Subcellular Alterations of Protein Kinase C Isozymes in the Rat Brain after Organophosphate Poisoning

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ABSTRACT

The protein kinase C (PKC) signaling pathway has been associated with modulation of N-methyl-D-aspartate receptor activity, motor behavior, learning, and memory, all of which are severely impaired in organophosphate (OP) intoxication. Nevertheless, the role of PKC in OP intoxication is largely unknown. The present study attempted to characterize alterations in the immunoreactivity levels of PKC isozymes expressed in different brain areas in the rat following exposure to the nerve agent sarin (1×LD50). Furthermore, possible neuroprotective effect of selective PKC regulating peptide after such insult was evaluated. The results indicated that a significant reduction in the immunoreactivity level of the conventional βII-PKC and the atypical ζ-PKC was observed in frontal cortex up to 24 h postsarin and in the striatum up to 5 days postsarin exposure. This reduction was in contrast to the increase in the immunoreactivity level of both isozymes seen in the hippocampus or thalamus. Treatment with the anticonvulsant midazolam (0.5 mg/kg) 10 min postsarin exposure markedly reduced ζ-PKC immunoreactivity level and βII-PKC in the membrane fractions in the hippocampus. βII-PKC peptide (380 ng/kg), known to inhibit PKC translocation and activation, attenuated sarin-induced neuropathology. These observations suggest a role for both conventional and atypical PKC isozymes in OP-induced neuropathy in the rat and further support their involvement in cell death.

The nerve agent sarin, a potent inhibitor of cholinesterase (ChE), is extremely toxic, mainly due to its interfering with the central and peripheral cholinergic nervous system (Taylor, 1985). The accumulation of acetylcholine at nerve terminals exhibits toxic manifestations of vital muscarinic and nicotinic sites such as salivation, muscle tremor, convulsions, respiratory center depression, and peripheral respiratory arrest. Sarin-induced convulsions are accompanied by increased release of excitatory amino acids, leading to a mass activation of the glutamate receptors (Sloviter and Dempster, 1985; Olney et al., 1986; McDonough and Shih, 1997). Substantial induction of both cholinergic and glutamatergic systems leads eventually to brain damage, associated with a disruption of intracellular Ca2+ homeostasis. Some of the Ca2+ mobilization from intracellular and extracellular stores occurs through the cleavage of phosphatidylinositol (4,5)-bisphosphate to yield inositol 1,4,5-trisphosphate and diacylglycerol (DAG) and triggers the activation of protein kinase C (PKC) to further continue a phosphorylating cascade. As emerging lately from the literature, PKC has been coupled with modulation of the NMDA receptor activity (Lan et al., 2001), appears to mediate the non-NMDA receptor Ca2+ mobilization (Connor et al., 1988; Alagarsamy et al., 2001) and also play different roles in epileptogenesis (Ono et al., 1994; Tang et al., 2004). Neurotoxic doses of glutamate induced a persistent subcellular redistribution of PKC isozymes in vitro (Favaron et al., 1990; Durkin et al., 1996), whereas its rigid structural analog, kainic acid (KA), was also shown to affect expression of PKC isozymes in vivo (Guglielmetti et al., 1997; McNamara et al., 1999). PKC inhibitors were found to delay the increase in intracellular Ca2+ and the neurotoxicity induced by glutamate receptor hyperstimulation (Favaron et al., 1990; Felipo et al., 1993).

The PKC family members consist of at least 12 serine/threonine kinases, activated indirectly by G-protein-coupled receptor stimulation. They differ in their distribution in the central nervous system, subcellular target receptors, substrate affinities, and second messenger activators (Tanaka and Nishizuka, 1994; Newton, 2001). Each PKC isozyme is

ABBREVIATIONS: ChE, cholinesterase; DAG, diacylglycerol; PKC, protein kinase C; NMDA, N-methyl-D-aspartate; KA, kainic acid; OD, optical density; TS, tris-sodium chloride.
expressed in a specific manner within different cells, and multiple PKC isozymes are expressed in a single cell (Mochly-Rosen, 1995). The PKC isozymes are grouped into three classes: conventional (α, β, and γ), which are Ca²⁺- and DAG-dependent; novel (δ, ε, θ, and η), which are DAG-dependent; and atypical (ζ, η, λ), which are insensitive to both Ca²⁺ and DAG. The accepted mechanism has been that these isozymes are cytosolic in the inactive state and translocate to the inner leaflet of the cellular membrane as part of their activation process (Sakai et al., 1997). Thus, classical and novel PKC isozymes become activated as a result of the association of the cytosolic enzyme with membranes containing phospholipids and elevations in intracellular Ca²⁺, both of which are considered to be elicited by hyperactivity of the central nervous system.

Several brain regions are vulnerable to OP intoxication. It has been found that in surviving rats following exposure to sublethal doses of OP, there was an enlargement of brain ventricles on the expense of other brain tissues, and mainly the piriform cortex, thalamus, and hippocampus are damaged, e.g., a significant decrease in CA1 and CA3 hippocampal cells was detected (Lemercier et al., 1983; Kadar et al., 1995). Pretreatment with carbamate ChE inhibitors, such as pyridostigmine, followed by atropine sulfate and oxime therapy (Sidell, 1974) could increase significantly the survival rate in rats. However, this treatment regimen does not eliminate sarin-induced seizures and convulsions (Gordon et al., 1978; Shih et al., 1999). Furthermore, the seizure activity may progress into status epilepticus and thus extend neuronal cell pathology (Lemercier et al., 1983). Anticonvulsant compounds such as benzodiazepines decrease seizure activity although incompletely, depending mostly on the administration time postexposure (Lallement et al., 1991; McDonough et al., 1995). The compounds included in the treatment regimen are characterized as receptor upstream effectors. Nevertheless, receptor-mediated downstream effectors were scarcely studied. Also, the molecular and cellular events responsible for the brain pathology following OP poisoning, including the role of PKC, were scantily investigated (Bodjarian et al., 1992). Therefore, the present study aimed to find a link between PKC activation and sarin-induced brain damage by characterization of the alterations in brain PKC isozymes expression in the rat. The immunoreactivity level in membrane and cytosolic fractions of individual PKC isozymes in different brain regions was examined during sarin-induced seizure activity. In addition, a possible neuroprotective effect of selective peptide regulators for PKC isozymes (Ron et al., 1995; Koponen et al., 2003) was evaluated following OP insult. The results suggested that up-regulation of PKC isozymes, e.g., β-II and ζ-PKC, might

Fig. 1. Immunoreactivity of conventional βII-PKC isozyme following different time points postsarin exposure. Rats exposed to sarin (90 mg/kg, ~1 LD₅₀ i.m.) were sacrificed by decapitation at the following time points: 10 and 30 min, 2 and 24 h, and 5 and 10 days postsarin exposure. Hippocampal, frontal cortex, thalamus, and striatum tissues were further microdissected, homogenized, separated to cytosolic and membrane fractions, and subjected to Western blot analysis. PKC isozyme-specific βII-PKC antibody was used to determine PKC isozyme expression. A, βII-PKC immunoreactivity in the rat frontal cortex. B, βII-PKC immunoreactivity in the rat hippocampus. C, βII-PKC immunoreactivity in the rat thalamus. D, βII-PKC immunoreactivity in the rat striatum. Autoradiograph from a representative Western blot illustrates membrane (M) and cytosolic (C) fractions from hippocampus and thalamus probed with anti-βII-PKC antibody from rats decapitated in different times indicated above. Data are mean ± S.E.M. (bars) values (n = 6 per time point), expressed as percentage of control (saline-treated) values (100%, dotted line). ∗, p < 0.05 compared with control (OD per microgram protein) values.
contribute to the histopathological sequelae produced by sarin.

Materials and Methods

Materials. Atropine sulfate, pyridostigmine hydrobromide, and other chemicals were purchased from Sigma Chemical (Poole, Dorset, UK). Sarin (isopropyl methylphosphono-fluoridate) was synthesized by the Department of Organic Chemistry (Israel Institute for Biological Research, Ness Ziona, Israel) and used in freshly prepared saline solutions. Sodium pentobarbitone (Nembutal) for anesthesia was purchased from CTS Ltd. (Tel-Aviv, Israel). The oxime 1-(4-aminocarbonylpyridinium) 1’-2’-pyridiniumaldoxime (dimethylether dichloride) was received as a gift from the German Ministry of Defense; Midazolam (Dormicum, 5 mg/ml solution) was purchased from Hoffman-La Roche (Nutley, NJ). Rabbit anticonventional PKC (1:100), βII (1:2000), and aPKC-ζ (1:1000) polyclonal antibodies, including their blocking peptides for control analysis and Western blotting luminol reagent (ECL) were purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). Fuji Super HRG film was purchased from Fuji Photo Film Co., Ltd. (Tokyo, Japan). Peptides regulators of PKC isozymes conjugated to antennapedia (translocation inhibitor of antennapedia (translocation inhibitor of (Tokyo, Japan). Peptides regulators of PKC isozymes were purchased from Bio-Rad Laboratories GmbH (Munchen, Germany).

Animal Model. The following experimental procedures were approved by the Institutional Animal Care and Use Committee at the Israel Institute for Biological Research and are in accordance with the principles enunciated in the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996. Adult male Sprague-Dawley rats (weighing 290–340 g; Charles River Laboratories, Inc., Wilmington, MA) were exposed to sarin in five experimental groups: 1, sarin exposure (90 mg/kg, –1 LD50 i.m.); 2, sarin exposure (108 mg/kg, –1.2 LD50 i.m.); 3, sarin exposure (108 mg/kg, –1.2 LD50 i.m.) with supportive treatment [oxime 1-(4-aminocarbonylpyridinium) 1’-2’-pyridiniumaldoxime (dimethylether dichloride, 5 mg/kg i.m.) and ChE inhibitor, pyridostigmine (0.1 mg/kg, i.m.) 20 min prior to sarin exposure and muscarinic antagonist, atropine (3 mg/kg i.m.) 1 min after exposure; 3, sarin exposure (108 mg/kg, –1.2 LD50 i.m.) with supportive treatment as in group number 2, including peptide regulators for PKC isozymes injected (380 ng/kg i.c.) 10 min postsarin-induced convulsive activity; 4, sarin exposure (108 mg/kg, –1.2 LD50 i.m.) with supportive treatment as in group number 2, including peptide regulators for PKC isozymes injected (380 ng/kg i.c.) 10 min postsarin-induced convulsive activity; and 5, naive or sham-operated rats served as a control group.

Subcellular Fractionation. Rats from groups 1 and 3 were sacrificed by decapitation at the following time points: 10 and 30 min, 2 and 24 h, and 5 and 10 days postsarin exposure. Brains were rapidly removed and further microdissected into frontal cortex, hippocampus, and thalamus components. Tissues were then homogenized with a freshly prepared homogenization buffer (20 mM HEPES, pH 7.5, 2 mM EDTA, 0.3 mg/ml dithioerythritol, 0.16 mg/ml phenylmethylsulfonyl fluoride, and 0.02 mg/ml aprotinin) and prepared in accordance with subcellular fractionation procedures.
(McNamara et al., 1999). Briefly, homogenates were centrifuged at 100,000g for 1 h at 4°C. The resulting supernatant represented cytosolic proteins. Membrane proteins were collected by solubilizing the remaining pellet in homogenization buffer containing 0.1% Triton X-100 on ice for 45 min and centrifuging at 50,000g for 30 min at 4°C. Both fractions were normalized to contain a final concentration of 0.05% Triton. Protein concentration was determined by the method of Bradford (1976). Samples were immediately boiled in SDS-polyacrylamide gel electrophoresis sample buffer for 3 min and stored at −80°C until used.

Western Blot Analysis. PKC isozyme-specific antibodies, βII- and γ-PKC, were used to determine PKC isozyme expression. Western blot analysis was performed by loading 40 μg of protein on 10% SDS-polyacrylamide gel electrophoresis and electrophoresing the samples for ~1.45 h at 100 V. The gels were transferred to a nitrocellulose membrane (0.45 μm) in a Trans-Blot Semi-Dry electrophoresis apparatus (Bio-Rad) at 10 V for 25 min. Following transfer, the blots were rinsed in TBS buffer (20 mM Tris, pH 7.5, and 500 mM NaCl), and nonspecific binding sites were blocked by incubating the blots in 5% dry milk in TBS buffer for 1 h at room temperature. The primary PKC isozyme-specific antisera were diluted in TBS buffer and titered to yield optimal specific binding. Blots were incubated over night with the primary antibodies at 4°C, washed three times for 15 min in TBS buffer containing 0.2% Tween 20 and subsequently incubated with the second antibody (anti-mouse or anti-rabbit IgG horse-radish peroxidase conjugate) for 1 h at room temperature. The blots were washed, incubated with the enhanced chemoluminescence reagent for 1 min, exposed to film, and developed. For graphing purposes, potential changes in PKC isozyme levels following sarin exposure were expressed as a percentage of control values that were electrophoresed on the same gels.

Surgery Procedure. Rats anesthetized with pentobarbitone (25 mg/kg) were shaved in the neck, and two small incisions were made on both the neck and the back. The right carotid artery was exposed through a 2-cm ventral neck incision, and its lower part was ligated. A cannula (PE-50; 1000 U/ml heparin in saline) was inserted to the artery and was pushed forward 1 cm toward the brain. By aspirating some blood, the position of the cannula was checked, and then the cannula was tightly sutured to the carotid and skin, flushed gently with 50 μl of heparinized saline solution, and closed with a pin. Finally, the cannula was tunneled subcutaneously from the ventral neck incision to emerge at the back of the neck, and both incisions were closed with 3-0 silk sutures. Seventy-two hours post-surgical processes, rats were exposed to sarin (as described in Animal Model, group 2). Peptide regulators for PKC isozymes were injected (380 ng/kg i.c.) 10 min postinjection of sarin-induced convulsions.

Histology. Rats from groups 4 and 5 were sacrificed by decapitation. Hippocampus tissues were further microdissected, homogenized, separated to cytosolic and membrane fractions, and subjected to Western blot analysis. A, βII-PKC immunoreactivity in the rat hippocampus. B, γ-PKC immunoreactivity in the rat hippocampus. Data are mean ± S.E.M. (bars) values (n = 6 per time point), expressed as percentage of control (saline-treated) values (100%). * p < 0.05 compared with control (OD per microgram protein) values.

Results

Expression of PKC Isozymes. In the present study, the expression of members of the PKC isozyme family was examined in cytosolic and membrane fractions derived from rat frontal cortex, striatum, hippocampus, and thalamus. The involvement of conventional PKC isozymes in sarin-induced brain damage was first detected by using a nonselective antibody for the α, β, and γ isotypes (data not shown). Since each conventional isotype contribution could not be evaluated with the nonspecific conventional PKC antibody, a specific antibody for the βII-PKC isotype was used. As can be seen in Fig. 1A, there was hardly a significant change in the immunoreactivity level either in the cytosolic or in the membrane fractions in the frontal cortex, except for a 30% reduction 2 h post-sarin exposure. On the contrary, the βII-PKC isotype was highly expressed (3- to 4-fold versus control) in both cytosolic and membrane fractions in the hippocampus (Fig. 1B) and thalamus (Fig. 1C), accompanied by a remarkable down-regulation (50%) in the striatum (Fig. 1D). The expression of the atypical protein member, γ-PKC isozyme, also changed significantly following sarin exposure. A dramatic increase (3- to 5-fold) of γ-PKC immunoreactivity was observed 5 and up to 10 days post-sarin exposure in the hippocampus (Fig. 2B) and thalamus (Fig. 2C), respectively. Quite the opposite was seen in the frontal cortex (Fig. 2A), whereas a decrease (30–50% of control values) in both fractions was found. No changes were seen in the striatum (Fig. 2D). Treatment with the anticonvulsant midazolam (0.5 mg/kg) 10 min post-sarin exposure attenuated seizure activity, causing rats an immediate sedation-like state. Rats admin-
istered midazolam and sacrificed 2 h post-sarin exposure showed a marked reduction in βII- and ζ-PKC expression in the membrane fraction in the hippocampus (Fig. 3, A and B, respectively).

**Clinical and Histopathological Observations.** Manifestation of symptoms shown in Table 1 in sarin-treated rats appeared within 2 to 4 min after injection. Animal follow-up included clinical observations for 24 h post-sarin exposure, weight monitoring, and histopathology evaluation 48 h following poisoning. Weight loss reached its maximal level 48 h post-intoxication (15%, p < 0.05, in 90% of exposed animals). Table 1 presents both clinical and histopathological scores graded from 0 to 5 according to the severity condition of each parameter.

**The Influence of βII-PKC Inhibitor Peptide on Sarin Neurotoxicity.** Since both βII- and ζ-PKC isotypes were overexpressed postsarin exposure, the effect of certain peptide regulator of these kinases has been determined. βII-PKC inhibitor peptide (380 ng/kg), known to inhibit PKC translocation and activation, was chosen as an example for such challenge. As can be seen in Figs. 4 to 6, βII-PKC inhibitor peptide attenuated sarin-induced neuropathology. Notice the viability of hippocampal CA1 cells in the histological brain sections stained with hematoxylin and eosin (Fig. 4C) in contrast to the neuronal damage postsarin exposure (Fig. 4B). By counting the intact cells in the CA1 layer, the neuroprotective effect of βII-PKC inhibitor peptide could be quantitated (Fig. 5). Also, as can be seen in Fig. 6, a fine correlation between clinical score and histological findings was observed.

**Discussion**

OP nerve agent studies were conducted during the past several years to define mechanisms of OP-induced convulsions to improve the currently utilized nerve agent antidotal regimen. Since subcellular and molecular events were scarcely studied, the purpose of the present study was to characterize specific messengers that might participate in OP-induced brain damage. In this study, we have shown that PKC isozymes, which have been implicated in different neuronal signaling processes, namely the conventional βII-PKC and the atypical ζ-PKC, were involved in sarin-induced brain damage. This was demonstrated by the following findings. Significant elevations (2- to 4-fold) in βII-PKC immunoreactivity were observed up to 10 days post-sarin exposure in the membrane fractions in the hippocampus and thalamus, but barely in the frontal cortex or as opposed to the striatum (50% decrease). A dramatic elevation (3- to 5-fold) in ζ-PKC immunoreactivity was found in the hippocampus and thalamus, 5 and, to a lesser extent, 10 days post-sarin exposure. ζ-PKC isoform also showed alterations in the frontal cortex, characterized by remarkable down-regulation immediately post-sarin intoxication and lasted up to 10 days, mostly in both the cytosol and membrane fractions. Anticonvulsive treatment following administration of the conventional antidotal regimen reduced, almost to baseline, the immunoreactivity of ζ-PKC as well as βII-PKC isozymes in the hippocampal membrane fractions postsarin poisoning. Injection of βII-PKC inhibitor peptide into the rat cardiotropic attenuated sarin-induced neuropathology. These observations support accumulating evidences that βII- and ζ-PKC isozymes play a role in promoting neuronal cell death (McNamara et al., 1999; Koponen et al., 2003; Tang et al., 2004).

In the present study, different patterns of protein expression for βII- and ζ-PKC isozymes were detected, emphasizing the diversity in the regulation of both PKC isozymes in different brain areas. Although not in the exact pattern in the hippocampus and thalamus, a significant up-regulation of both βII-PKC (Fig. 1, B and C) and ζ-PKC isozymes (Fig. 2, B and C) has occurred. The high expression in both the cytosolic and membrane fractions is likely not due to translocation, which is characterized by an increase in content in the membrane, accompanied by a reduction of a similar magnitude in the cytosol. Instead, such increase could be attributed to a reduction in proteolytic degradation or a rapid induction in synthesis. A combination of both processes could explain our observations. Young et al. (1987) reported that synthesis of PKC takes minutes following phorbol ester treatment, verifying the possibility of de novo synthesis. On the other hand, the reduction in the PKC isozymes observed minutes after sarin exposure (Fig. 1D; Fig. 2, A and D), in the absence of apparent translocation from the other fraction, indicates a modulation in proteolytic degradation processes. Our data are in accord with the findings of McNamara et al. (1999), which demonstrated a redistribution of PKC isozymes following KA administration in the rat hippocampus. No translocation was observed, whereas remarkable changes in isozymes expression were found. Also, a dramatic up-regulation of ζ-PKC occurred 5 days post-KA administration, similar to the present data (Fig. 2, B and C). Albeit a different

**TABLE 1**

<table>
<thead>
<tr>
<th>Score</th>
<th>Clinical Symptoms</th>
<th>Histopathological Evaluation</th>
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<tbody>
<tr>
<td>0</td>
<td>Normal behavior</td>
<td>Normal morphology</td>
</tr>
<tr>
<td>1 (minimal)</td>
<td>Grooming and piloerection</td>
<td>Enlargement of ventricles, few pyknotic cells</td>
</tr>
<tr>
<td>2 (mild)</td>
<td>Salivation and tremor</td>
<td>Typical OP damage (loss of cells and vacuolization) in one of three vulnerable regions (piriform cortex, hippocampus, thalamus)</td>
</tr>
<tr>
<td>3 (moderate)</td>
<td>Impairments in motor activity and weight loss</td>
<td>Typical OP damage in two of three vulnerable regions (see above)</td>
</tr>
<tr>
<td>4 (moderate to severe)</td>
<td>Dyspnea</td>
<td>Typical OP damage in the three vulnerable regions</td>
</tr>
<tr>
<td>5 (severe)</td>
<td>Convulsions</td>
<td>Degeneration over 50% of cells in the three vulnerable regions including additional brain regions such as septum and amygdala</td>
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mechanism of action, similarity between KA and OP in seizure-induced neuropathology can now be presented in the subcellular level, involving the modulation of PKC isozymes, alone with histopathological studies (O’Shaughnessy and Gerber, 1986; T. Kadar and S. Chapman, personal communication). Our results described an up-regulation of PKC isozyme immunoreactivity in the hippocampus and thalamus, the brain regions that are known to be vulnerable to OP intoxication (Lemercier et al., 1983; Kadar et al., 1995). On the contrary, no significant changes or down-regulation had occurred in the frontal cortex or striatum (Fig. 1, A and D; Fig. 2, A and D). This regional correlation between isozyme high expression and vulnerability to OP intoxication might underscore the contribution of βII- and ζ-PKC isozymes to neuronal cell death. However, the precise mechanism of action and how the decrease in isozyme expression might be related to cell survival remains to be elucidated.

Protein kinase C has been implicated in the delayed neurotoxic effects of glutamate that is known to participate in OP intoxication. Previous studies have suggested a role for PKC isozymes in brain damage and outlined a correlation among sustained glutamate release, PKC activity, and downstream neuronal damage (Nakane et al., 1998; Alagarsamy et al., 2001; Skeberdis et al., 2001). Although there are conflicting results concerning its activation or inhibition, PKC plays a role in excitotoxic neuronal death (Favaron et al., 1990; Felipo et al., 1993; Durkin et al., 1996; Wagey et al., 2001). For example, Felipo et al. (1993) claimed that inhibitors of PKC are able to protect cultured cerebellar neurons from excitotoxic...
death. In contrast, Wagey et al. (2001) showed that preexposure to PMA significantly augmented death of NMDA-treated transfected HEK cells. Lately, Koponen et al. (2003) have suggested a role for $\eta$-PKC in NMDA excitotoxicity demonstrated by confocal microscopic photographs. They showed that inhibition of $\xi$-PKC, but not $\delta$-PKC, confers protection to neuron-astrocyte cultures exposed to a level of NMDA, which produced excitotoxic cell death. Taking advantage of commercially available specific antibodies for PKC isotypes and previously characterized peptides, which inhibit different PKC isozymes translocation (Ron et al., 1995), it was possible to study their involvement in sarin neurotoxicity in vivo. In the present study, inhibition of $\beta$II-PKC translocation, by a specific peptide, not only attenuated the clinical manifestations following sarin-intoxication (Fig. 5) but also increased neuronal survival as was detected by histopathological studies, supported by morphometric analysis (Fig. 4). These rationally designed peptides were found to selectively inhibit PKC isoform translocation and induce cardioprotection (Chen et al., 2001; Inagaki et al., 2003) and neuroprotection (Koponen et al., 2003; Bright et al., 2004). Even though the above studies described PKC involvement in the development of ischemia and apoptotic neuronal death, which are not the primary mechanisms related to OP exposure, it is reasonable to assume that both ischemia and OP-induced excitotoxicity share related downstream processes. All together, these findings support the hypothesis of a modulation role for PKC in NMDA receptor-mediated neuropathology and its possible clinical value. Completing the study with $\eta$-PKC inhibitor and others will verify PKC involvement in OP poisoning and provide a potentially new target for drug development and treatment.

According to the results presented in this study, and others (Weissman and Raveh, 2003; Gilat et al., 2004), we propose a possible sequence of neurochemical events following OP-induced seizures. The inhibition of ChE by OP causes a remarkable elevation of acetylcholine that is responsible for the initiation of crucial events (McDonough and Shih, 1997). The earlier cholinergic phase, which is started as a result of massive accumulation of ACh and its binding to the muscarinic and nicotinic receptors, probably induces PLC activation and PKC isozyme mobilization and activation in different brain areas. This PKC activation is not necessarily the one that is related to the degenerative processes. However, due to the progressive events that include the disruption of catecholamine and excitatory amino acid neurotransmitters following the cholinergic phase, there is maintenance of the vast seizure activity leading to brain damage. The excitatory amino acid phase post-OP exposure may involve PKC isoforms in a Ca$^{2+}$-dependent and -independent manner. Parallel or sequenced activation of different pathways including phospholipases (A and D) and their metabolites (such as cyclooxygenase), reactive oxygen and nitrogen species, kinases, proteases, and cytokines may further account for the neurodegenerative processes.

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