TOXICOLOGICAL SCIENCES **109(2)**, 276–285 (2009)
doi:10.1093/toxsci/kfp068
Advance Access publication April 6, 2009

The Homeostasis of Phosphatidylcholine and Lysophosphatidylcholine in Nervous Tissues of Mice was not Disrupted after Administration of Tri-*o*-cresyl Phosphate

Wei-Yuan Hou,*,† Ding-Xin Long,* and Yi-Jun Wu*,[1]

*Laboratory of Molecular Toxicology, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, P.R. China; and †Graduate School of the Chinese Academy of Sciences, Beijing 100039, P.R. China

Received January 6, 2009; accepted March 17, 2009

Neuropathy target esterase (NTE) is proven to act as a lysophospholipase (LysoPLA) in mice and phospholipase B (PLB) in cultured mammalian cells. In sensitive species, organophosphate (OP)–induced delayed neurotoxicity is initiated when NTE is inhibited by > 70% and then aged. It is hypothesized that homeostasis of phosphatidylcholine (PC) and/or lysophosphatidylcholine (LPC) in mice might be disrupted by the OPs since NTE and other phospholipases could be inhibited. To test this hypothesis, we treated mice using tri-*o*-cresyl phosphate (TOCP), which can inhibit and age NTE. Phenylmethylsulfonyl fluoride (PMSF), which inhibits NTE but cannot age, was used as a negative control. Effects on activity of NTE, LysoPLA, and PLB, the levels of PC, LPC, and glycerophosphocholine (GPC), and the aging of NTE in the brain, spinal cord, and sciatic nerve were examined. The results showed that the activities of NTE, NTE-LysoPLA, LysoPLA, NTE-PLB, and PLB were significantly inhibited in both TOCP- and PMSF-treated mice, and the inhibition of NTE and NTE-LysoPLA or NTE-PLB showed a high correlation coefficient. The NTE inhibited by TOCP was of the aged type, while nearly all NTE inhibited by PMSF was of the unaged type. Although the GPC level was remarkedly decreased, no significant change of PC and LPC levels was observed. However, the inhibition of these enzymes in mice by TOCP exhibited different characteristics from the TOCP-treated hens that we previously reported, which indicates that these enzymes were inhibited and then recovered more rapidly in mice than in hens. All results suggest that PC and LPC homeostasis was not disrupted in mice after exposure to TOCP. Differences in inhibition of NTE, LysoPLA, and PLB activities by TOCP between mice and hens may elucidate why these two species display different signs after exposure to the same neuropathic OPs.

Key Words: **organophosphate; homeostasis; phosphatidylcholine; lysophosphatidylcholine; mouse; neuropathy target esterase.**

Neurotoxic organophosphates (OPs) constitute active components of the major classes of pesticides, industrial additives, and chemical warfare agents. Human exposure to these OPs may lead to acute toxicity due to the inhibition of acetylcholinesterase (Karczmar, 1984; Solberg and Belkin, 1997). Certain OPs such as tri-*o*-cresyl phosphate (TOCP), one of the three isomers (i.e., *o*-, *m*-, or *p*-cresyl) of tricresyl phosphate which have been widely used as plasticizers, plastic softeners, flame-retardants, and jet oil additive in industry (Craig and Barth, 1999; Winder and Balouet, 2002), are able to induce chronic neurological disorders known as OP-induced delayed neurotoxicity (OPIDN). OPIDN is an axonopathy characterized by distal degeneration of some long and large-diameter axons in the peripheral nerves and the spinal cord (Johnson, 1993; Lotti, 1992). Inhibition and aging of neuropathy target esterase (NTE) has been proposed as the initial effect of OPs that induce OPIDN (Glynn, 2000; Johnson, 1990; Lotti, 1992). NTE is a neural protein with esterasic activity which is anchored to the cytoplasmic face of the endoplasmic reticulum (Akassoglou *et al*., 2004; Li *et al*., 2003). NTE is defined as the paraoxon-resistant and mipafox-sensitive esterase with phenyl valerate–hydrolyzing activity (Johnson, 1977). It was reported that an OP dose sufficient to inhibit NTE activity by > 70% would predictably cause clinical signs of neuropathy 1–3 weeks later (Johnson, 1969, 1975).

A species specific susceptibility to OPIDN has been demonstrated. Adult hens have been widely used as the animal model for experimental studies of OPIDN due to their sensitivity and the development of clinical signs similar to those seen in humans (Barrett *et al*., 1985; Schwab and Richardson, 1986). In contrast, rodents, particularly mice, are considered to be insensitive to clinical expression of OPIDN though the pathological changes undoubtedly occur (Abou-Donia and Lapadula, 1990; Veronesi *et al*., 1991). However, the mouse model has some features in common with OPIDN in hens. It has been reported that chronic dosing with TOCP can induce OPIDN in mice (Lapadula *et al*., 1985). Moreover, mice given 2-octyl-4*H*-1,3,2-benzodioxaphosphorin 2-oxide and ethyl

[1] To whom correspondence should be addressed at Institute of Zoology, CAS, Datunlu Road, Beijing 100101, P.R. China. Fax: +86-10-64807099. E-mail: wuyj@ioz.ac.cn.

octylphospho-nofluoridate sometimes display ataxia and paralysis 1–3 days after early transient cholinergic signs, though the delay prior to onset of this neurotoxicity differs from OPIDN in hens and apparently correlates with NTE inhibition, but does not require NTE aging (Wu and Casida, 1996). Unfortunately, the mechanism responsible for neurotoxicity of OPs and different presentation of signs in mice and hens that are exposed to neuropathic OPs has not been elucidated.

Recently great progress has been made in understanding the biochemical function of NTE and has provided new clues to investigate these unresolved problems. NTE is suggested to be as a type of lysophospholipase (LysoPLA) with lysophosphatidylcholine (LPC) as its physiological substrate in mouse (Quistad *et al*., 2003). Further study demonstrated that NTE also has phospholipase B (PLB) activity and its biochemical function is involved with membrane phosphatidylcholine (PC) homeostasis in mammalian cell lines and yeast (Read *et al*., 2007; Zaccheo *et al*., 2004). NTE is a member of the very large class of serine hydrolases that includes esterases, phospholipases, amidases, peptidases and proteases (Glynn, 2005). Serine hydrolases are considered sensitive targets of OPs (Casida and Quistad, 2005; Quistad *et al*., 2006; Vose *et al*., 2007). Not only paraoxon-resistant and mipafox-sensitive LPC-hydrolyzing activity (NTE-LysoPLA) but also total LysoPLA activity is inhibited by several OPs *in vitro* and *in vivo* (Quistad and Casida, 2004). Together, these early studies indicate that the homeostasis of PC and/or LPC might be disrupted, which could be related to the neurotoxicity in mice after certain OPs exposure due to the inhibition of NTE or other phospholipases.

PC is the most abundant phospholipid of eukaryotic cells (Cui and Houweling, 2002). PC homeostasis is regulated by a balance between the opposing actions of hydrolysis and synthesis (Baburina and Jackowski, 1999). PC is mainly synthesized from the cytidine-5′-diphosphocholine (CDP)-choline pathway via a phosphorylcholine intermediate for condensation with diacylglycerol to produce PC (Cornell and Northwood, 2000; Jackowski and Fagone 2005; Kent, 2005). PC can be hydrolyzed by phospholipase A (PLA), PLB, and phospholipase D (PLD) (Adibhatla *et al*., 2006). NTE is a devoted PLB only hydrolyzing PC derived from the CDP-choline pathway, whereas PC molecules formed through the methylation pathway are not degraded (Glynn, 2005; Zaccheo *et al*., 2004). LPC is a normal constituent of mammalian plasma, where it constitutes 5–20% of the total phospholipids (Nelson, 1967). An elevated LPC level is deleterious to normal cells. For example, LPC can induce neuronal sheath demyelination, together with a variable degree of axonal degeneration (Hall, 1972; Jean *et al*., 2002), which is similar to the pathological changes in OPIDN. LPC is mainly produced from PC deacylation catalyzed by PLA. LysoPLAs are the principal enzymes for removing LPC from cell membranes (Ross and Kish, 1994; Wang and Dennis, 1999) (see Fig. 1 for the pathway of NTE participating in PC and LPC metabolism).

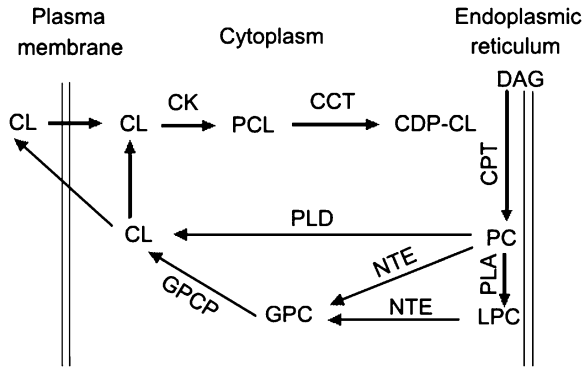

FIG. 1. The pathway of NTE participating in PC metabolism. Choline (CL) is transported into cells and then phosphorylated by choline kinase (CK). Phosphocholine (PCL) reacts with cytidine triphosphate (CTP) in the pathway's rate-limiting step catalyzed by CTP:phosphocholine cytidylyltransferase (CCT) forming CDP-choline (CDP-CL). CDP-CL reacts with diacylglycerol (DAG) and is catalyzed by choline phosphotransferase (CPT) to form membrane-associated PC. PC can be hydrolyzed by PLD, forming phosphatidic acid and CL. PC can also be deacylated by NTE at the cytoplasmic face of the endoplasmic reticulum to form soluble products: free fatty acids and GPC. In addition, PLA and NTE can sequentially deacylate PC to GPC. GPC is hydrolyzed by glycerophosphorylcholine phosphodiesterase (GPCP), forming CL and glycerophosphate. CL can be phosphorylated or secreted from the cell. The above information is combined from Quistad *et al*. (2003), Anfuso *et al*. (2003), Zaccheo *et al*. (2004), and Read *et al*. (2007).

As might be expected from Figure 1, PC and LPC could accumulate after OPs exposure since NTE or other phospholipases are inhibited. In the present study, we examined the effect of TOCP on activities of NTE, NTE-LysoPLA, LysoPLA, NTE-PLB, PLB, and the levels of PC, LPC, and glycerophosphocholine (GPC) *in vivo* in brain, spinal cord, and sciatic nerve of mice to investigate the relationship between alteration of NTE-LysoPLA or PLA and NTE-PLB or PLB activities and the alteration of the homeostasis of the PC, LPC, and GPC. These data were then compared with our previously reported results obtained from TOCP-treated hens to look for possible clues to elucidate the mechanism for the different signs between mice and hens after TOCP exposure.

MATERIALS AND METHODS

Reagents. TOCP (purity > 99%) was purchased from BDH Chemicals Co. Ltd (Poole, UK). Coomassie brilliant blue G-250 and phenylmethylsulfonyl fluoride (PMSF) were purchased from Fluka Chemika (Buchs, Switzerland). Mipafox and phenyl valerate were synthesized in our laboratory as described previously (Johnson, 1982). Benzenesulphonyl fluoride, paraoxon, *sn*-glycero-3-phosphocholine phosphodiesterase, choline oxidase, peroxidase (horseradish), PC, LPC, and GPC were purchased from Sigma (St Louis, MO). 3-(N-ethyl-3-methylanilino)-2-hydroxypropanesulfonic acid sodium salt was obtained from Nanjing Robiot Company (Nanjing, China). 4-Aminoantipyrine was obtained from Beijing Xizhong Chemical Factory (Beijing, China) and silica gel 60 F_{254} plates (20 × 20, 0.25 mm thick) were purchased from Merck (Darmstadt, Germany).

Animals. Adult male Kunming (KM) mice (8 weeks old and 20–25 g in size) used in this study were obtained from the Animal Center, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences. The mice

were housed in groups of three per cage and maintained under standard conditions with access to water and food *ad libitum*. The animals were acclimatized for 1 week prior to the start of the experiment. All animal procedures were performed in accordance with current China legislation and approved by the Institute of Zoology Animal and Medical Ethics Committee.

Animal administration. Seventy-two mice were divided into three groups (Control, TOCP, and PMSF treated) with 24 in each group. The TOCP-group mice were given a single dose of 900 mg/kg TOCP dissolved in dimethyl sulfoxide (DMSO) orally, while 12 mice in control group were given DMSO vehicle only. The PMSF-group mice were injected with PMSF (200 mg/kg, sc) dissolved in DMSO, while the other twelve mice in the control group received injected DMSO vehicle only. Beginning on the first day postexposure, the mice were examined twice daily (in the morning and late afternoon) for the delayed neurotoxic signs until the 14th day. On completion of evaluations at 4 h, 4, 7, and 14 days postdosing, six mice from each group at each time point were sacrificed, respectively, by cervical decapitation. The whole brain, spinal cord, and sciatic nerve were quickly dissected and frozen in liquid nitrogen before storing at −80°C.

NTE activity assay. Nervous tissues were homogenized in TE buffer (50mM Tris-HCl, 0.2mM ethylenediaminetetraacetic acid [EDTA], pH 8.0) and centrifuged at 100 × g at 4°C for 2 min. NTE activity in the supernatant fraction was determined from colorimetric assay of the phenol formed by the absorbance difference for phenyl valerate hydrolysis between samples exposed to 40μM paraoxon and those with both 40μM paraoxon plus 50μM mipafox according to Johnson (1977) with modification for reduced volume microassay as previously described in our lab and expressed as nanomoles of phenol formed per minute per milligram of protein with phenol as the standard (Chang *et al*., 2006). Concentration of protein was measured by the method of Bradford (1976) using bovine serum albumin as standard.

NTE aging measurement. The aging measurement procedure was previously described by Kellner *et al*. (2000) with modifications for reduced volume. Nerve tissue samples were homogenized in 10% (wt/vol) TE buffer. Fresh solutions of both potassium chloride (KCl) and potassium fluoride (KF) reactivation buffer (250mM in 50mM Tris-Citrate, 0.2mM EDTA, pH 5.2) were prepared; and reactivation was initiated by adding 0.25 ml nerve tissue homogenate to 1.5 ml of KF reactivation buffer. Another 0.25-ml aliquot was added to 1.5 ml of KCl-containing buffer in plastic test tubes (buffer prewarmed to 37°C in shaking water bath). After a 30-min incubation at 37°C, tubes were cooled on ice and 4.25 ml of ice-cold distilled water was added to each tube to slow the reaction. The cooled tubes were centrifuged at 27, 000 × g for 60 min, the supernatant was discarded, and the pellet was resuspended in 1 ml of TE buffer. NTE activity was determined as described above except using benzenesulphonyl fluoride (250μM, final concentration) instead of paraoxon (Johnson *et al*., 1985).

NTE-LysoPLA, NTE-PLB, total LysoPLA, and PLB activities assay. The procedures to measure NTE-LysoPLA and total LysoPLA were modified from a method for analysis of LPC in human plasma and described by Quistad *et al*. (2003) and expressed as mAU/min (Kishimoto *et al*., 2002; Quistad *et al*., 2003). NTE-LysoPLA was defined as the paraoxon-resistant and mipafox-sensitive LPC-hydrolyzing activity. Briefly, two reagents (A and B) were used. Reagent A contains 3-(N-ethyl-3-methylanilino)-2-hydroxysulfonate (3mM), peroxidase (10 units/ml), *sn*-glycero-3-phosphocholine phosphodiesterase (0.1 units/ml), and choline oxidase (10 units/ml). Reagent B contains 5mM 4-aminoantipyrine. All reactants were dissolved in 100mM Tris buffer (pH 8.0) containing 1mM calcium chloride and 0.01% Triton X-100. The supernatant fraction (700 × g, 10 min, 4°C) of nervous tissues homogenate (nervous tissues were homogenized in 20% [wt/vol] TE buffer) was used as the enzyme sample. Four reaction groups (I, II, III, and IV) with six replicates each were designed. The total reaction volume of each group was 320 μl. One hundred and twenty microliters of reagents A, 80 μl of reagent B, and 15 μl of the sample were added to individual wells containing Tris buffer in a 96-well polystyrene plate. Then 40μM (final concentration) paraoxon and 50μM (final concentration) mipafox plus paraoxon were added, respectively, in groups III and IV. After a 20-min incubation at 25°C, LPC (250μM, final concentration) was introduced in all groups except excluding group I, to which Tris buffer added instead. The enzyme activity was measured by kinetic assay of absorbance at 570 nm for 10 min at 25°C using a microplate reader with group I as the blank. Total LysoPLA was measured from group II. The difference of LPC-hydrolysis activity between groups III and IV is the NTE-LysoPLA activity as defined. The NTE-PLB and total PLB were determined with the same procedure except that the substrate was PC instead of LPC.

Lipids and choline metabolites extraction. Lipids and choline metabolites were extracted from nervous tissues with a chloroform/methanol mixture (2:1, vol/vol) using the procedure of Folch *et al*. (1957) with some modifications. Briefly, tissue samples were weighted, and then homogenized in 10% (wt/vol) TE buffer at 4°C. The homogenate was centrifugated at 1240 × g for 10 min, then the supernatant (S_1) and the sediment (P_1) were separated. Fivefold the S_1 volume of chloroform/methanol mixture (2:1, vol/vol) was introduced and stirred vigorously, and then the upper aqueous phase (U_1) and the lower organic phase (L_1) were recovered after centrifugation (140 × g, 5 min). The P_1 was homogenized again in 20-fold volume chloroform/methanol mixture (2:1, vol/vol) of the tissue sample weight at 4°C, and then centrifugated at 1240 × g for 10 min. The supernatant (S_2) and the sediment (P_2) were separated. The S_2 was recovered. The P_2 was re-extracted in 10-fold volume chloroform/methanol mixture (2:1, vol/vol) of the tissue sample weight and supernatant (S_3) was recovered after centrifugation (1240 × g, 10 min). S_2 and S_3 was combined, and then 0.2-fold combined S_2 and S_3 volume of 0.1M KCl was introduced and vigorously mixed. The upper aqueous phase (U_2) and lower organic phase (L_2) were recovered after centrifugation (140 × g, 5 min). Then U_1 and U_2, L_1, and L_2 were combined. Organic extracts were evaporated to dryness under nitrogen stream. Water-soluble choline metabolites were evaporated under nitrogen stream to evaporate methanol, then freeze-dried. After evaporation, the lipid extract and choline metabolites were redissolved in a small volume of chloroform/methanol (1/1, vol/vol) and methanol/water (1/1, vol/vol), respectively, for thin-layer chromatography (TLC).

Separation of phospholipids and choline metabolites by TLC. Analysis of phospholipids was achieved by one-dimensional TLC on silica gel 60 plates using chloroform/methanol/acetic acid/acetone/water (40/25/7/4/2, vol/vol) as the solvent, according to the method of Wang and Gustafson (Wang and Gustafson, 1992). The choline metabolites were fractionated by TLC in methanol/0.6% NaCl/25% aqueous ammonia (10/10/1, vol/vol) (Williams and McMaster, 1998). The individual phospholipids and choline metabolites were visualized on TLC plates by staining with iodine vapor. The bands corresponding to PC, LPC, and GPC were scraped into glass tubes for quantification.

Measurement of inorganic phosphorus. Inorganic phosphorus in each phospholipid and choline metabolite fraction was measured using the method of Vaskovsky *et al*. (1975) with some modifications. Briefly, 0.2 ml of 72% perchloric acid was added to the scrapings, and the tubes were digested for 20 min at 190°C in an electrically heated metal block, then the temperature was elevated to 220°C to allow the perchloric acid to evaporate completely. After cooling, 0.3 ml of water and 2.7 ml of working solution were added and mixed thoroughly with a vibration mixer, then heated in a boiling water bath for 5 min. After centrifugation (310 × g, 5 min), the absorbance of the supernatant at 815 nm was measured against a blank. The concentrations of inorganic phosphorus were calculated using a standard phosphorus curve. The amount of each phospholipid and choline metabolite was given using the amount of recovered phosphate in each spot and the results were expressed as nanomoles or micromoles of phosphorus per gram nerve tissue wet weight.

Statistical analysis. Data were generally expressed as mean ± standard error values and groups of data were compared by ANOVA. A difference between means was considered significant at $p < 0.05$. Correlation analysis between NTE and NTE-LysoPLA, NTE, and NTE-PLB were made by linear correlation using SPSS 13.0 (Chicago, IL).

TABLE 1
The Activities of NTE (nmol phenol/min/mg protein), NTE-LysoPLA (mAU/min), and NTE-PLB (mAU/min) after TOCP and PMSF Administration in Mouse Nervous Tissues

Tissue	Enzyme	4 h		4 days		7 days		14 days	
		TOCP	PMSF	TOCP	PMSF	TOCP	PMSF	TOCP	PMSF
Brain	NTE	3.46 ± 0.23 (63)	0.25 ± 0.10 (97)	4.53 ± 0.31 (52)	5.95 ± 0.41 (37)	4.86 ± 0.46 (48)	6.76 ± 0.35 (28)	7.24 ± 0.41 (23)	8.00 ± 0.19 (15)
	NTE-LysoPLA	1.66 ± 0.24 (62)	0.30 ± 0.20 (93)	2.00 ± 0.13 (54)	1.95 ± 0.28 (55)	2.22 ± 0.28 (49)	2.71 ± 0.08 (38)	3.15 ± 0.14 (28)	3.14 ± 0.25 (28)
	NTE-PLB	1.87 ± 0.08 (40)	1.21 ± 0.20 (61)	2.16 ± 0.21 (31)	1.75 ± 0.18 (44)	2.24 ± 0.14 (29)	2.16 ± 0.09 (31)	2.53 ± 0.11 (19)	2.59 ± 0.20 (17)
Spinal cord	NTE	1.19 ± 0.20 (68)	0.13 ± 0.06 (96)	1.47 ± 0.10 (61)	2.04 ± 0.12 (46)	2.05 ± 0.29 (45)	2.50 ± 0.19 (33)	2.63 ± 0.19 (30)	2.99 ± 0.25 (20)
	NTE-LysoPLA	0.90 ± 0.20 (75)	0.27 ± 0.16 (92)	1.46 ± 0.13 (59)	1.25 ± 0.22 (65)	1.84 ± 0.22 (48)	2.19 ± 0.09 (38)	2.87 ± 0.09 (18)	2.55 ± 0.20 (27)
	NTE-PLB	0.76 ± 0.08 (67)	0.63 ± 0.15 (72)	1.34 ± 0.11 (41)	1.00 ± 0.13 (56)	1.58 ± 0.10 (31)	1.70 ± 0.10 (25)	1.88 ± 0.07 (17)	1.95 ± 0.08 (15)
Sciatic nerve	NTE	0.39 ± 0.05 (67)	0.02 ± 0.01 (98)	0.55 ± 0.04 (54)	0.60 ± 0.04 (50)	0.71 ± 0.09 (42)	0.76 ± 0.06 (37)	0.83 ± 0.06 (31)	0.98 ± 0.05 (18)
	NTE-LysoPLA	0.57 ± 0.13 (75)	0.13 ± 0.15 (94)	1.11 ± 0.12 (50)	0.74 ± 0.12 (67)	1.53 ± 0.08 (32)	1.21 ± 0.10 (46)	1.78 ± 0.10 (20)	1.61 ± 0.06 (28)
	NTE-PLB	0.56 ± 0.07 (53)	0.39 ± 0.09 (68)	0.71 ± 0.06 (41)	0.51 ± 0.07 (57)	0.90 ± 0.04 (25)	0.76 ± 0.05 (36)	1.02 ± 0.04 (14)	1.07 ± 0.04 (10)

Note. Data are expressed as mean ± SE ($n = 3$). The numbers in parentheses represent the inhibition rates (%). Control NTE activities in brain, spinal cord and sciatic nerve were defined as 9.42 ± 0.78, 3.75 ± 0.54, and 1.21 ± 0.18 nmol phenol/min/mg protein, respectively, control NTE-LysoPLA activities were 4.39 ± 0.28, 3.52 ± 0.17, and 2.24 ± 0.25 mAU/min, respectively, and control NTE-PLB activities were 3.14 ± 0.41, 2.28 ± 0.36, and 1.19 ± 0.33 mAU/min, respectively.

RESULTS

Mice are Resistant to Signs of OPIDN by TOCP Treatment

We know that TOCP has been the prototype delayed neurotoxicity-inducing agent as it can inhibit and age NTE, while PMSF does not initiate delayed neurotoxicity because NTE inhibited by PMSF does not undergo the aging reaction (Johnson, 1990). To examine the effect of TOCP on the NTE esterase activity-related metabolism in mice, we first orally administrated the mice with a single dose of TOCP at 900 mg/kg or PMSF which was used as the negative control. The mice given TOCP or PMSF appeared free of overt signs of acute cholinergic poisoning at all postexposure observation times. It has been showed that mice dosed orally with 225 mg/kg TOCP daily for 270 days displayed OPIDN signs such as muscle wasting, weakness, and ataxia, which then progressed to severe hind limb paralysis; however, the administration of two single 1000 mg/kg doses of TOCP at a 21-day interval produced no observable adverse effects (Lapadula *et al*., 1985). Consistently, we did not observe any sign of delayed neurotoxicity in mice treated either with a single dose of TOCP or PMSF (data not shown).

TOCP and PMSF Inhibit NTE, NTE-LysoPLA and NTE-PLB Activities in Mice

To examine the effect of TOCP and PMSF on NTE, NTE-LysoPLA, and NTE-PLB activities in mice, crude extracts were prepared from the brain, spinal cord, and sciatic nerve of mice treated with TOCP, PMSF and controls for 4 h, 4, 7, and 14 days, respectively, and the NTE, NTE-LysoPLA, and NTE-PLB activities were measured. As shown in Table 1, both TOCP and PMSF significantly inhibited the NTE, NTE-LysoPLA, and NTE-PLB activities at the four chosen time points in all three nervous tissues. Remarkably, the inhibition of NTE and NTE-LysoPLA after TOCP and PMSF exposure in brain, spinal cord, and sciatic nerve showed high correlation with an R^2 of 0.94, 0.87, and 0.85, respectively (Figs. 2A–C). Similarly, the inhibition of NTE and NTE-PLB by the two chemicals also showed high correlation with an R^2 of 0.75, 0.80, and 0.79, respectively (Figs. 2D–F). The inhibition of NTE, NTE-LysoPLA, and NTE-PLB by TOCP and PMSF showed similar trends in the three different nervous tissues with the peak inhibition occurring at 4 h after dosing.

TOCP Induces mice NTE Aging *In Vivo*

It is known that TOCP can inhibit and age NTE, while PMSF inhibits but cannot age NTE, though it is a potent NTE inhibitor. To confirm this observation, the aging of NTE was measured in the brain, spinal cord, and sciatic nerve at 4 h, 4, 7, and 14 days after exposure to TOCP and PMSF. As shown in Table 2, the mice given TOCP showed that almost all inhibited NTE was of the aged type in the brain, spinal cord, and sciatic nerve. As expected, the opposite result was observed in mice administered with PMSF, with almost all of the inhibited NTE unaged in the three nervous tissues.

TOCP and PMSF Inhibit Total LysoPLA and Total PLB Activities in Mice

To investigate the effect of TOCP and PMSF on total LysoPLA and total PLB activities, we assayed the activities of these two enzymes extracted from brain, spinal cord, and sciatic nerve of mice at 4 h, 4, 7, and 14 days after single doses of TOCP or PMSF. As a result, the activities of the total LysoPLA (Figs. 3A–C) and total PLB (Figs. 3D–F) in the three nervous

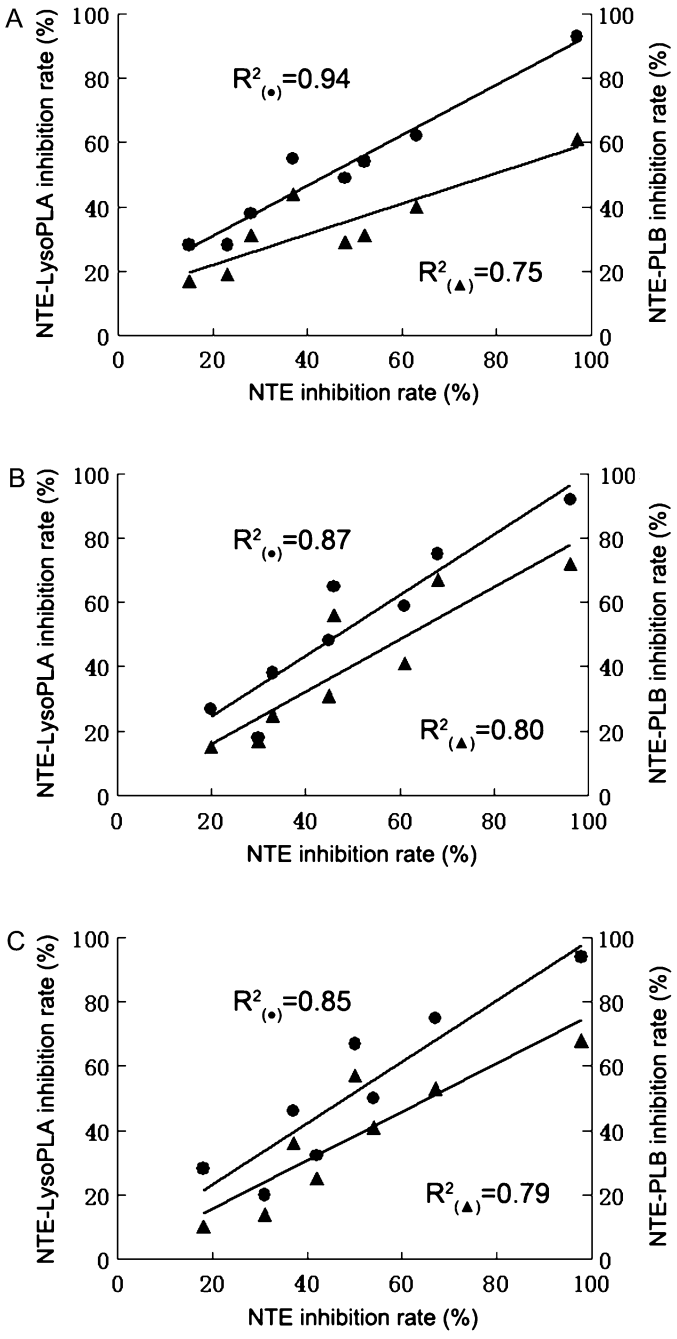

FIG. 2. The correlation between NTE and NTE-LysoPLA or NTE-PLB activity inhibition in nervous tissues of mice exposed to TOCP and PMSF. The inhibition rates of NTE, NTE-LysoPLA (closed circle) and NTE-PLB (closed triangle) in mouse brain (A), spinal cord (B), and sciatic nerve (C) were analyzed by linear correlation.

tissues significantly decreased at the four chosen time points after dosing. The inhibition of total LysoPLA or total PLB by TOCP and PMSF exhibited similar characteristic and similar trends in the three different nervous tissues with the peak inhibition occurring at 4 h after dosing. Furthermore, PMSF was a more potent inhibitor of total LysoPLA than TOCP.

TOCP and PMSF Do Not Alter the Levels of PC and LPC but Decrease the Level of GPC in Mice

To investigate whether the inhibition of NTE, NTE-LysoPLA, NTE-PLB, total LysoPLA, and total PLB could affect the levels of PC, LPC, and GPC in nervous tissues of mice, and whether the aged and unaged NTE had different effects, the levels of PC, LPC, and GPC were determined in brain, spinal cord, and sciatic nerve at 4 h, 4, 7, and 14 days after TOCP and PMSF exposure. Surprisingly, the levels of PC (Figs. 4A–C) and LPC (Figs. 4D–F) were not significantly altered in any of the three nervous tissues at the four chosen time points, although NTE, NTE-LysoPLA, NTE-PLB, total LysoPLA, and total PLB activities were clearly inhibited. However, a remarkable decrease of GPC level (Figs. 4G–I) was observed in the nervous tissues at the four chosen time points after TOCP and PMSF exposure. The trends for GPC levels in all three nervous tissues after exposure were generally similar except that GPC was lowest at 4 days after PMSF exposure and 7 days after TOCP exposure, respectively, and then gradually recovered although it was clearly decreased until 14 days postdosing.

DISCUSSION

Interspecies differences in sensitivity to OPIDN are well known. The hen is traditionally used as a model for OPIDN, while mouse is less susceptible to the ataxia characteristic of OPIDN (Glynn, 2000). In hens, OPs such as TOCP, a prototype delayed neurotoxicity-inducing agent, that inhibit brain NTE by 70% and are capable of aging present the requisite conditions for the initiation of OPIDN. However, inhibition of NTE in the brain of mice never exceeded 68% even when exposed to high dosages of TOCP, such as 3480 mg/kg and no clinical signs were produced (Veronesi *et al.*, 1991). Here, we confirmed these early observations. Our data showed that both TOCP and PMSF significantly inhibited NTE activity in brain, spinal cord, and sciatic nerve of mice. Nevertheless, the NTE inhibition was not more than 68% in the three nervous tissues at any of the four time points after TOCP exposure. NTE inhibited by TOCP underwent the aging reaction, while NTE inhibited by PMSF did not. The mice treated with TOCP or PMSF did not exhibit any sign of OPIDN. However, our previous date showed that a single 750 mg/kg dose of TOCP could induce complete OPIDN in adult hens (Hou *et al.*, 2008). This provided further evidence of difference in susceptibility to OPIDN between mice and hens.

The mechanisms responsible for this differential sensitivity in mice and hens exposed to neuropathic OPs are not well understood. Nevertheless, significant progress has been achieved on the biochemical function of NTE during the last years with the discovery that NTE act as a LysoPLA in mouse brain and PLB in cultured mammalian cells, which has provided new insights into these unresolved problems (Quistad

TABLE 2
Aged and Unaged NTE Activities (nmol phenol/min/mg protein) in Nervous Tissues from TOCP- and PMSF-Treated Mice

Tissue	NTE activity	4 h		4 days		7 days		14 days	
		TOCP	PMSF	TOCP	PMSF	TOCP	PMSF	TOCP	PMSF
Brain	Inhibited	5.94 ± 0.45	9.29 ± 0.34	5.17 ± 0.41	3.28 ± 0.46	4.78 ± 0.10	2.47 ± 0.43	2.23 ± 0.32	1.31 ± 0.30
	Unaged	0.14 ± 0.35	9.06 ± 0.20	0.16 ± 0.14	3.13 ± 0.33	0.07 ± 0.17	2.44 ± 0.26	0.34 ± 0.28	1.18 ± 0.31
	Aged	5.80 ± 0.51	0.23 ± 0.39	5.01 ± 0.60	0.15 ± 0.36	4.71 ± 0.35	0.03 ± 0.35	1.89 ± 0.42	0.13 ± 0.33
Spinal cord	Inhibited	2.54 ± 0.07	3.61 ± 0.14	2.32 ± 0.06	1.58 ± 0.16	1.68 ± 0.11	2.09 ± 0.15	1.23 ± 0.13	0.70 ± 0.17
	Unaged	0.06 ± 0.16	3.58 ± 0.16	0.03 ± 0.05	1.53 ± 0.09	0.05 ± 0.10	2.01 ± 0.13	0.07 ± 0.10	0.65 ± 0.30
	Aged	2.48 ± 0.16	0.03 ± 0.18	2.29 ± 0.09	0.05 ± 0.14	1.63 ± 0.13	0.08 ± 0.17	1.16 ± 0.14	0.05 ± 0.14
Sciatic nerve	Inhibited	0.77 ± 0.05	1.19 ± 0.04	0.69 ± 0.03	0.59 ± 0.06	0.50 ± 0.05	0.42 ± 0.08	0.41 ± 0.09	0.24 ± 0.11
	Unaged	0.01 ± 0.04	1.17 ± 0.06	0.01 ± 0.05	0.56 ± 0.07	0.02 ± 0.04	0.41 ± 0.10	0.02 ± 0.08	0.20 ± 0.08
	Aged	0.76 ± 0.05	0.02 ± 0.07	0.68 ± 0.06	0.03 ± 0.08	0.48 ± 0.05	0.01 ± 0.07	0.39 ± 0.08	0.04 ± 0.09

Note. Data are expressed as mean ± SE ($n = 3$). Unaged NTE, the NTE activity after treatment with KF subtracted the NTE activity after treatment with KCl; aged NTE, control NTE activity subtract the NTE activity after treatment with KF, inhibited NTE activity, aged NTE activity, unaged NTE activity (see the details of aging measurement described in ''Materials and Methods''). Control NTE activities in brain, spinal cord, and sciatic nerve were, respectively, defined as 9.42 ± 0.78, 3.75 ± 0.54, and 1.21 ± 0.18 nmol phenol/min/mg protein.

et al., 2003; Zaccheo *et al*., 2004). Firstly, our present results strengthen these findings with two different toxicants in three different nervous tissues of mice. The inhibition of NTE and NTE-LysoPLA or NTE-PLB in brain, spinal cord and sciatic nerve after TOCP and PMSF exposure showed high correlation. These observations indicate that the three enzymes display very similar inhibitor sensitivity and specificity profiles in the three different nervous tissues, which provides further evidence to support the claim that NTE functions as LysoPLA or PLB. These results were consistent with the result of our previous study in TOCP- and PMSF-treated hens (Hou *et al*., 2008).

It was observed that susceptible species were generally larger and hence had longer axons than resistant species, while animals with relatively low levels of NTE were relatively resistant to OPIDN (Glynn, 2000; Johnson, 1987). Furthermore, in mice, a particularly resistant species, NTE appears to turn over faster ($t_{1/2} = 2$ days) than in chickens ($t_{1/2} = 4$–5 days) (Johnson, 1974; Meredith and Johnson, 1988). Here, our results were consistent with these early observations. The basal NTE activity in brain, spinal cord, and sciatic nerve of hens was much higher than that in mice. Moreover, NTE inhibited by TOCP recovered more rapidly in mice than in hens. This would indicate a mechanism, whereby a certain absolute level of OP-modified NTE must be achieved and then maintained for a finite period in order to initiate OPIDN. Remarkably, compared to our previous results in hens, the present data also shows that the basal activities of LysoPLA and PLB in nervous tissues of mice were much lower than those in hens, and these two enzymes were inhibited and recovered more rapidly in mice than in hens after TOCP exposure. This indicates that a certain absolute level of OP-modified LysoPLA and PLB may be also achieved and then maintained for a finite period in order to initiate OPIDN. This difference in time for maintenance of OP-modified LysoPLA and PLB between in mice and hens may elucidate why these two species display different signs after exposure to the same neuropathic OP. NTE, LysoPLA, and PLB all belong to serine hydrolase families which are considered sensitive targets of OPs (Casida and Quistad, 2005; Vose *et al*., 2007). Each serine hydrolase has a specific function and every OP has a unique inhibitory profile. Thus, clarifying whether other serine hydrolases are modified and different duration of modification between in mice and hens after neuropathic OPs exposure may be useful to better understand the interspecies differences in susceptibility to OPIDN.

A number of literatures suggested that interspecies differences in susceptibility to OPIDN were related to the diversity of OP absorption and distribution (Abou-Donia, 1983), bioactivation (Hansen, 1983; Veronesi, 1984), nervous system structure (Johnson, 1975), and/or neuronal repair mechanisms (Glynn, 2000). Of the above variables, pharmacokinetics and metabolism of OPs are thought to play prominent roles in OP sensitivity (Abou-Donia, 1983; Hansen, 1983). Insensitive animals may detoxify and excrete the OPs more rapidly and completely than sensitive animals (Novak and Padilla, 1986). Our present results showed that the activities of NTE, LysoPLA, and PLB were inhibited and recovered more rapidly in nervous tissues of mice than those of hens, which may reflect more active metabolism and elimination of the neuropathic chemical or its metabolite in mice than in hens. It also suggests a mechanism for differences in neurotoxicity induced by the same neuropathic OP in these two species.

NTE is known to be a LysoPLA or PLB (Quistad *et al*., 2003; Zaccheo *et al*., 2004). This indicates that inhibition of NTE may be able to disrupt the PC and LPC homeostasis. We originally hypothesized that PC and LPC homeostasis is disrupted and this disruption is related to the different signs observed between mice and hens after TOCP exposure.

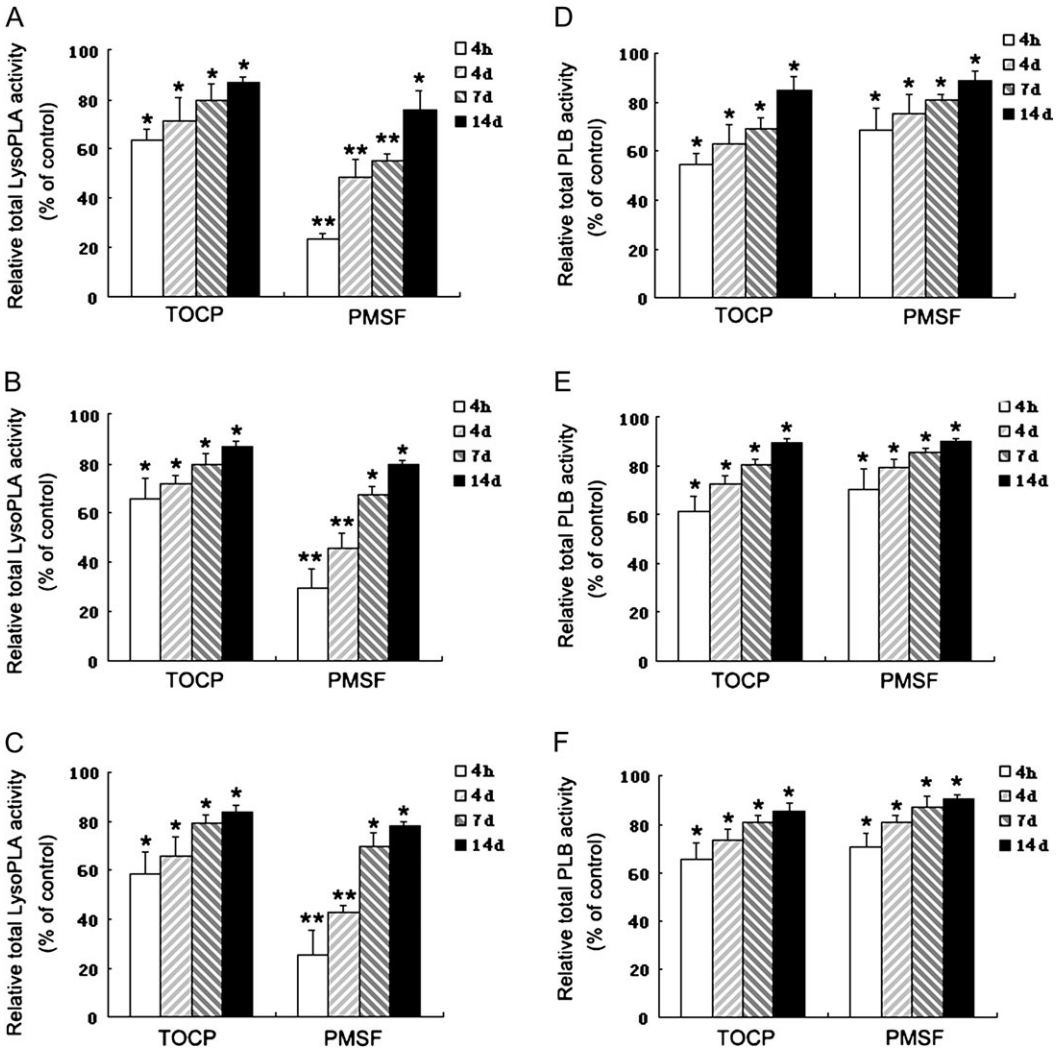

FIG. 3. The alteration of total LysoPLA activity (left panel) and total PLB activity (right panel) in mouse brain (A, D), spinal cord (B, E) and sciatic nerve (C, F) after 4 h, 4, 7 and 14 days administration with TOCP and PMSF. Data are presented as a percentage of control total LysoPLA or total PLB activities. The control total LysoPLA activity in brain, spinal cord, and sciatic nerve were defined as 28.12 ± 2.76, 17.68 ± 2.51, and 12.81 ± 3.42 mAU/min, respectively. The control total PLB activities in brain, spinal cord, and sciatic nerve were, respectively, defined as 10.41 ± 1.47, 8.72 ± 1.59, and 5.37 ± 1.31 mAU/min. Data are expressed as mean ± SE and were compared by ANOVA between control and administration groups, $*p < 0.05$, $**p < 0.01$, $n = 3$.

However, similar to that in hens, surprising results were obtained indicating that levels of PC and LPC were not significantly altered in brain, spinal cord and sciatic nerve of mice, though NTE, total LysoPLA and total PLB activities were clearly inhibited. This suggests that TOCP and PMSF administration did not disrupt PC and LPC homeostasis and the aged and unaged NTE have no difference on the homeostasis of PC and LPC in mice nervous tissues. This also indicated that the disruption of PC and LPC homeostasis may not be a mechanism involved in the different signs observed in mice and hens exposed to TOCP. We speculate that there may be feedback mechanisms in mice to maintain the homeostasis of PC and LPC by reducing their synthesis and/or activating other degradation pathways such as PC-specific phospholipase C or PLD (Adibhatla *et al*., 2006). Nevertheless, the level of GPC, the product of PC or LPC, remarkably decreased. The trends of GPC levels were generally similar after TOCP and PMSF exposure in all three nervous tissues except that the GPC levels were lowest on day 7 after TOCP exposure, while on day 4 after PMSF exposure. This may indicate that NTE inhibition and recovery by PMSF was much more rapid than by TOCP, however, it was not related to whether the NTE is aged or not. Comparison of the results obtained from TOCP-treated mice and hens showed that GPC levels in both species decreased significantly after TOCP exposure. This suggests that the decrease in GPC level is unrelated to the different signs in mice compared to hens after TOCP exposure. However, the decrease of GPC showed a different characteristic between these two species of animals. In hens, the GPC levels decreased gradually reaching the lowest level at 14 days postdosing. This may be

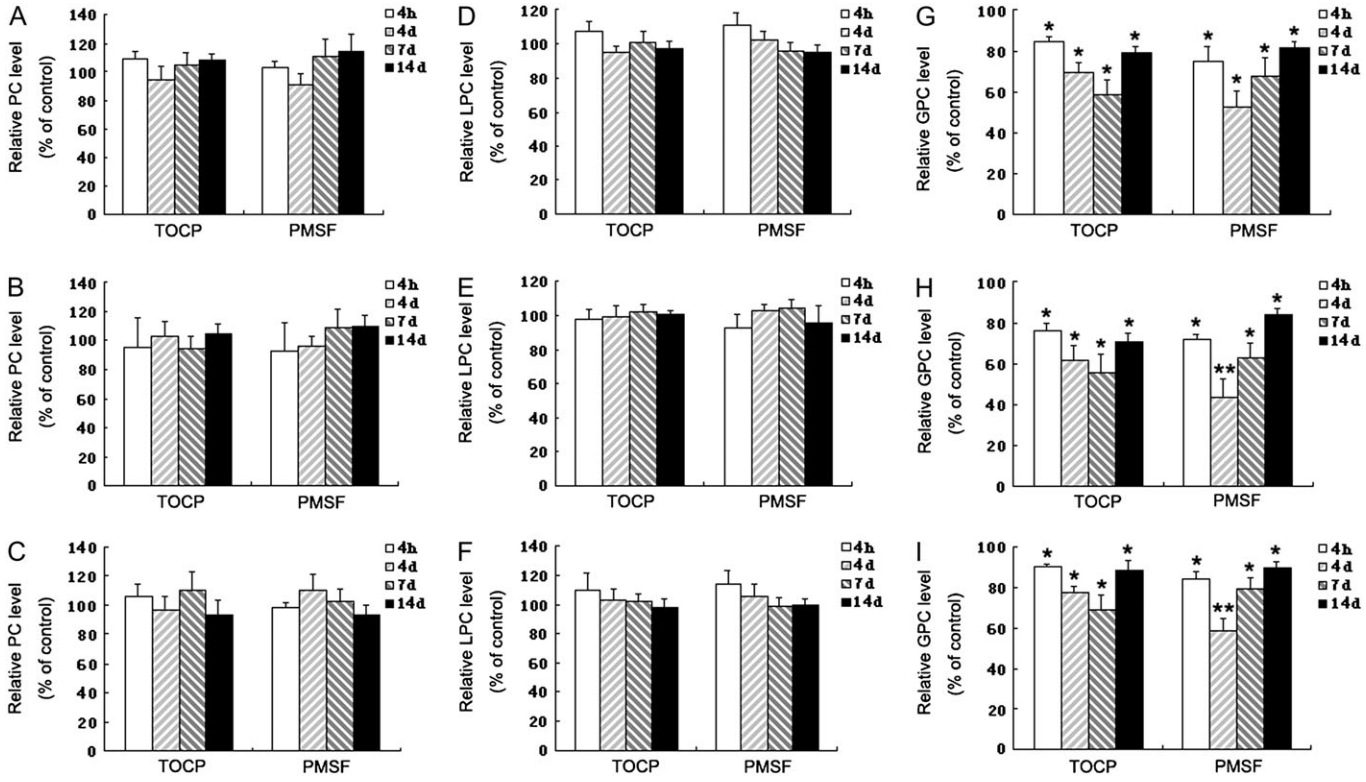

FIG. 4. The alteration of levels of PC (left panel), LPC (middle panel), and GPC (right panel) in mouse brain (A, D, G), spinal cord (B, E, H), and sciatic nerve (C, F, I) after 4 h, 4, 7, and 14 days administration with TOCP and PMSF. Data are presented as a percentage of control PC, LPC, and GPC levels in nervous tissues. The control PC levels in brain, spinal cord, and sciatic nerve were, respectively, defined as 19.23 ± 1.02 μmol/g wet brain weight, 18.36 ± 0.62 μmol/g wet spinal cord weight, and 22.29 ± 2.25 μmol/g wet sciatic nerve weight. The control LPC levels in brain, spinal cord, and sciatic nerve were, respectively, defined as 420.91 ± 21.13 nmol/g wet brain weight, 702.94 ± 31.86 nmol/g wet spinal cord weight, and 683.94 ± 28.73 nmol/g wet sciatic nerve weight. The control GPC levels in brain, spinal cord, and sciatic nerve were, respectively, defined as 476.52 ± 29.39 nmol/g wet brain weight, 412.37 ± 38.55 nmol/g wet spinal cord weight, and 378.62 ± 21.24 nmol/g wet sciatic nerve weight. Data are expressed as mean ± SE and were compared by ANOVA between control and administration groups, $^{*}p < 0.05$, $^{**}p < 0.01$, $n = 3$.

due to different inhibition dynamics of NTE, LysoPLA, and PLB between mice and hens after TOCP and PMSF exposure. The role of GPC in the neurotoxicity has been controversial; for examples, an increased level of GPC has been observed in some neurodegenerative diseases such as Alzheimer's disease (Bárány *et al.*, 1985; Blusztajn *et al.*, 1990). While a decreased level of GPC was seen in cultured primary mouse neurons exposed to saligenin phosphate, mipafox (two neuropathic NTE inhibitors) and phenyl dipentylphosphinate (a non-neuropathic NTE inhibitor). However, only saligenin phosphate caused cytotoxicity. This suggests that reduction of GPC is not the cause of neural cytotoxicity (Read *et al.*, 2007). In fact, we have reported that reduction of GPC levels is not directly related to OPIDN in hens, although both TOCP and PMSF exposure could lead to a decrease of GPC levels (Hou *et al.*, 2008).

In summary, we investigated the activities of NTE, LysoPLA and PLB, the levels of PC, LPC, and GPC in mice from the new viewpoint that NTE may act as a LysoPLA or PLB and may affect PC and LPC homeostasis. Our data are the first to show that the difference in the inhibition of LysoPLA, and PLB activities by TOCP between mice and hens may be why these two species display different signs after exposure to the same neuropathic OP. However, the results also suggested that the disruption of PC and LPC homeostasis and the decrease of GPC level may not be the primary mechanism for this difference. Altogether, this study contributes to a better understanding of the mechanism of neurotoxicity in mice and interspecies differences in susceptibility to OPIDN.

FUNDING

National Nature Science Foundation of China (30470228, 30870537); and Ministry of Science & Technology of China (2006AA06Z423, 2006BAK02A02).

ACKNOWLEDGMENTS

We would like to thank Ms Jill Wentzell for polishing the English of the revised manuscript.

REFERENCES

Abou-Donia, M. B. (1983). Toxicokinetics and metabolism of delayed neurotoxic organophosphorus esters. *Neurotoxicology* **4,** 113–129.

Abou-Donia, M. B., and Lapadula, D. M. (1990). Mechanisms of organophosphorus ester-induced delayed neurotoxicity: Type I and type II. *Annu. Rev. Pharmacol. Toxicol.* **30,** 405–440.

Adibhatla, R. M., Hatcher, J. F., Larsen, E. C., Chen, X., Sun, D., and Tsao, F. H. (2006). CDP-choline significantly restores phosphatidylcholine levels by differentially affecting phospholipase A2 and CTP: Phosphocholine cytidylyltransferase after stroke. *J. Biol. Chem.* **281,** 6718–6725.

Akassoglou, K., Malester, B., Xu, J., Tessarollo, L., Rosenbluth, J., and Chao, M. V. (2004). Brain-specific deletion of neuropathy target esterase/Swiss cheese results in neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* **101,** 5075–5080.

Anfuso, C. D., Sipione, S., Lupo, G., Ragusa, N., and Alberghina, M. (2003). Characterization of glycerophosphocholine phosphodiesterase activity and phosphatidylcholine biosynthesis in cultured retinal microcapillary pericytes. Effect of adenosine and endothelin-1. *Lipids* **38,** 45–52.

Baburina, I., and Jackowski, S. (1999). Cellular responses to excess phospholipids. *J. Biol. Chem.* **274,** 9400–9408.

Bárány, M., Chang, Y. C., Arús, C., Rustan, T., and Frey, W. H. 2nd. (1985). Increased glycerol-3-phosphorylcholine in post-mortem Alzheimer's brain. *Lancet* **1,** 517–517.

Barrett, D. S., Oeheme, F. W., and Kruckenberg, S. M. (1985). The review organophosphorus ester-induced delayed neurotoxicity. *Vet. Hum. Toxicol.* **27,** 22–37.

Blusztajn, J. K., Lopez Gonzalez-Coviella, I., Logue, M., Growdon, J. H., and Wurtman, R. J. (1990). Levels of phospholipid catabolic intermediates, glycerophosphocholine and glycerophosphoethanolamine, are elevated in brains of Alzheimer's disease but not of Down's syndrome patients. *Brain Res.* **536,** 240–244.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Casida, J. E., and Quistad, G. B. (2005). Serine hydrolase targets of organophosphorus toxicants. *Chem. Biol. Interact.* **157-158,** 277–283.

Chang, P. A., Wu, Y. J., Li, W., and Leng, X. F. (2006). Effect of carbamate esters on neurite outgrowth in differentiating human SK-N-SH neuroblastoma cells. *Chem. Biol. Interact.* **159,** 65–72.

Cornell, R. B., and Northwood, I. C. (2000). Regulation of CTP:phosphocholine cytidylyl-transferase by amphitropism and relocalisation. *Trends Biochem. Sci.* **25,** 441–447.

Craig, P. H., and Barth, M. L. (1999). Evaluation of the hazards of industrial exposure to tricresyl phosphate: A review and interpretation of the literature. *J. Toxicol. Environ. Health B Crit. Rev.* **2,** 281–300.

Cui, Z., and Houweling, M. (2002). Phosphatidylcholine and cell death. *Biochim. Biophys. Acta* **1585,** 87–96.

Folch, J., Lees, M., and Sloane Stanley, G. H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509.

Glynn, P. (2000). Neural development and neurodegeneration: Two faces of neuropathy target esterase. *Prog. Neurobiol.* **61,** 61–74.

Glynn, P. (2005). Neuropathy target esterase and phospholipid deacylation. *Biochim. Biophys. Acta* **1736,** 87–93.

Hall, S. M. (1972). The effect of injections of lysophosphatidyl choline into white matter of the adult mouse spinal cord. *J. Cell Sci.* **10,** 535–546.

Hansen, L. G. (1983). Biotransformation of organophosphorus compounds relative to delayed neurotoxicity. *Neurotoxicology* **4,** 97–111.

Hou, W. Y., Long, D. X., Wang, H. P., Wang, Q., and Wu, Y. J. (2008). The homeostasis of phosphatidylcholine and lysophosphatidylcholine was not disrupted during tri-o-cresyl phosphate induced delayed neurotoxicity in hens. *Toxicology* **252**(1–3), 56–63.

Jackowski, S., and Fagone, P. (2005). CTP:phosphocholine cytidylyltransferase: Paving the way from gene to membrane. *J. Biol. Chem.* **280,** 853–856.

Jean, I., Allamargot, C., Barthelaix-Pouplard, A., and Fressinaud, C. (2002). Axonal lesions and PDGF-enhanced remyelination in the rat corpus callosum after lysolecithin demyelination. *Neuroreport* **13,** 627–631.

Johnson, M. K. (1969). The delayed neurotoxic action of some organophosphorus compounds. Identification of the phosphorylation site as an esterase. *Biochem. J.* **114,** 711–717.

Johnson, M. K. (1974). The primary biochemical lesion leading to the delayed neurotoxic effects of some organophosphorus esters. *J. Neurochem.* **23,** 785–789.

Johnson, M. K. (1975). The delayed neuropathy caused by some organophosphorus esters: Mechanism and challenge. *CRC. Crit. Rev. Toxicol.* **3,** 289–316.

Johnson, M. K. (1977). Improved assay of neurotoxic esterase for screening organophosphates for delayed neurotoxicity potential. *Arch. Toxicol.* **37,** 113–115.

Johnson, M. K. (1982). The target for initiation of delayed neurotoxicity by organophosphorus esters: Biochemical studies and toxicological applications. *Rev. Biochem. Toxicol.* **4,** 141–212.

Johnson, M. K. (1987). Receptor or enzyme: The puzzle of NTE and organophosphate-induced delayed polyneuropathy. *Trends Pharmacol. Sci.* **8,** 174–179.

Johnson, M. K. (1990). Organophosphates and delayed neuropathy—Is NTE alive and well? *Toxicol. Appl. Pharmacol.* **102,** 385–399.

Johnson, M. K. (1993). Symposium introduction: Retrospect and prospects for neuropathy target esterase (NTE) and the delayed polyneuropathy (OPIDP) induced by some organophosphorus esters. *Chem. Biol. Interact.* **87,** 339–346.

Johnson, M. K., Read, D. J., and Benschop, H. P. (1985). Interaction of the four stereoisomers of soman (pinacolyl methylphosphonofluoridate) with acetylcholinesterase and neuropathy target esterase of hen brain. *Biochem. Pharmacol.* **34,** 1945–1951.

Karczmar, A. G. (1984). Acute and long lasting central actions of organophosphorus agents. *Fundam. Appl. Toxicol.* **4,** S1–17.

Kellner, T., Sanborn, J., and Wilson, B. (2000). In vitro and in vivo assessment of the effect of impurities and chirality on methamidophos-induced neuropathy target esterase aging. *Toxicol. Sci.* **54,** 408–415.

Kent, C. (2005). Regulatory enzymes of phosphatidylcholine biosynthesis: A personal perspective. *Biochim. Biophys. Acta* **1733,** 53–66.

Kishimoto, T., Soda, Y., Matsuyama, Y., and Mizuno, K. (2002). An enzymatic assay for lysophosphatidylcholine concentration in human serum and plasma. *Clin. Biochem.* **35,** 411–416.

Lapadula, D. M., Patton, S. E., Campbell, G. A., and Abou-Donia, M. B. (1985). Characterization of delayed neurotoxicity in the mouse following chronic oral administration of tri-o-cresyl phosphate. *Toxicol. Appl. Pharmacol.* **79,** 83–90.

Li, Y., Dinsdale, D., and Glynn, P. (2003). Protein domains, catalytic activity, and subcellular distribution of neuropathy target esterase in mammalian cells. *J. Biol. Chem.* **278,** 8820–8825.

Lotti, M. (1992). The pathogenesis of organophosphate delayed polyneuropathy. *Crit. Rev. Toxicol.* **21,** 465–487.

Meredith, C., and Johnson, M. K. (1988). Neuropathy target esterase: Rates of turnover in vivo following covalent inhibition with phenyl di-n-pentylphosphinate. *J. Neurochem.* **51,** 1097–1101.

Nelson, G. J. (1967). The phospholipid composition of plasma in various mammalian species. *Lipids* **2,** 323–328.

Novak, R., and Padilla, S. (1986). An in vitro comparison of rat and chicken brain neurotoxic esterase. *Fundam. Appl. Toxicol.* **6,** 464–471.

Quistad, G. B., Barlow, C., Winrow, C. J., Sparks, S. E., and Casida, J. E. (2003). Evidence that mouse brain neuropathy target esterase is a lysophospholipase. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 7983–7987.

Quistad, G. B., and Casida, J. E. (2004). Lysophospholipase inhibition by organophosphorus toxicants. *Toxicol. Appl. Pharmacol.* **196,** 319–326.

Quistad, G. B., Liang, S. N., Fisher, K. J., Nomura, D. K., and Casida, J. E. (2006). Each lipase has a unique sensitivity profile for organophosphorus inhibitors. *Toxicol. Sci.* **91,** 166–172.

Read, D. J., Langford, L., Barbour, H. R., Forshaw, P. J., and Glynn, P. (2007). Phospholipase B activity and organophosphorus compound toxicity in cultured neural cells. *Toxicol. Appl. Pharmacol.* **219,** 190–195.

Ross, B. M., and Kish, S. J. (1994). Characterization of lysophospholipid metabolizing enzymes in human brain. *J. Neurochem.* **63,** 1839–1848.

Schwab, B. W., and Richardson, R. J. (1986). Lymphocyte and brain neurotoxic esterase: Dose and time dependence of inhibition in the hen examined with three organophosphorus esters. *Toxicol. Appl. Pharmacol.* **83,** 1–9.

Solberg, Y., and Belkin, M. (1997). The role of excitotoxicity in organophosphorous nerve agents central poisoning. *Trends Pharmacol. Sci.* **18,** 183–185.

Vaskovsky, V. E., Kostetsky, E. Y., and Vasendin, M. (1975). A universal reagent for phospholipid analysis. *J. Chromatogr.* **14,** 129–141.

Veronesi, B. (1984). Effect of metabolic inhibition with piperonyl butoxide on rodent sensitivity to tri-ortho-cresyl phosphate. *Exp. Neurol.* **85,** 651–660.

Veronesi, B., Padilla, S., Blackmon, K., and Pope, C. (1991). Murine susceptibility to organophosphorus-induced delayed neuropathy (OPIDN). *Toxicol. Appl. Pharmacol.* **107,** 311–324.

Vose, S. C., Holland, N. T., Eskenazi, B., and Casida, J. E. (2007). Lysophosphatidylcholine hydrolases of human erythrocytes, lymphocytes, and brain: sensitive targets of conserved specificity for organophosphorus delayed neurotoxicants. *Toxicol. Appl. Pharmacol.* **224,** 98–104.

Wang, A., and Dennis, E. A. (1999). Mammalian lysophospholipases. *Biochim. Biophys. Acta* **1439,** 1–16.

Wang, W. Q., and Gustafson, A. (1992). One-dimensional thin-layer chromatographic separation of phospholipids and lysophospholipids from tissue lipid extracts. *J. Chromatogr.* **58,** 1139–1142.

Williams, J. G., and McMaster, C. R. (1998). Scanning alanine mutagenesis of the CDP-alcohol phosphotransferase motif of saccharomyces cerevisiae cholinephosphotransferase. *J. Biol. Chem.* **273,** 13482–13487.

Winder, C., and Balouet, J. C. (2002). The toxicity of commercial jet oils. *Environ. Res.* **89,** 146–164.

Wu, S. Y., and Casida, J. E. (1996). Subacute neurotoxicity induced in mice by potent organophosphorus neuropathy target esterase inhibitors. *Toxicol. Appl. Pharmacol.* **139,** 195–202.

Zaccheo, O., Dinsdale, D., Meacock, P. A., and Glynn, P. (2004). Neuropathy target esterase and its yeast homologue degrade phosphatidylcholine to glycerophosphocholine in living cells. *J. Biol. Chem.* **279,** 24024–24033.