardini, V.; Meneguz, A.; Amorico, L.; De Acetis, L.; Bignami, G. Behaviorally augmented tolerance during chronic cholinesterase reduction by paraoxon. Neurobehav. Toxicol. Teratol. 4:335–345; 1982.
16. Glow, P. H.; Rose, S. Effects of reduced acetylcholinesterase levels on extinction of a conditioned response. Nature 206:475–477; 1965.
17. Jett, D. A.; Abdallah, E. A. M.; El-Fakahany, E. E.; Eldefrawi, M. E.; Eldefrawi, A. T. High-affinity activation by paraoxon of a muscarinic receptor subtype in rat brain striatum. Pest. Biochem. Physiol. 39:149–157; 1991.
18. Karczmar, A. G. Acute and long lasting actions of organophosphorus agents. Fundam. Appl. Toxicol. 4:S1–S17; 1984.
19. Kozar, M. D.; Overstreet, D. H.; Chippendale, T. C.; Russell, R. W. Changes of acetylcholinesterase activity in three major brain areas and related changes in behaviour following acute treatment with diisopropyl fluorophosphate. Neuropharmacology 15:291–298; 1976.
20. Kulkarni, A. P.; Hodgson, E. Metabolism of insecticides by mixed function oxidase systems. Pharmacol. Ther. 8:379–475; 1980.
21. Landauer, M. R.; Romano, J. A. Acute behavioral toxicity of the organophosphate sarin in rats. Neurobehav. Toxicol. Teratol. 6:239–243; 1984.
22. Lehotzky, K. Effects of pesticides on central and peripheral nervous system function in rats. Neurobehav. Toxicol. Teratol. 4:665–669; 1982.
23. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275; 1951.
24. Lynch, M. R.; Rice, M. A.; Robinson, S. E. Dissociation of locomotor depression and ChE activity after DFP, soman, and sarin. Pharmacol. Biochem. Behav. 24:941–947; 1986.
25. MacPhail, R. C. Effects of pesticides on schedule-controlled behavior. In: Behavioral pharmacology: The current status. New York: Alan R. Liss, Inc.; 1985:519–535.

26. Moser, V. C.; MacPhail, R. C. Cholinergic involvement in the action of formetanate on operant behavior in rats. Pharmacol. Biochem. Behav. 26:119–121; 1987.
27. Neal, R. A. A comparison of the *in vitro* metabolism of parathion in the lung and liver of the rabbit. Toxicol. Appl. Pharmacol. 23: 123–130; 1972.
28. Overstreet, D. H.; Russell, R. W.; Vasquez, B. J.; Dalglish, F. W. Involvement of muscarinic and nicotinic receptors in behavioral tolerance to DFP. Pharmacol. Biochem. Behav. 2:45–54; 1974.
29. Raslear, T. G.; Leu, J. R.; Simmons, L. The effects of diisopropyl phosphofluoridate (DFP) on inter-response time and circadian patterns of lever-pressing in rats. Neurobehav. Toxicol. Teratol. 8:655–658; 1986.
30. Reiter, L.; Talens, G.; Woolley, D. Acute and subacute parathion treatment: Effects on cholinesterase activities and learning in mice. Toxicol. Appl. Pharmacol. 25:582–588; 1973.
31. Reiter, L. W.; Talens, G. M.; Woolley, D. Parathion administration in the monkey: Time course of inhibition and recovery of blood cholinesterases and visual discrimination performance. Toxicol. Appl. Pharmacol. 33:1–13; 1975.
32. Roney, P. L., Jr.; Costa, L. G.; Murphy, S. D. Conditioned taste aversion induced by organophosphate compounds in rats. Pharmacol. Biochem. Behav. 24:737–742; 1986.
33. Ruppert, P. H.; Cook, L. L.; Dean, K. F.; Reiter, L. W. Acute behavioral toxicity of carbaryl and propoxur in adult rats. Pharmacol. Biochem. Behav. 18:579–584; 1983.
34. Russell, R. W.; Overstreet, D. H. Mechanisms underlying sensitivity to organophosphorus anticholinesterase compounds. Prog. Neurobiol. 28:97–129; 1987.
35. Sandberg, K.; Sanberg, P. R.; Hanin, I.; Fisher, A.; Coyle, J. T. Cholinergic lesion of the striatum impairs acquisition and retention of a passive avoidance response. Behav. Neurosci. 98:162–165; 1984.
36. Whitehouse, L. W.; Ecobichon, D. J. Paraoxon formation and hydrolysis by mammalian liver. Pest. Biochem. Physiol. 5:284–322; 1975.
37. Wolthuis, O. L.; Vanwersch, R. A. P. Behavioral changes in the rat after low doses of cholinesterase inhibitors. Fundam. Appl. Toxicol. 4:S195–S208; 1984.