FUNDAMENTAL AND APPLIED TOXICOLOGY 23, 578–584 (1994)

Phosphotriesterase—A Promising Candidate for Use in Detoxification of Organophosphates

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Received June 24, 1993; accepted March 10, 1994

Phosphotriesterase—A Promising Candidate for Use in Detoxification of Organophosphates. TUOVINEN, K., KALISTE-KORHONEN, E., RAUSHEL, F. M., AND HÄNNINEN, O. (1994). *Fundam. Appl. Toxicol.* 23, 578–584.

The effect of phosphotriesterase (PTE) on cholinesterase (ChE) activities was studied with exposures to different organophosphates in mice. Paraoxon (PO) (1.0 mg/kg, ip) almost totally inhibited serum ChE activity. This activity, however, recovered to the normal level within 24 hr. The PTE pretreatment (16.8 U/animal, 2.5 μg/10 g body wt, iv 10 min before the organophosphate) accelerated this reactivation. The same phenomenon was also seen *in vitro*. *In vitro* with human serum, there was only minimal reactivation of the inhibited ChE. PTE, however, reactivated it significantly. The PTE-pretreated mice (168 U/animal, 30 μg/10 g body wt, iv) tolerated even 50 mg/kg of PO without showing any remarkable signs of intoxication. In PTE-untreated animals, however, PO doses as low as 1.0 and 1.5 mg/kg caused severe signs of poisoning. PTE (16.8 U/animal, 4 μg/10 g body wt, iv) reduced the inhibition of brain and serum ChE activities after PO and diisopropyl fluorophosphate exposure. In sarin and soman intoxications, PTE decreased only slightly the inhibition of ChE activities. The results indicate that PTE pretreatment given iv prevents the inhibition of ChE activities after certain organophosphates and it also hastens the recovery of activities after PO poisoning.

Organophosphates (OPs) are widely used as insecticides. In addition OPs, like isopropyl methylphosphonofluoridate (sarin), pinacolyl methylphosphonofluoridate (soman), and dimethylamido ethoxy phosphoryl cyanide (tabun), are important chemical warfare agents (McGeorge, 1987). Although their production and testing have been forbidden by international agreements, their manufacture still continues. In the treatment of OP poisonings, the combination of atropine and oximes (e.g., obidoxime, pralidoxime, bis-pyridinium oxime HI-6) has been used. The ability of oximes to reactivate the OP-inhibited cholinesterases (ChE) is not, however, satisfactory in all cases (Dunn and Sidell, 1989). Moreover, oximes may bind with nerve agents and form phosphonylated oximes which can be as toxic as the OPs themselves (Durham and Hayes, 1962; Namba *et al.*, 1971; Sawyer *et al.*, 1991). Exogenous butyrylcholinesterase and acetylcholinesterase have recently been successfully tested as new possible therapeutic agents against OP toxicity (Wolfe *et al.*, 1987; Ashani *et al.*, 1991a; Wolfe *et al.*, 1991, 1992). Their effects are due to their ability to bind the OPs.

OP hydrolyzing enzymes, such as phosphotriesterase (PTE), could, however, be even more effective in the detoxification of OPs. PTE can hydrolyze several OPs. The enzyme is present in the soil bacteria, *Pseudomonas diminuta* and *Flavobacterium* sp. The PTE coding gene from *P. diminuta* has been transferred into *Escherichia coli* (Serdar and Gibson, 1985; Serdar *et al.*, 1989) and it has also been subcloned into a baculovirus expression system (Dumas *et al.*, 1990a). The LD50 for diethyl-*p*-nitrophenyl phosphate (paraoxon, PO) increased by 250-fold in the fall armyworm larvae infected with this recombinant baculovirus (Dumas *et al.*, 1990a). Active enzyme has also been produced in *Spodoptera frugiperda* (sf9) cells after injection with the recombinant baculovirus. Furthermore, expression of the PTE coding gene in *Drosophila melanogaster* has been reported (Phillips *et al.*, 1990). *In vitro*, PTE hydrolyzes preferentially PO, but also diethyl-*p*-nitrophenyl phosphorothioate (parathion) diisopropyl fluorophosphate (DFP), diethyl fluorophosphate, sarin, soman, *p*-nitrophenyl ethylphenyl phospohinate, and alpha (1-ethylpropylmethyl phosphonofluoridate) (Dumas *et al.*, 1989a,b, 1990b; Ashani *et al.*, 1991b; Raveh *et al.*, 1992). *In vivo*, this enzyme protected mice against tabun toxicity (Ashani *et al.*, 1991b).

Due to the broad substrate specificity of PTE, it seems to be a promising new candidate for use in detoxification of OPs. In previous work, we studied the effect of PTE in PO intoxication in mice (Kaliste-Korhonen *et al.*, 1993). The purpose of this study was to clarify further the effects of

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0272-0590/94 $6.00

PTE on serum and brain ChE activities with different OP exposures. To our surprise, we noticed that PTE also had a reactivating effect on the ChE activities inhibited by PO.

METHOD

Chemicals. The PTE coding gene from *P. diminuta* has been expressed in *E. coli* and a protein preparation has been purified by Dumas *et al.* (1989b) at Texas A & M University. The enzyme was in 50 mM Triethanolamine buffer, pH 9.0, 0.105 mg/ml with the PO hydrolyzing activity 168 U/ml (1600 U/mg protein). In *in vitro* experiments we used PTE preparation which has been purified by Omburo *et al.* (1992). It was in 50 mM Hepes buffer, pH 8.5, 0.861 mg/ml with the PO hydrolyzing activity 6905 U/ml (8020 U/mg protein). PO was purchased from Ehrenstorfer (Augsburg, FRG) and DFP from Fluka AG (Buchs, Switzerland). Sarin and soman were obtained from the Research Centre of The Finnish Defense Forces (Lakiala, Finland) and stored in ethylacetate solution (1 mg/ml) at −20°C. Acetylthiocholine and propionylthiocholine iodide, bovine albumin, atropine sulfate, dithiobis(2-nitrobenzoic acid) (DTNB), and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. Specific pathogen-free CD_2F_1 (7–10 weeks old; weight, 21–27 g) or Han:NMRI (12 or 18 weeks old and 34–38 or 38–44 g, respectively) male mice obtained from the National Laboratory Animal Center, University of Kuopio, Finland, were used. Animals were housed in groups of six in stainless-steel shoebox cages with aspen bedding (FinnTapvei, Kaavi, Finland) and under a controlled alternate schedule of 12 hr light and dark (07.00–19.00). The ambient temperature and the relative humidity of the animal rooms were 22 ± 1°C and 50 ± 10%, respectively. The animals received Rat and Mouse No. 1 Maintenance diet (Special Diets Services Limited, Essex, England). Mice had free access to food and tap water.

For comparative studies, human sera were collected from three men and three women (35–55 years old). The mouse sera were obtained from CD_2F_1 male mice. Samples of sera were stored at −80°C until measurements.

In vivo experiments. The effect of PTE pretreatment on the recovery of the PO hydrolyzing and ChE activities after PO exposure was studied in 18 week old Han:NMRI mice. The animals received iv 0.1 ml PTE preparation (16.8 U, approx 2.5 μg/10 g body wt) or saline (controls) and 10 min later ip the PO dose of 1.0 mg/kg or olive oil (controls). The animals were terminated 24 hr later. Heparinized blood samples (0.1 ml) were drawn from retroorbital sinus into capillary tubes 1, 3, 6, and 24 hr after the PO administration. Sera were separated with a hematocrit centrifuge. The brains were dissected after termination and stored with sera at −80°C until measurements.

The maximum protective effect of PTE in PO exposure was studied in 12 week old Han:NMRI mice. PTE (168 U/animal, 30 μg/m10 g body wt) was given iv 10 min before the ip injection of PO and the animals were observed for possible signs for up to 24 hr. PTE-pretreated mice were terminated either 4 or 24 hr after the PO administration. The mice without PTE pretreatment were terminated when severe signs occurred. Sera were separated and brains were dissected and stored at −80°C until measurements.

In studies of the effects of PTE in different acute OP intoxications, CD_2F_1 mice received iv PTE (16.8 U/animal, 4 μg/10 g body wt) into the tail vein and 10 min later ip different doses of PO, DFP, sarin, or soman in 0.5 or 0.6 ml olive oil. The control animals received saline and olive oil. Doses of OPs were selected to cause a clear inhibition of ChE activities in brain and serum. The animals also received atropine in saline sc, 50 mg/kg, immediately after the OP injection. After 2 hr the animals were terminated. At the end of experiments, animals were anesthetized with CO_2 and blood samples (0.4–1.0 ml) were taken by cardiac puncture and centrifuged at 1000*g* for 15 min at 4°C to separate sera. Brains were dissected and stored with sera at −80°C until measured.

In vitro experiments. The effect of PTE on the mouse and human serum ChE activities inhibited with PO was followed for up to 24 hr. Samples of sera (125 μl) were incubated at 37°C with 0.7 mM PO in 50 mM sodium phosphate buffer (50 μl) before the addition of PTE (50 μl).

Assay of serum PO hydrolyzing activity. PO hydrolyzing activity was measured from serum samples. In the assay, 10 μl of serum was added into 890 μl of 0.0125 M borate buffer (pH 7.5) containing 0.3 mM $CaCl_2$. After preincubation for 2 min, the substrate (100 μl of 3 mM PO) was added and the absorbance was spectrophotometrically monitored at 405 nm (37°C).

Assay of brain and serum ChE activities. ChE activities in brain and serum were measured using the method of Ellman *et al.* (1961). Whole brain samples were homogenized with a Heidolph homogenizer in 10 vol of ice-cold 50 mM sodium phosphate buffer, pH 7.7. Brain homogenates were further diluted with 4 vol of 0.2% Triton in the same buffer and solubilized for 1 min. Then the homogenates were centrifuged at 1500*g* for 30 min at 4°C. The samples of supernatants (50 μl) were preincubated with 1.0 ml of 10 mM DTNB in 50 mM sodium phosphate buffer for 2 min at 37°C. The substrate, 50 μl of 60 mM acetylthiocholine iodide, was added and the change in absorbance was measured spectrophotometrically at 410 nm. Serum ChE activity was assayed using the same method but with propionylthiocholine iodide as the substrate.

Protein. The protein content in the samples were measured using bovine serum albumin as the reference (Lowry *et al.*, 1951).

Statistical analysis. The data are presented as means ± SD. Comparisons of enzyme activities between groups were calculated using one-way analysis of variance followed by Scheffe's test. The p value of 0.05 was used as the level of statistical significance. All calculations were performed with an StatView 512 + programmed Macintosh computer.

RESULTS

Effect of PTE in Vivo

In vivo, the amount of PTE used increased the PO hydrolyzing activity in mouse serum by up to sixfold when measured 1 hr after the administration (Fig. 1A). The half-life of PTE in the circulation was approximately 5 hr, and the activity returned to the normal level by 24 hr. After the PO administration (1.0 mg/kg) the serum ChE activity had totally recovered within 24 hr, both in PTE-pretreated and untreated animals. However, this recovery was faster in PTE-treated mice during the first 6 hr, when the PO hydrolyzing activity in serum was high. Moreover, serum ChE activity was less inhibited in PTE-treated than untreated animals. After 24 hr, the brain ChE activity was reduced to 36% of the control in the animals without PTE treatment, whereas in PTE-treated mice it was reduced only to 60%.

When the PTE amount was increased by 10-fold, the serum PO hydrolyzing activity was 13–16 times higher than control activity 4 hr after administration (Table 1). Even after 24 hr this activity was still 2-fold elevated. In PTE-untreated animals, PO doses of 1.0 and 1.5 mg/kg inhibited the serum and brain ChE activities almost completely within a few hours. The PTE pretreatment clearly protected the brain ChE activities; even the largest PO dose reduced

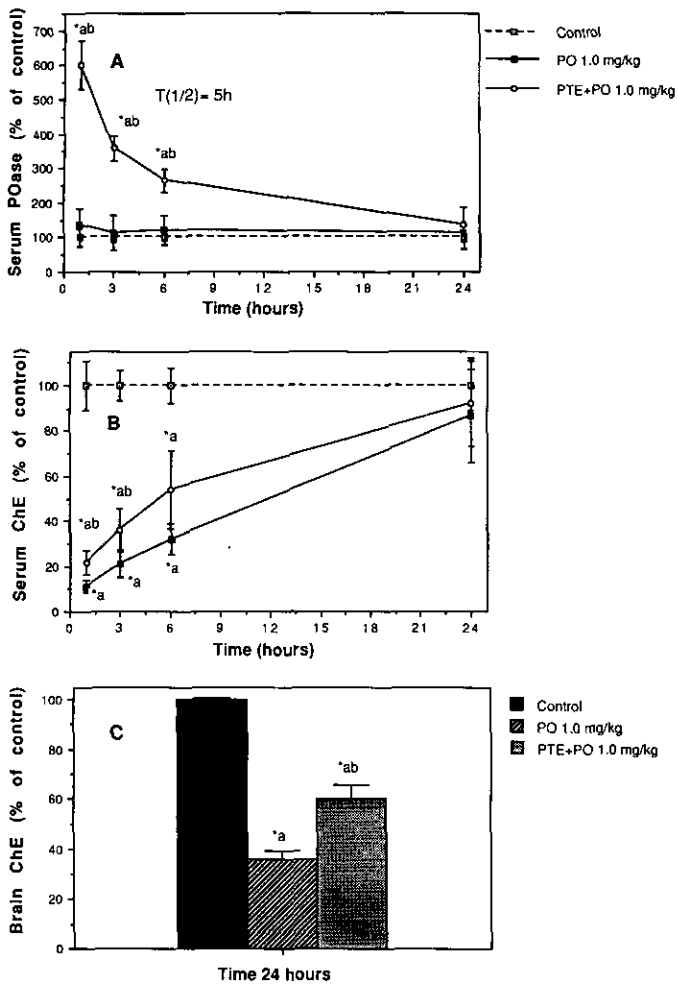

FIG. 1. The effect of phosphotriesterase (PTE, iv 16.8 U/mouse) on serum paraoxon hydrolyzing (POase) (A), serum ChE (B), and brain ChE activities (C) in PO (1.0 mg/kg)-treated mice ($n = 4$). The animals received PTE iv 10 min before the ip injection of PO and atropine sc (50 mg/kg) immediately after PO. Control activities were: serum PO hydrolyzing activity 387 ± 32 nmol/min × ml, serum ChE 5407 ± 275 nmol/min × ml, and brain ChE activity 157 ± 2 nmol/min × mg protein. Means ± SD are given. $^{*a}p < 0.05$ when compared to the control activity. $^{*b}p < 0.05$ when compared to the animals that received PO without PTE.

the ChE activity in brain by only 40% in 4 hr. A similar degree of protection was seen in serum ChE activity with a PO dose of 15 mg/kg. PTE did not, however, protect the serum ChE activities against the higher PO doses. Brain ChE activities seemed to be able to recover, because 24 hr after PO administrations of 30 and 35 mg/kg the activities were at the normal level, although a PO dose of 15 mg/kg caused a clear reduction in activity at 4 hr. Serum ChE activity has still not recovered at 24 hr. The animals without PTE treatment showed severe signs of poisoning already at PO doses of 1.0 and 1.5 mg/kg (Table 2). In the PTE-treated animals, however, only minor and transient signs were observed.

The effects of PTE (16.8 U/mouse) on the serum and brain ChE activities with different OP administrations are shown in Figs. 2 and 3. PTE pretreatment caused a fourfold increase in the serum PO hydrolyzing activity measured 2 hr after administration (data not shown). The PTE treatment reduced the inhibition of brain ChE activity after PO exposure (Fig. 2A). A fourfold higher PO dose, 2.0 mg/kg, was needed to cause a similar inhibition of brain ChE activity in the PTE-treated animals as that seen after a PO dose of 0.5 mg/kg in mice without PTE. PTE treatment also protected, though to a lesser extent, serum ChE activities. In DFP exposure, PTE also protected the brain ChE activity (Fig. 2B). The serum ChE activities were very sensitive to the inhibitory effect of DFP and only slight protection by PTE was observed.

The sarin dose of 0.15 mg/kg significantly decreased the brain ChE activity, whereas in PTE-treated animals a higher dose (0.20 mg/kg) caused no significant inhibition (Fig. 3A). Moreover, 0.50 mg/kg of sarin did not cause greater inhibition of brain and serum ChE activity in PTE-treated animals than a dose of 0.30 mg/kg in untreated animals. PTE did not protect the ChE activities in brain or serum against soman (Fig. 3B). However, the soman dose of 0.30 mg/kg in PTE-pretreated animals did not cause a greater inhibition of the activities than the soman dose of 0.20 mg/kg without PTE.

Effect of PTE in Vitro

PTE in amounts of 3.45, 34.5, and 345 U increased the PO hydrolyzing activity by 10- to 1000-fold in the incubation system (Fig. 4A). Furthermore, with the highest PTE amount tested, the activity notably increased during the first 3 hr and after that it started to be inactivated. PO effectively inhibited the mouse serum ChE activity (Fig. 4B). However, the activity recovered spontaneously from 4 to 37% of the control level during 24 hr. ChE activities were less inhibited in PTE-added than in control serum. PTE also hastened the recovery of ChE activity. The inhibited ChE activity recovered due to PTE from 25–31 to 76–100% of the control activity in 24 hr. Increasing the amount of PTE caused only minor changes in the recovery of the ChE activity.

PTE was more stable in human serum than in mouse serum (Fig. 5A). PTE had the same reactivating effect on the inhibited ChE activity as that in mouse serum (Fig. 5B). In the presence of PTE, the PO-inhibited ChE activity recovered from 4 to 16% of the control level in 24 hr. In contrast to mouse serum, only minimal spontaneous reactivation of human serum ChE occurred. The reactivation was also considerably faster in mouse serum during 24 hr.

DISCUSSION

The ChE activity in mouse serum had recovered to the normal level within 24 hr after a moderate PO administra-

TABLE 1
Brain ChE, Serum ChE, and Paraoxon (PO) Hydrolyzing Activity (% of Control, $n = 1$ at Each PO Dose) in Mice

Doses	Time of termination	Brain ChE (% of control)	Serum ChE (% of control)	Serum paraoxon hydrolyzing activity (% of control)
PO 1.0 mg/kg + saline	2 hr 40 min	21	6	100
PO 1.5 mg/kg + saline	1 hr	12	11	103
PO 15.0 mg/kg + PTE	4 hr	77	51	1659
PO 50.0 mg/kg + PTE	4 hr	60	11	1302
PO 30.0 mg/kg + PTE	24 hr	98	—	241
PO 35.0 mg/kg + PTE	24 hr	99	20	192

Note. The animals received phosphotriesterase (PTE, 168 U/animal) iv before the ip injection of PO, and samples were collected 4 or 24 hr after the PO injection. The mice which received PO without PTE treatment were sacrificed when severe signs occurred. Control activities (means ± SD, $n = 3$) were: brain ChE, 143 ± 3 nmol/min × mg protein; serum ChE, 6359 ± 15 nmol/min × ml; and PO hydrolyzing activity, 389 ± 84 nmol/min × ml.

tion (Fig. 1). This occurred both in PTE-pretreated and in untreated animals, but PTE clearly accelerated the recovery. Also, *in vitro* mouse serum ChE activity which was almost completely inhibited showed a recovery both spontaneously and in the presence of PTE (Fig. 4). The spontaneous reactivation may be due to the endogenous serum PO hydrolyzing enzyme in mouse serum. The reactivation of OP-inhibited ChE by PO hydrolyzing enzyme (or PTE) can happen in two ways: the enzyme breaks the ChE–OP complex; or, if this is spontaneously broken, the enzyme hydrolyzes the OP molecule before it can inhibit a new ChE molecule. In light of the studies of Chambers and Chambers (1989), this latter alternative would seem more probable. According to them, PO inhibited the brain AChE activity in two steps in rats. The first stage was reversible, whereas the second, irreversible step caused an aging of the inhibited ChE. The aging gradually increased and was complete after 4 days. After that no spontaneous reactivation took place, and the recovery of ChEs was due exclusively to *de novo* synthesis of enzyme. The rate of aging depends on the OP. For example, the half-time for the aging of sarin–ChE complex is 5 hr, but for soman–ChE complex it is only about 2 min (Dunn and Sidell, 1989). This would explain the poor detoxifying effect of PTE in mice exposed with soman in the present study.

In human serum, the negligible spontaneous reactivation of the PO-inhibited ChE may be due to the lower endogenous activity and/or the different properties of the PO hydrolyzing enzyme in human serum (Fig. 5B). Also, the stability of the ChE–OP bond can be the reason for the slow reactivation. PTE could, however, effectively but incompletely reactivate the human serum ChE activity which had been inhibited by PO.

In *in vitro* incubations, the PO hydrolyzing activity of PTE (345 U) increased during the first 3 hr with both human and mouse sera. This could be due to the high levels of PO in the incubation system during the first 3 hr, which may have an inhibitory effect on the activity.

TABLE 2
Signs of Mice ($n = 1$ at Each PO Dose) when the Animals Received Saline or Phosphotriesterase (PTE, 168 U/animal) iv before the ip Injection of Paraoxon (PO)

Doses (time of termination after PO)	Symptoms (hr)				
	1	2	3	4	24
PO (1.0 mg/kg + saline) (2 hr 40 min)	Piloerection, decreased mobility 15 min after administration	Tremors, disorders of balance	—	—	—
PO (1.5 mg/kg + saline) (1 hr)	Piloerection, disorders of balance, strong convulsions 15–20 min after treatment. No mobility	—	—	—	—
PO 15.0 mg/kg + PTE (4 hr)	Decreased mobility, piloerection	Piloerection, normal mobility, eating	ns	ns	—
PO 50.0 mg/kg + PTE (4 hr)	Decreased mobility, piloerection	Decreased mobility	ns	ns	—
PO 30.0 mg/kg + PTE (24 hr)	Decreased mobility, piloerection	Decreased mobility	ns	ns	ns
PO 35.0 mg/kg + PTE (24 hr)	Decreased mobility, piloerection	Decreased mobility	ns	ns	ns

Note. The mice which received PO without PTE treatment were sacrificed when severe signs occurred. ns, no signs, normal behavior.

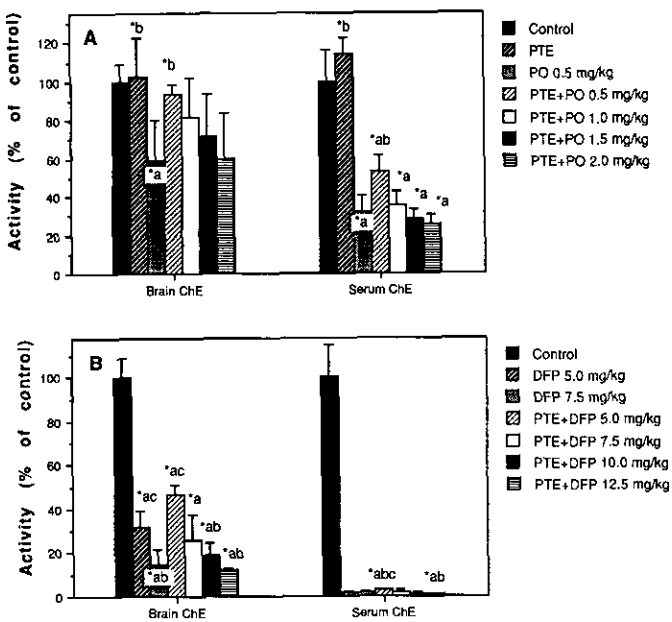

FIG. 2. The effect of phosphotriesterase (PTE, iv 16.8 U/mouse) on brain and serum ChE activities in paraoxon (PO)- (A) or DFP- (B) treated mice (n = 5 or 6). The animals received PTE iv 10 min before the ip injection of OP and atropine sc (50 mg/kg) immediately after OP, and the samples were collected 2 hr after the OP injection. Means ± SD are given. $^{*a}p < 0.05$ when compared to the control group. $^{*b}p < 0.05$ when compared to the animals administered PO or DFP at a dose of 5.0 mg/kg without PTE. $^{*c}p < 0.05$ when compared to the animals administered 7.5 mg/kg DFP without PTE.

When the amount of PTE was increased by 10-fold, the animals could tolerate a PO dose as high as 50 mg/kg without revealing any clear signs of toxicity (Table 1). With this PO dose, the inhibition of brain ChE activity remained at 40% of normal in 4 hr, whereas the serum ChE activity was inhibited by 90%. Brain ChE activity seemed to be able to recover, because 24 hr after the PO doses of 30 and 35 mg/kg it was at a normal level. Mouse brain also possesses endogenous PO hydrolyzing activity, which may reactivate the ChE enzyme. However, brain ChE activity did not recover to the normal level in PTE (16.8 U) group after a PO dose of 1.0 mg/kg at 24 hr. The recovery could also be due to the PTE in brain. However, we did not find any increase in the PO hydrolyzing activity in brain after the smaller (16.8 U) PTE treatment (data not shown). Despite this, the larger amount of PTE could have been able to increase this activity in brain. Although the brain ChE activity totally recovered in 24 hr, the activity in serum did not. Presumably, PO was stored in peripheral tissues from where it was released back into the circulation and bound with the serum ChE inhibiting the activity once more. The half-life of PTE in mouse blood circulation is only 5 or 6 hr (Kaliste-Korhonen *et al.*, 1993; Fig. 1A). Therefore exposure to large doses of OP would need several treatments with PTE before all the OP had been detoxified. On the basis of Fig. 1B, it can be estimated that repeated administration of PTE every 3 hr could accelerate the reactivation of ChE activity by twofold, and the normal activity could be attained within 12 hr.

The PTE pretreatment protected the ChE activities in brain and serum in mice exposed to OPs (Figs. 2 and 3). The results correlated with the OP hydrolyzing activity of PTE *in vitro*. PTE can best hydrolyze PO (k_{cat}, 2070/sec), whereas the hydrolysis of DFP, sarin, and soman is much slower (k_{cat}, 56, 41, and 4.8/sec, respectively; Dumas *et al.*, 1990b). In this study with CD_2F_1 mice, PTE was able to protect brain and serum ChE against PO and DFP. In our previous studies with NMRI mice, we did not find any protective effect of the same amount of PTE on the serum ChE activity against a PO dose of 0.5 mg/kg (Kaliste-Korhonen *et al.*, 1993). The explanation for this difference is presumably the different properties of the strains. PTE offered no protective effect on the ChE activities with lower doses of sarin and soman. The highest doses of these tested, however, did not increase the inhibition of the ChE activities in PTE-pretreated animals. This indicates that PTE hydrolyzes these compounds effectively only when the substrate concentrations are sufficiently high. This assumption is supported by the finding of Broomfield *et al.* (1992). They

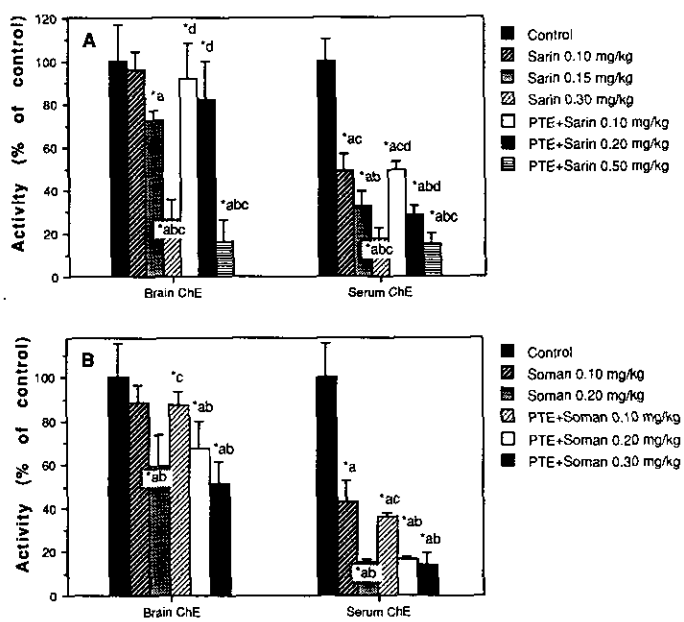

FIG. 3. The effect of phosphotriesterase (PTE, iv 16.8 U/mouse) on brain and serum ChE activities in sarin (A)- or soman (B)-treated mice (n = 4–6). For explanations see the legend to Fig. 1. Means ± SD are given. $^{*a}p < 0.05$ when compared to the control group. $^{*b}p < 0.05$ when compared to the animals treated with the lowest OP dose without PTE. $^{*c}p < 0.05$ when compared to the animals treated with the middle OP dose without PTE. $^{*d}p < 0.05$ when compared to the animals treated with the highest OP dose without PTE.

have reported that mice treated with PTE survived doses of soman which were more than double the lethal dose of the untreated animals.

It was important that PTE treatment was given before or immediately after OP administration due to the quite low amount of PTE in this study. In a previous study, PTE given iv (0.1 ml, PO hydrolyzing activity, 1.5 μmol/min) prevented the decrease in brain ChE activity by PO and when PTE was injected 15 min after PO it still significantly prevented the reduction of the brain ChE activity when compared to the PTE-untreated animals or animals treated 1 hr after PO (Kaliste-Korhonen *et al.*, 1993). When the larger amount of PTE (2400 U/mouse, 64–69 μg/10 g body wt) was administered 1 1/2 hr after the PO injection, it significantly prevented inhibition of ChE activities in brain, lung, and blood when measured 24 hr after intoxication (author's unpublished observation). Because the rate of aging is quite slow for ChE–PO complex, it is possible that PTE pretreatment could be much more important in DFP, sarin, and soman intoxications.

Our results indicate that PTE is a promising candidate for use in detoxification of OPs. In acute poisoning, the PTE-pretreated animals tolerated even a 50-fold higher amount of PO when compared to the untreated animals.

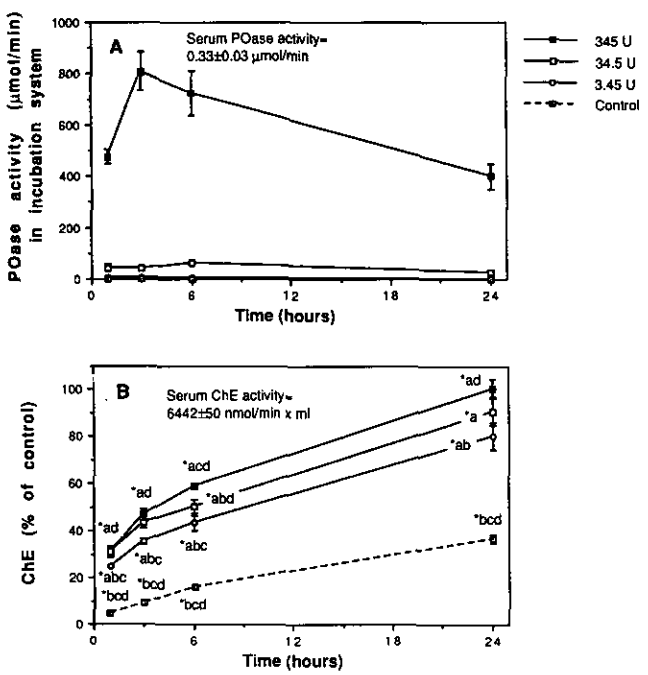

FIG. 4. The effect of PTE on the paraoxon hydrolyzing activity (POase) (A) and the recovery of serum ChE activity (B) *in vitro*. Mouse serum was incubated with 0.7 mM PO and PTE at 37°C. PTE was added into the incubation system immediately after the addition of PO. Means ± SD are given ($n = 6$). $^{*a}p < 0.05$ when compared to the control group. $^{*b}p < 0.05$ when compared to the 345 U of PTE. $^{*c}p < 0.05$ when compared to the 34.5 U of PTE. $^{*d}p < 0.05$ when compared to the 3.45 U of PTE.

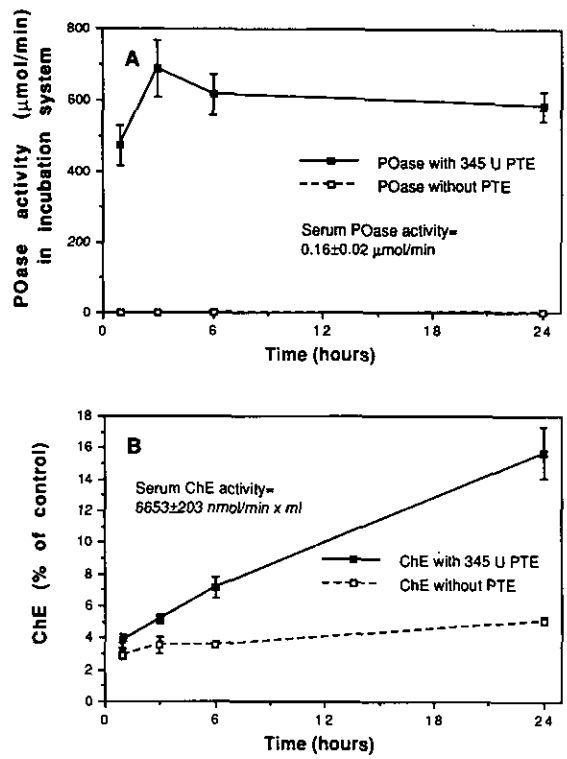

FIG. 5. The effect of PTE on the paraoxon hydrolyzing activity (POase) (A) and the recovery of serum ChE activity (B) *in vitro*. Human serum was incubated with 0.7 mM PO and PTE at 37°C. PTE (345 U) was added into the incubation system immediately after the addition of PO. Means ± SD are given ($n = 6$).

Moreover, PTE seemed to have ChE-reactivating properties. The importance of this phenomenon is likely greater for humans because of the poor spontaneous recovery of ChE activity in human serum after its inhibition by PO.

ACKNOWLEDGMENTS

This study was financially supported by MATINE/Finnish Scientific Committee for National Defense (contract 61/Mdd 341/92). The authors are grateful to Dr. Ewen MacDonald for checking the language of the manuscript.

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