TOXICOLOGY AND APPLIED PHARMACOLOGY **103**, 66–76 (1990)

Serum Paraoxonase and Its Influence on Paraoxon and Chlorpyrifos-oxon Toxicity in Rats[1]

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Received June 26, 1989; accepted November 21, 1989

Serum Paraoxonase and Its Influence on Paraoxon and Chlorpyrifos-oxon Toxicity in Rats. COSTA, L. G., MCDONALD, B. E., MURPHY, S. D., OMENN, G. S., RICHTER, R. J., MOTULSKY, A. G., AND FURLONG, C. E. (1990). *Toxicol. Appl. Pharmacol.* **103**, 66–76. Paraoxon and chlorpyrifos-oxon, the active metabolites of the organophosphorus insecticides parathion and chlorpyrifos, respectively, are hydrolyzed by an "A"-esterase, paraoxonase, which is present in the sera of several mammalian species. In this study, we investigated whether levels of serum paraoxonase activity in laboratory animals can influence the *in vivo* toxicity of paraoxon and chlorpyrifos-oxon. Paraoxonase was found to be 7-fold higher in rabbit serum than in rat serum. The dose of paraoxon required to produce similar signs of toxicity and similar degrees of cholinesterase inhibition in rats and rabbits (0.5 and 2.0 mg/kg, respectively) differed by 4-fold. Paraoxonase was then purified from rabbit serum and 8.35 units was injected in the tail veins of rats, increasing the peak hydrolytic activity of rat serum by 9-fold toward paraoxon and by 50-fold toward chlorpyrifos-oxon. The increase in serum paraoxonase/chlorpyrifos-oxonase activity was long-lasting, with a 2- and 10-fold increase, respectively, still present after 24 hr. Thirty minutes following enzyme injection, rats were challenged with an acute dose of paraoxon or chlorpyrifos-oxon given by the intravenous, intraperitoneal, dermal, or oral route. Cholinesterase activities were measured in plasma, red blood cells, brain, and diaphragm after 4 hr. Rats pretreated with paraoxonase exhibited less inhibition of cholinesterase than vehicle-treated controls following identical doses of paraoxon, particularly when the organophosphate was given iv or dermally. A very high degree of protection, particularly toward brain and diaphragm cholinesterase, was provided by paraoxonase pretreatment in animals challenged with chlorpyrifos-oxon by all routes. These results indicate that levels of serum paraoxonase activity can affect the toxicity of paraoxon and chlorpyrifos-oxon.

Parathion (diethyl-*p*-nitrophenyl phosphorothioate), a widely used organophosphorus insecticide, is involved in a large number of acute poisonings of agricultural workers every year (Hayes, 1982; Murphy, 1986). Parathion is bioactivated *in vivo* via oxidative desulfuration to form its oxygen analog and active metabolite paraoxon (diethyl *p*-nitrophenyl phosphate), which is a potent inhibitor of the enzyme acetylcholinesterase and is responsible for the cholinergic crisis typical of parathion poisoning. Paraoxon is detoxified predominantly by hydrolysis, catalyzed by the A-esterase paraoxonase (EC 3.1.1.2), to generate *p*-nitrophenol and diethylphosphoric acid (Zech and Zurcher, 1974). Paraoxonase is present in several tissues including serum. Measurement of paraoxonase in serum

[1] Presented in part at the 27th annual meeting of the Society of Toxicology, Dallas, TX, February 15–19, 1988 (*Toxicologist* **8**, 173, 1988).

0041-008X/90 $3.00

from human subjects of European origin (i.e., Caucasian) has revealed a bimodal or trimodal distribution (reviewed in Geldmacher-von Mallinkrodt and Diepgen, 1988). On the basis of enzymatic tests, humans can be divided into three serum paraoxonase phenotypes: A (low activity), AB (intermediate activity), and B (high activity), with about 50% of the studied populations of European origin displaying low activity (Eckerson *et al.*, 1983). Studies on a large number of populations of Asian and African origin, which indicated strong interethnic differences, have also been conducted (Geldmacher-von Mallinckrodt and Diepgen, 1987, 1988). There is at least a 40-fold difference in serum paraoxonase activity between the extremes and a 5-fold difference between the medians of the low- and high-activity groups (Furlong *et al.*, 1989).

Interest in paraoxonase arises from the hypothesis that individuals with low serum activity of this enzyme would be expected to have a diminished ability to metabolize paraoxon and, therefore, might be more sensitive to the toxicity of paraoxon, its parent compound parathion, and of other organophosphates (Omenn, 1987; La Du and Eckerson, 1984; Geldmacher-von Mallinckrodt and Diepgen, 1988). These individuals also might require a longer recovery time after any given exposure. This hypothesis implies that serum paraoxonase has a pivotal role in the detoxification of paraoxon following *in vivo* administration of the organophosphate. This has already been noted, as birds have very low to undetectable serum paraoxonase activity (Brealey *et al.*, 1980) and are more sensitive than various mammals to the acute toxicity of paraoxon, diazinon-oxon, and pirimiphos-oxon (Machin *et al.*, 1976; Brealey *et al.*, 1980). Evidence of a differential sensitivity to paraoxon or other organophosphate toxicity in mammals, in relationship to different activity of serum paraoxonase, is not clear (Geldmacher-von Mallinkrodt and Diepgen, 1988). Before epidemiological studies testing the hypothesis are conducted, it would be useful to determine in mammalian animal models whether differential levels of serum paraoxonase activity lead to differential sensitivity to paraoxon toxicity.

The present study, therefore, was designed to compare the toxicity of paraoxon in two mammalian species, the rabbit and the rat, whose serum paraoxonase activities differ by severalfold, and to determine whether administration to rats of paraoxonase purified from rabbit serum would alter paraoxon toxicity. Furthermore, the protective effect of rabbit serum paraoxonase in rats exposed to chlorpyrifos-oxon was also investigated, since we recently found that chlorpyrifos-oxon, the active metabolite of chlorpyrifos, in addition to being hydrolyzed by liver A-esterases (Sultatos and Murphy, 1983), is hydrolyzed by serum paraoxonase at a high rate, and that serum chlorpyrifos-oxonase levels are 50-fold higher in rabbit serum than in rat serum (Furlong *et al.*, 1988; 1989).

MATERIALS AND METHODS

Animals and treatments. Male Sprague–Dawley-derived rats (200–250 g) and male New Zealand white rabbits (approximately 3.0 kg) were obtained from Tyler Laboratories (Bellevue, WA). Animals were housed in the University of Washington Vivarium and had food (Rodent Blox, No. 8604-00, Continental Grain Co., Chicago, IL, and Manna Pro Rabbit food (03-4690-01), Manna Pro Corp., Los Angeles, CA) and water available *ad libitum*. Paraoxon (diethyl *p*-nitrophenyl phosphate; >99% pure) was obtained from ICN Biomedicals Inc. (Plainview, NY), while chlorpyrifos-oxon (*O,O*-diethyl *O*-[3,5,6-trichloro-2-pyridyl] phosphate; analytical grade) was furnished by the Dow Chemical Co. (Midland, MI). Both compounds were dissolved in corn oil and administered in a volume of 1 ml/kg of body wt. Paraoxon was administered by ip injection to rabbits and both organophosphates were given by the dermal, iv, ip, and oral routes to rats. When given dermally, paraoxon and chlorpyrifos-oxon were dissolved in acetone, and a volume of approx 0.1 ml (0.5 ml/kg) was applied on a previously shaven 4.9-cm^2 area at the base of the neck. For iv injections, both organophosphates were dissolved in saline and injected in the tail vein in a volume of 0.5 ml/kg. When pesticides were given by the oral route, ani-

mals were fasted overnight. In all experiments, control animals received the same amount of vehicle. Blood was drawn from the ear vein in the rabbits and from the tail vein and/or the eye vein in the rats. Animals were then terminated with carbon dioxide, and brain and diaphragm were rapidly removed on ice and stored at −80°C until assayed (within 48 hr). Blood samples were always assayed shortly after they were drawn.

Assay of paraoxonase activity. Blood was centrifuged and activity of paraoxonase was determined in plasma by a novel assay that measured only arylesterase-associated paraoxonase activity and not albumin-catalyzed hydrolysis of paraoxon (Furlong *et al.*, 1988). The paraoxonase assay contained 760 μl of assay buffer (0.132 M Tris–HCl, pH 8.5, 1.32 mM $CaCl_2$, and 2.76 M NaCl), 40 μl of sample containing 2–10 μl of plasma in water, and 200 μl of 6 mM freshly prepared paraoxon substrate solution. A stock substrate solution of 120 mM paraoxon in acetone was stable for approximately 3 weeks. The stock solution was diluted 1:20 with 50 mM Tris–HCl (pH 8.5) for each set of assays. The assay at 37°C was initiated by the addition of the substrate solution, and the absorbance was continuously monitored at 405 nm. Paraoxonase activity is expressed as units/liter (unit = μmol paraoxon hydrolyzed/min).

The activity of plasma paraoxonase toward chlorpyrifos-oxon was measured by a new method developed in our laboratory (Furlong *et al.*, 1988, 1989). The assay contained 0.1 M Tris–HCl (pH 8.5), 2.0 M NaCl, 0.5 mM $CaCl_2$, and 10 μl of plasma in a final volume of 1 ml at 37°C. The reaction was started by the addition of 32 μl of a 10 mM solution of chlorpyrifos-oxon in acetone. The appearance of 3,5,6 trichloro-2-pyridinol was continuously monitored at 310 nm.

Assay of acetylcholinesterase activity. Activity of acetylcholinesterase in brain areas and diaphragm was assayed according to the method of Ellman *et al.* (1961) as modified by Benke *et al.* (1974). Briefly, tissues were homogenized in 0.1 M sodium phosphate buffer (pH 8.0 at 25°C). An aliquot of tissue homogenate (equivalent to 1 mg of tissue for the diaphragm and 0.77 mg for the brain), 5 μl of 1.0 M acetylthiocholine (ATC; Sigma Chemical Co., St. Louis, MO), and 50 μl of 0.1 M 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB; Sigma Chemical Co.) were added to an appropriate volume of sodium phosphate buffer to make a final volume of 5 ml. The absorbance was read immediately after the addition of the substrate ATC and after a 30-min incubation at 27°C. The initial absorbance and a reagent blank absorbance were subtracted from the final reading. The change in absorbance during the incubation is due to formation of 5-thio-2-nitrobenzoate from DTNB and thiocholine, the hydrolytic product of ATC. Acetylcholinesterase activity is expressed as micromoles ATC hydrolyzed/minute/milligrams of tissue or of protein (measured by the method of Lowry *et al.*, 1951).

Plasma and red blood cell cholinesterase activities were measured according to the procedure of Voss and Sachsse (1970) with minor modifications. Briefly, heparinized blood was diluted (1:247 v/v) with 0.1 M sodium phosphate buffer (pH 8.0) and divided into two aliquots, A and B. Total blood cholinesterase was assayed in tube A by adding DTNB and the substrate ATC. After a 10-min incubation at 27°C, the reaction was stopped by the addition of 25 μl of physostigmine (1 mg/ml). Both tubes were centrifuged for 10 min to separate the plasma. Plasma cholinesterase was assayed in the supernatant of tube B. After the addition of ATC and DTNB a first reading of absorbance was taken at 412 nm followed by a second reading after a 10-min incubation at 27°C. The first absorbance was subtracted from the second to calculate the activity of plasma cholinesterase (expressed as μmole ATC hydrolyzed/min/ml). Acetylcholinesterase in erythrocytes was calculated by subtracting total plasma absorbance (including the blank) from the absorbance of tube A (total blood).

Partial purification of paraoxonase from rabbit serum. Rabbit serum was precipitated as described by Main (1956). The final dialysis against tap water was omitted. The precipitate was resuspended in a minimal volume of cold 1% NH_4OH. The redissolved material was fractionated by Sephadex-G200 chromatography (column buffer, 5% glycerol, 20 mM imidazole, pH 8.0, 10 mM $CaCl_2$). The active fractions were pooled and concentrated by ultrafiltration. Before use, enzyme was exchanged into injection buffer and refractionated on a Sephadex-G75 column (injection buffer, 136 mM NaCl, 0.1 mM $CaCl_2$). The active fractions were concentrated to >17 units (μmol paraoxon hydrolyzed/min) of paraoxonase/milliliter in an Amicon pressure cell fitted with a YM-10 membrane. (See Table 1.)

Data analysis. Data were analyzed for statistical significance by analysis of variance followed by Newman–Keuls test or by Student's *t* test (Snedecor and Cochran, 1980).

RESULTS

Serum paraoxonase and paraoxon toxicity. Measurements of the activity of paraoxonase in serum from rats and rabbits showed a sevenfold difference between the activity in rats (140 ± 8 units/liter) and that in rabbits (1005 ± 48 units/liter) (mean ± SEM; $n = 16$; $p < 0.01$). A series of experiments were conducted, therefore, to determine whether these two species displayed a differential sensitivity to paraoxon toxicity. We determined that the minimal effective doses (ip) of paraoxon,

TABLE 1

PURIFICATION OF PARAOXONASE FROM RABBIT SERUM

	Protein (mg/ml)	ml	Paraoxonase activity[a]	Specific activity[b]	Fold purification	Percentage yield
Whole serum	52.8	300	1.57	0.029	—	—
After precipitation	64.7	110	3.89	0.060	2.1	90
After Sephadex G-200	7.2	78	0.94	0.131	4.5	73
After Sephadex G-75/ concentration	95.0	5	20.01	0.210	7.2	113

[a] Activity is described as units/ml serum. One unit hydrolyzes 1 μmol of substrate in 1 min.
[b] Specific activity is described as units/milligram protein.

causing some sign of cholinergic intoxication were 0.5 mg/kg in the rat and 2.0 mg/kg in the rabbit.

To confirm that these different doses of paraoxon had similar biochemical effects, cholinesterase activity was measured in various tissues of control and paraoxon-treated rats and rabbits. As shown in Table 2, doses of paraoxon of 0.5 mg/kg in the rat and 2.0 mg/kg in the rabbit caused a similar degree of cholinesterase inhibition. Decreases of cholinesterase activity of such degree were accompanied by only slight signs of cholinergic intoxication.

Since serum paraoxonase activity, but not other parameters, differed between these two animal species (Aldridge, 1953; Chemnitius *et al.*, 1983; Costa *et al.*, 1987), we designed experiments to determine whether increasing paraoxonase activity in serum would protect rats against the toxicity of paraoxon. Paraoxonase was partially purified from rabbit serum (see Materials and Methods), and 8.35 units (in a volume of 0.5 ml) was injected in the tail veins of rats. At various intervals after injection, blood was drawn from the tail vein and paraoxonase activity was measured. In several experiments, blood was also drawn from the postorbital vein of the eye to ensure that the enzyme had reached the general circulation. There was no difference in paraoxonase activity between blood drawn from the

TABLE 2

EFFECT OF PARAOXON (PO) ON CHOLINESTERASE ACTIVITY IN VARIOUS TISSUES OF RAT AND RABBIT

	Cholinesterase activity (μmol ATC/min/mg tissue (ml))					
	Rat			Rabbit		
Tissue	Control	PO-treated	Percentage of control	Control	PO-treated	Percentage of control
Diaphragm	0.90 ± 0.11	0.31 ± 0.07	34	0.62 ± 0.04	0.13 ± 0.01	21
Plasma	0.41 ± 0.03	0.12 ± 0.05	29	0.67 ± 0.08	0.23 ± 0.08	34
Hippocampus	4.66 ± 0.41	2.81 ± 0.15	60	7.97 ± 0.44	4.75 ± 1.18	60
Cortex	5.91 ± 0.43	3.56 ± 0.43	60	7.85 ± 0.38	4.61 ± 1.37	60

Note. Paraoxon was dissolved in corn oil and administered ip at the doses of 0.5 (rat) or 2.0 mg/kg (rabbit). Controls were administered corn oil only and the injection volume was 1 ml/kg. Animals were terminated 1 hr after injection. Results are the means (±SEM) of three to six animals.

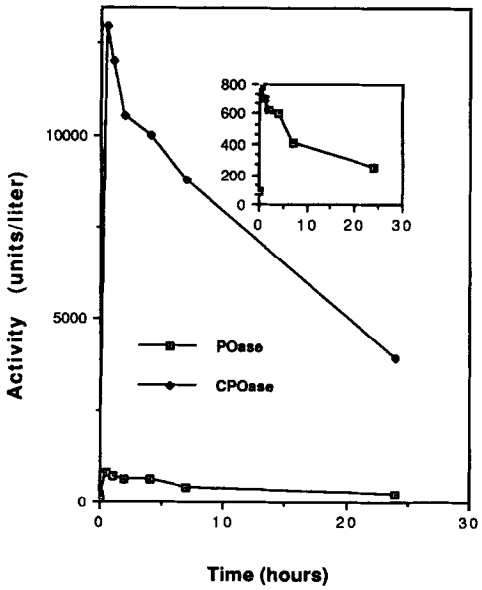

FIG. 1. Time course of plasma paraoxonase activity toward paraoxon (POase) and chlorpyrifos-oxon (CPOase) in rats following iv administration of 8.35 units of paraoxonase (measured with paraoxon as a substrate) partially purified from rabbit serum. Each point represents the mean (±SEM) of at least 15 rats.

tail or the eye. Administration of rabbit paraoxonase elevated rat serum paraoxonase activity by ninefold (Fig. 1). The activity decreased slowly over 4 hr and at 24 hr was still more than twice the basal activity.

To determine whether rats whose serum paraoxonase activity was increased by paraoxonase administration would be less sensitive to the toxic effects of paraoxon, paraoxon was given by four different routes: intravenous (iv), intraperitoneal (ip), dermal, and oral. Doses of paraoxon (0.1 mg/kg, iv; 0.5 mg/kg, ip; 0.3 mg/kg, dermal; 2 mg/kg, oral) were selected so that minor signs of cholinergic intoxication and a relatively similar degree of cholinesterase inhibition would be elicited by paraoxon in control animals with each route of administration. In each experiment, three groups of rats were used: a control group, which received an iv saline injection, followed after 30 min by the vehicle for the paraoxon; a paraoxon group, which received iv saline, followed by paraoxon; and a paraoxonase + paraoxon group, which was administered 8.35 units of paraoxonase (iv), followed at 30 min by paraoxon. In each experiment, serum paraoxonase activity was measured 30 min and 4.5 hr following enzyme administration to ensure that the activity had risen to the expected levels. In all these experiments, administration of rabbit paraoxonase to rats increased activity in serum by 8- to 10-fold. Cholinesterase activity in plasma, red blood cells, brain, and diaphragm was measured as an index of paraoxon toxicity 4 hr after its administration. Results are shown in Fig. 2. After iv administration of paraoxon (0.1 mg/kg) cholinesterase activity was significantly less inhibited in rats which were pretreated with paraoxonase, suggesting that the increased serum paraoxonase activity had offered protection against paraoxon toxicity (Fig. 2). Paraoxonase offered partial protection when paraoxon (0.5 mg/kg) was given by the ip route. There were no differences in plasma or red blood cell (RBC) cholinesterase activity, while cholinesterase in brain and diaphragm was less affected in paraoxonase-pretreated rats. Similar results were observed when paraoxon was given dermally (0.3 mg/kg), although significant protection was observed only in brain tissue. When paraoxon was given orally (2.0 mg/kg), preadministration of paraoxonase offered no protection against its effects on cholinesterases.

Paraoxonase and chlorpyrifos-oxon toxicity. We also examined the effect of administration to rats of exogenous paraoxonase (partially purified from rabbit serum as described under Materials and Methods) on the toxicity of chlorpyrifos-oxon, which is a very good substrate for this enzyme (Furlong *et al.*, 1988; 1989). The experimental design was identical to that utilized for paraoxon. Administration of 8.35 units of the enzyme by iv injection to rats increased their serum paraoxonase activity toward chlorpyrifos-oxon by 50-fold (Fig. 1). Thirty minutes after

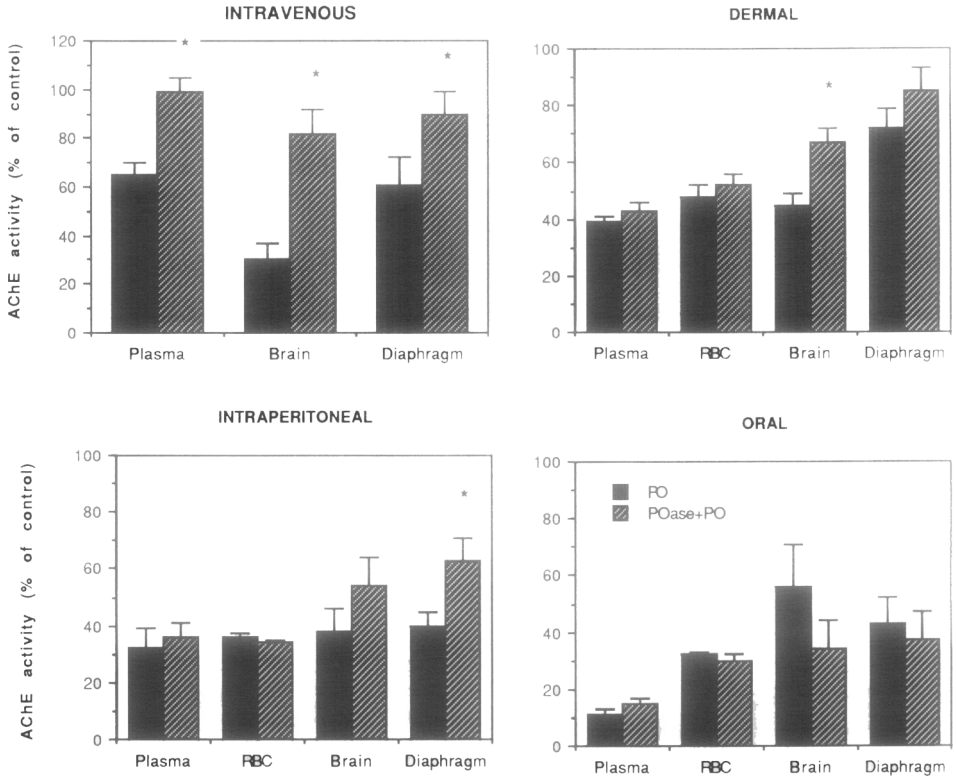

FIG. 2. Inhibition of cholinesterase in various tissues of rats following intravenous, intraperitoneal, dermal, or oral administration of paraoxon (0.1, 0.5, 0.3, and 2.0 mg/kg, respectively). Dark bars represent animals which were injected with paraoxon (PO) alone, while shaded bars indicate animals that were injected iv with 8.35 units of partially purified rabbit paraoxonase (POase) followed after 30 min by paraoxon. Rats were terminated 4 hr following administration of paraoxon. Results are expressed as percentage of control (vehicle-treated) animals and represent the means (±SEM) of 6 to 10 rats. Control AChE activities were (μmol ATC/min/mg of tissue (ml)): 7.21 ± 1.15 (brain); 1.01 ± 0.16 (diaphragm); 0.40 ± 0.03 (plasma); 1.30 ± 0.06 (RBC). *Significantly different from paraoxon alone, $p < 0.05$.

administration of paraoxonase, rats were challenged with an acute dose of chlorpyrifos-oxon given by the iv (0.4 mg/kg), dermal (15 mg/kg), ip (12 mg/kg), or oral (40 mg/kg) route. These doses were shown in preliminary experiments to induce relatively similar degrees of cholinesterase inhibition in plasma, red blood cells, brain, and diaphragm. Animals were terminated 4 hr after chlorpyrifos-oxon administration. As shown in Fig. 3, cholinesterase activity in tissues was significantly less inhibited following chlorpyrifos-oxon in rats which had been pretreated with the enzyme. Maximal protection was seen in brain and diaphragm, independent of the route of administration of chlorpyrifos-oxon. On the other hand, a reduction in the inhibition of plasma and RBC cholinesterase activity was observed only when chlorpyrifos-oxon was given intravenously or applied dermally (Fig. 3).

DISCUSSION

The results in this paper indicate that raising the serum paraoxonase activity in rats decreases their sensitivity to the toxicity of

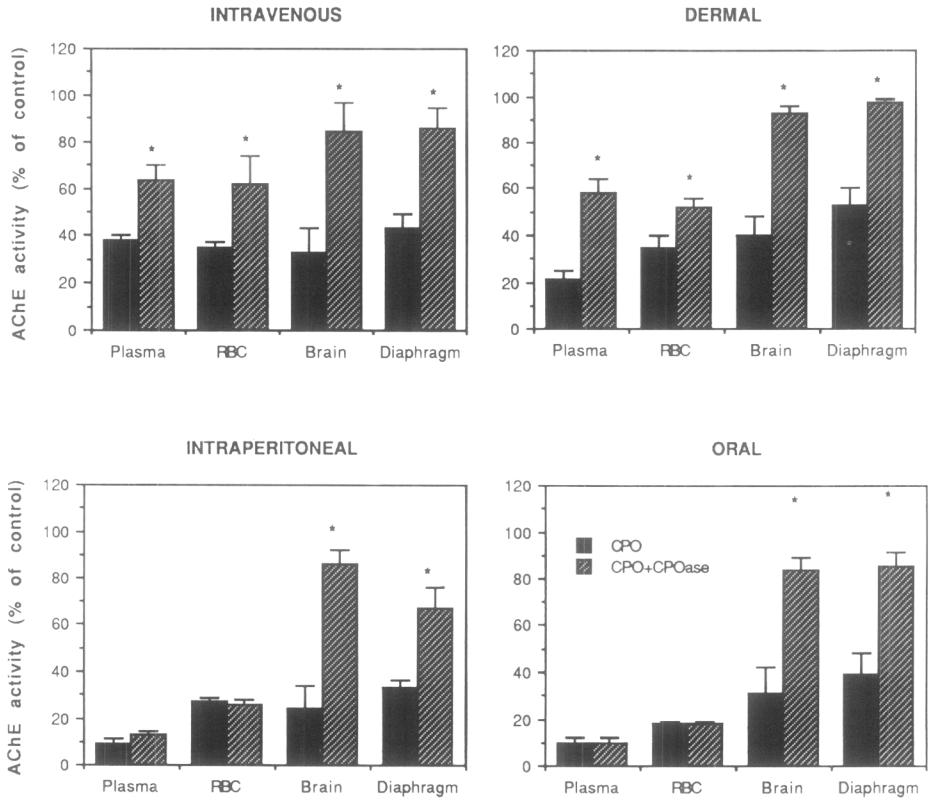

FIG. 3. Inhibition of cholinesterase in various tissues of rats following intravenous, intraperitoneal, dermal, or oral administration of chlorpyrifos-oxon (0.4, 12, 15, and 40 mg/kg, respectively). Dark bars represent animals that were injected with chlorpyrifos-oxon (CPO) alone, while shaded bars indicate animals that were injected iv with 8.35 units of partially purified rabbit paraoxonase (indicated here as CPOase) followed after 30 min by chlorpyrifos-oxon. Rats were terminated 4 hr following administration of chlorpyrifos-oxon. Results are expressed as percentage of control (vehicle-treated) animals and represent the means (±SEM) of five to eight rats. Control AChE activities were (μmol ATC/min/mg of tissue (ml)): 8.09 ± 0.98 (brain); 1.12 ± 0.19 (diaphragm); 0.49 ± 0.04 (plasma); 1.16 ± 0.06 (RBC). *Significantly different from chlorpyrifos-oxon alone, $p < 0.05$.

paraoxon and chlorpyrifos-oxon, the active metabolites of two widely used organophosphorus insecticides.

Despite the recognition that A-esterases can detoxify organophosphates, relatively little is known concerning the role of these enzymes in determining the *in vivo* toxicity of organophosphorus insecticides. The most striking example of how a difference in serum paraoxonase activity could influence selective toxicity of organophosphates is shown by the comparison of birds and mammals (Brealey *et al.*, 1980; Walker and Mackness, 1987). Several organophosphorus insecticides are 10- to 100-fold more toxic to birds than mammals, and this differential sensitivity has been ascribed to differences in serum A-esterase activity (Brealey *et al.*, 1980; Machin *et al.*, 1976; Walker and Machness, 1987). For example, the LD50 of pirimiphos-methyl-oxon is 1–2 g/kg in various mammalian species (rat, mouse, guinea pig), but only 30–60 mg/kg in chickens and 140 mg/kg in the Japanese quail (Brealey *et al.*, 1980). The pirimiphosmethyl-oxonase activity is about 2000 nmol/min/ml of plasma in rats, while it

is undetectable in serum from various avian species (Brealey *et al.*, 1980). Less attention has been given to the role of serum paraoxonase activity in the differential sensitivity of organophosphate toxicity in mammals. Yet, the genetically determined large variation in serum paraoxonase activity in the human population (Playfer *et al.*, 1976; Carro-Ciampi *et al.*, 1981; Eckerson *et al.*, 1983; Mueller *et al.*, 1983; La Du and Eckerson 1984; Ortigoza-Ferado *et al.*, 1984; Geldmacher-von Mallinkrodt and Diepgen, 1987; 1988; Furlong *et al.*, 1988; 1989) suggests that individuals with different serum paraoxonase activity might be differentially susceptible to the toxicity of at least some organophosphates.

In this study, we conducted a series of investigations to determine whether serum paraoxonase levels can regulate the toxicity of paraoxon and chlorpyrifos-oxon. Since the variability of this enzyme's activity in the rat is very low (less than 1.5-fold), we conducted an initial series of experiments using two animal species, rat and rabbit, which had been shown to have different levels of serum paraoxonase activity. The 7-fold difference that we found between these two species falls within the range previously reported (3- to 18-fold) using different assay conditions (Aldridge, 1953; Main, 1956; Brealey *et al.*, 1980; Chemnitius *et al.*, 1983). A difference of 65-fold between rat and rabbit, however, had been reported by Zech and Zurcher (1974). By carefully titrating paraoxon *in vivo*, we determined an approximately 4-fold difference between the doses of paraoxon causing similar degrees of cholinesterase inhibition and minimal clinically visible signs of cholinergic intoxication in the two species. We had shown previously that cholinesterase from both species had the same *in vitro* sensitivity to inhibition by paraoxon, and that both species had similar density of brain muscarinic receptors and a similar functional response upon receptor activation by cholinergic agonists on phosphoinositide metabolism (Costa *et al.*, 1987). Since paraoxonase levels in other tissues (liver, kidney, spleen, brain) do not differ significantly between rabbit and rat (Aldridge 1953; Chemnitius *et al.*, 1983), it is possible, therefore, that the differential sensitivity to paraoxon toxicity between rat and rabbit may be related to the different levels of serum paraoxonase. Possible differences in noncritical binding sites between these two species, however, might also play a role in this differential toxicity (Lauwerys and Murphy, 1969; Butler *et al.*, 1985).

Following an iv injection of 0.15 mg/kg paraoxon in the rabbit, serum paraoxonase was found to hydrolyze as much as 41% of the injected dose within the first 30 sec (Butler *et al.*, 1985). If a similarly important role for paraoxonase is present in the rat, then increasing the activity of the rat's serum enzyme might decrease the toxicity of paraoxon. To test this hypothesis, partially purified rabbit serum paraoxonase was administered by iv injection to rats in order to elevate their total serum paraoxonase activity by 8- to 10-fold. More than 30 years ago Main (1956) injected semipurified rabbit paraoxonase in the saphenous veins of rats and raised their plasma paraoxonase activity by 4- to 5-fold and made them more resistant than controls to the acute toxicity of paraoxon given intravenously (Main, 1956). Our results confirmed and extended these findings, showing that when paraoxon was given by the iv route, its toxicity (measured as inhibition of cholinesterase in various tissues) was significantly decreased in rats pretreated with paraoxonase (Fig. 1).

Additionally, since most human exposures to organophosphates are expected to occur via the oral route (in case of accidental or intentional poisoning), or dermally (the most common route of occupational exposure), we also tested the toxicity of paraoxon administered via these other routes. In all experiments rat serum paraoxonase was elevated by 8- to 10-fold over normal activity, but there was only partial protection against inhibition

of brain or diaphragm cholinesterase following dermal or intraperitoneal administration of paraoxon. No protection was seen when paraoxon was administered orally. Thus, at least in the rat, route of exposure to paraoxon plays an important role in determining whether elevated serum paraoxonase might affect its toxicity. In his study, Main (1956) found that when rats were fed the organochlorine pesticide aldrin for 250 days, paraoxonase activity in serum decreased by 50%, while activity in the liver doubled. Under these conditions, the toxicity of iv paraoxon was slightly increased, in accordance with the lower serum paraoxonase activity; on the other hand, when paraoxon was given orally, its toxicity was decreased by 3-fold. Thus, when paraoxon is given intravenously, its toxicity appears to be regulated by serum paraoxonase (this study; Main, 1956; Butler *et al.*, 1985) and is independent of liver paraoxonase activity, particularly when LD50 doses are used. When paraoxon is administered orally, however, the activity of paraoxonase in the liver, which is three to five times higher than in serum, seems to control the toxicity of the organophosphate (Main, 1956).

A novel finding was also provided by our studies which showed that the toxicity of chlorpyrifos-oxon was decreased by pretreatment of rats with exogenous rabbit paraoxonase. Rabbit paraoxonase hydrolyzes chlorpyrifos-oxon at a much higher rate than paraoxon (Furlong *et al.*, 1988; 1989) and, therefore, administration of the same amount of enzyme to rats caused a 50-fold increase in the hydrolytic capacity of rat serum toward chlorpyrifos-oxon (compared to the 8- to 10-fold for paraoxon). This may explain the finding that the degree of protection toward chlorpyrifos-oxon was greater than that toward paraoxon, particularly following ip and oral administration.

The finding that a substantial protective effect is present when paraoxon and chlorpyrifos-oxon exposure occurs by the intravenous route (which is most similar to the inhalation route) and by the dermal route warrants further investigation on the toxicological significance of serum paraoxonase activity. In particular, although this enzyme has been shown to be different from that hydrolyzing diisopropylfluorophosphate or soman (Chemnitius *et al.*, 1983; De Bisschop *et al.*, 1987), and is also possibly different from the serum enzyme which hydrolyzes DDVP (*O,O*-dimethyl-2,2-dichlorovinyl phosphate; Traverso *et al.*, 1989), paraoxonase can hydrolyze other organophosphates in addition to paraoxon and chlorpyrifos-oxon, including diazinon-oxon (Machin *et al.*, 1976), pirimiphosmethyl-oxon (Brealey *et al.*, 1980), methyl paraoxon (Geldmacher-von Mallinkrodt *et al.*, 1973), and EPN-oxon (Geldmacher-von Mallinckrodt and Diepgen, 1988). A critical question yet to be addressed is the role that different levels of serum paraoxonase play in regulating the toxicity of phosphorothioates, such as parathion and chlorpyrifos, which are the actual compounds applied as pesticides. Such studies are needed before any definite inference can be drawn on the role that serum paraoxonase levels have in the susceptibility to these insecticides. This information would be particularly useful in large-scale epidemiological studies to test the hypothesis that genetic variation in serum paraoxonase activity is a major determinant of susceptibility to poisoning of workers involved in the manufacturing or use of organophosphorus pesticides, and important in setting guidelines for reentry intervals and cholinesterase monitoring.

Finally, the physiological role of paraoxonase is still not known (Mackness, 1989). Its elucidation would shed new light on other potential implications of the human polymorphism with regard to possible inherited susceptibility to certain metabolic or cardiovascular disorders (Mackness, 1989).

ACKNOWLEDGMENTS

This work was supported in part by grants from the Charles A. Dana Foundation and the National Institutes

of Health (ES-03424, ES-04696, OH-00054, and GM-15253). We thank the Dow Chemical Co. for providing chlorpyrifos-oxon and Claudia Thomas for her valuable secretarial assistance.

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