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Seizure-related Brain Injuries in Organophosphate Poisoning

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ABSTRACT *: The features of seizure-related brain injuries in rats poisoned i.p. with diisopropylfluorophosphate were investigated. Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg), which are centrally inactive, were pretreated i.m. 30 min and 10 min, respectively, before diisopropylfluorophosphate (10 mg/kg, $2LD_{50}$) poisoning to reduce the mortality and eliminate peripheral signs. Diisopropylfluorophosphate induced severe limbic seizures, and early necrotic and delayed apoptotic brain injuries. The necrotic brain injury, which was closely related to seizure intensity, was exerted as early as 1 hr predominently in hippocampus and piriform cortex, showing spongiform change (malacia) of neurophils in severe cases, in contrast to a typical apoptotic (TUNEL-positive) pattern after 12 hr in thalamus, and a mixed type in amygdala. Nitric oxide content in cerebrospinal fluid significantly increased after 2 hr, reaching a maximal level at 6 hr. Pretreatment with $_L$-N^G-nitroarginine, an inhibitor of nitric oxide synthase, reduced nitric oxide content and attenuated only apoptotic brain injury in all four brain regions examined without affecting seizure intensity and necrotic injury. Taken together, early necrotic and delayed apoptotic brain injuries induced by diisopropylfluorophosphate poisoning in rats may be related to seizure intensity and nitric oxide production, respectively.*

Key Words *: Organophosphates, Seizures, Brain injuries, Nitric oxide, Apoptosis*

I. INTRODUCTION

Earlier (Olney *et al.*, 1983), seizure-related brain injury induced by cholinergic agents such as acetylcholine agonists and cholinesterase inhibitors was reported. Thereafter, some features of neural and cardiac injuries in pinacolylmethylphosphonofluoridate (soman)-poisoned rats have been demonstrated (McDonough *et al.*, 1987 and 1995; Tryphonas and Clement, 1995). Recently, it was reported that excitatory amino acids increased during seizures following organophosphate intoxications (Lallement *et al.*, 1991), and that excitatory amino acids and their receptor agonists (excitotoxins) were proposed to play a causative role in the injury of central nervous tissues (Choi *et al.*, 1987; Garthwaite and Garthwaite, 1989; Shih *et al.*, 1991; Sparenborg *et al.*, 1992). Accordingly, antagonists of excitatory amino acid receptors, especially N-methyl-$_D$-aspartate subtype of glutamate receptor, are expected to be potentially useful to prevent seizures and neuronal injuries induced by organophosphate poisoning (Shih *et al.*, 1991).

Furthermore, the involvement of nitric oxide in cholinergic models of epilepsy was proved, although controversial results were reported with nitric oxide synthase inhibitors, since nitric oxide played a role as a proconvulsant (Bagetta *et al.*, 1992) as well as an anticonvulsant (Starr and Starr, 1993) molecule. In the central nervous system, the activation of nitric oxide synthase, following stimulation of N-methyl-$_D$-aspartate receptor, produces nitric oxide which functions as a signalling or cytotoxic molecule (Dawson *et al.*, 1991; Lancaster, 1992). As a retrograde messenger, nitric oxide induces the release of several neurotransmitters including excitatory amino acid $_L$-glutamate (Montague *et al.*, 1994), which deranges neurotransmitter balance and affects neuronal excitability. In a previous paper, we demonstrated the involvement of nitric oxide in or-

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ganophosphate-induced seizures and the effectiveness of nitric oxide synthesis inhibitors in the prevention of seizures (Kim *et al.*, 1997a).

On the other hand, nitric oxide induces apoptotic death of a variety of cells including neural, immune and inflammatory cells (Blanco *et al.*, 1995; Bolanos *et al.*, 1997; Bonfoco *et al.*, 1995; Fehsel *et al.*, 1995; Illera *et al.*, 1993; Sarih *et al.*, 1993). Apoptosis is an active process of cell destruction with specifically-defined morphological and molecular features, which is considered a beneficial process whereby organisms eliminate "unwanted", i.e. old, damaged, precancerous or excessive, cells without further nearby tissue injuries shown in necrosis (Thompson, 1995). However, in central nervous tissues that have a limited capacity for self-renewal, apoptotic cell death may result in physiological or pathological disorders, which may underlie the etiology of neurodegenerative diseases (Thompson, 1995). In fact, apoptotic cell death has been evidenced in experimental traumatic, ischemia-reperfusion and excitotoxic brain injuries (Ankarcrona *et al.*, 1995; Bonfoco *et al.*, 1995; Pollard *et al.*, 1994; Charriaut-Marlangue *et al.*, 1996; Okamoto *et al.*, 1993; Rink *et al.*, 1995). In a recent report (Kim *et al.*, 1997b), we demonstrated nitric oxide-mediated neural apoptosis in organophosphate-poisoned rats. Subsequently, the present study shows early necrotic and delayed apoptotic brain injuries, which may be related to seizure intensity and nitric oxide production, respectively, following diisopropylfluorophosphate poisoning.

II. MATERIALS AND METHODS

1. Materials

Various chemicals and reagents, including pyridostigmine bromide, atropine methylnitrate and $_L$-N^G-nitroarginine, were from Sigma Chemical Co. (St. Louis, MO, USA), except diisopropylfluorophosphate which was procured from Adrich Chemical Co. (Milwakee, WI, USA). *In Situ* Apoptosis Detection Kit-Peroxidase (ApopTag TUNEL assay kit) was obtained from Oncor Co. (Gaithersberg, MD, USA)

2. Seizure induction

Adult Sprague-Dawley female rats (200-300 g) were poisoned i.p. with 4-10 mg/kg (0.8 - 2LD$_{50}$) of diisopropylfluorophosphate. Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg), which are centrally inactive (Shih *et al.*, 1991), were pretreated i.m. 30 min and 10 min, respectively, before diisopropylfluorophosphate poisoning to reduce the mortality and eliminate peripheral signs for the clear demonstration of seizure activity (Kim *et al.*, 1997a). $_L$-N^G-Nitroarginine, an inhibitor of nitric oxide synthase, was i.p. administered 30 min prior to diisopropylfluorophosphate challenge. The intensity of seizures was evaluated using 5-point scores (De Sarro *et al.*, 1993) as follows; 0, no response; 1, myoclonic jerks of the contralateral forelimb; 2, mouth and facial movements (i.e., facial myoclonus, clonus of the jaw and vibrissae) and head nodding with or without mild forelimb clonus; 3, severe forelimb clonus; 4, rearing and severe forelimb clonus; 5, rearing and falling. All compounds were dissolved in saline, except diisopropylfluorophosphate which was diluted in distilled water. Diisopropylfluorophosphate, pyridostigmine bromide and atropine methylnitrate were administered in a volume of 1 ml/kg, and $_L$-N^G-nitroarginine was injected in a volume of 5 ml/kg.

3. Cell death-type determination

Whole brain was removed after fixation by *in situ* intracardial perfusion with 10% neutral formalin solution containing 2 IU heparin/ml under ether anesthesia. For the identification of apoptotic injury, paraffin-embedded brain sections (4 μm in thickness) were stained immunohistochemically using terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) of DNA fragments with diaminobenzidine as chromogenic substrate, and counterstained with methyl green (Rink *et al.*, 1995). The injury type of morphologically-dead cells was determined based on the staining patterns of nucleus; brown staining as apoptosis versus green as necrosis. The degree of brain injury was evaluated using 5-point scores based on the approximate percentage of tissue in-

volvement according to the grading system of McDonough *et al.* (1995) with slight modification; 0, no lesion; 1, minimal, 1-10%; 2, mild, 11-25%; 3, moderate, 26-45%; 4, severe, 46-60%; 5, extreme,>60%.

4. Nitrite/nitrate determination

The ventral interspinal foramen between atlas and axis cervical spines were exposed after sacrifice of rats with deep ether anesthesia. Cerebrospinal fluid was collected carefully not to be contaminated with blood. An aliquot (100 μl) of cerebrospinal fluid was incubated with 10 μl NADPH (final 40 μM) and 10 μl nitrate reductase (final 28 mU/0.1 ml) solutions in a 96-well plate at room temperature for 10 min, and further 10 min after addition of an equal volume (120 μl) of Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% H_3PO_4) (Connor *et al.*, 1995; Sarih *et al.*, 1993). Nitrite was determined by reading the absorbance at 540 nm using a Titertek multiskan (Flow Laboratories, Australia) with sodium nitrite as standard.

Results were expressed as the mean±S.E. of 8-10 determinations. Tests of significance were performed using Duncan's multiple range test, with $P<0.05$ as a criterion of difference. The experiments were conducted according to the "Guide Principles in the Use of Animals in Toxicology" which had been adopted by the Society of Toxicology in 1989.

3. RESULTS AND DISCUSSION

Diisopropylfluorophosphate induced severe limbic seizures, displaying early (15-90 min) tonic-

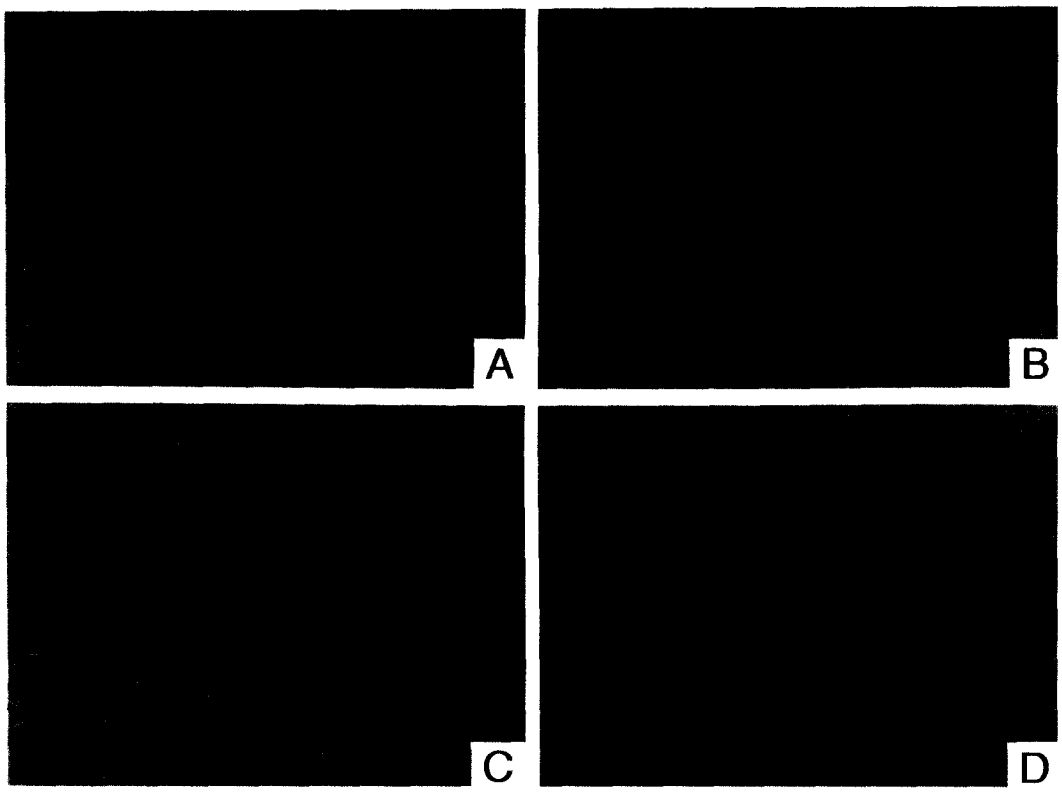

Fig. 1. Brain injuries induced by diisopropylfluorophosphate poisoning. Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 10 mg/kg ($2LD_{50}$) diisopropylfluorophosphate. At the indicated time after diisopropylfluorophosphate poisoning, whole brain was removed. Paraffin-embedded sections were stained using TUNEL technique as described in Methods section. Note the dark degeneration of hippocampal pyramidal cells (A, X100) at 24 hr and spongiform change (malacia) of piriform cortex (B, X100) at 12 hr, and the typical TUNEL-positive shrunken cells in dorsal thalamic nuclei (C, X100; D, X400) at 24 hr.

clonic seizures and later prolonged mild clonic epilepsy, which led to early necrotic and delayed apoptotic brain injuries (Fig. 1). Necrotic injury was observed as early as 1 hr predominently in hippocampal CA1 and CA3 regions and piriform/entorhinal cortices, showing dark degeneration of neuronal cells (Fig. 1A) and spongiform change (malacia) of neurophils (Fig. 1B). In severe cases, the malacia spread to all hippocampal formation (CA2, CA4 and dentate gyrus) and to auditory and somatosensory cortices (McDonough *et al.*, 1987 and 1995; Tryphonas and Clement, 1995). Interestingly, the degree of necrotic injury in hippocampus was determined within 1 hr and maintained constant, which was closely related to early seizure intensity (McDonough *et al.*, 1995), while that of piriform cortex, thalamus and amygdala increased after 4 hr, demonstrating an additional delayed effect (Fig. 2). In contrast, typical TUNEL-positive (apoptotic) cells appeared at 12 hr most predominently in thalamus (Fig. 1C and 1D), which reached a maximal level at 24 hr (Fig. 3). A

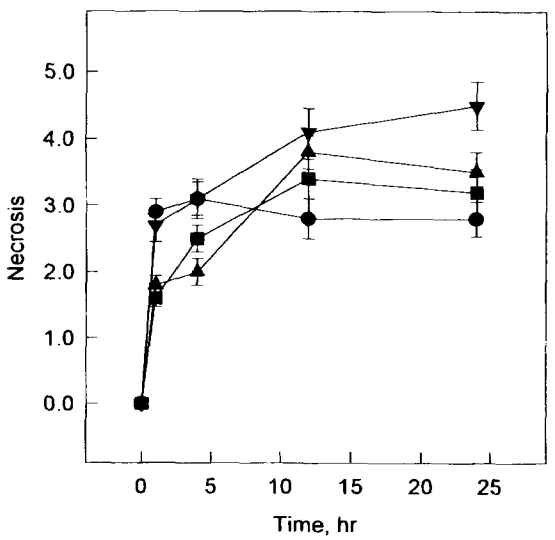

Fig. 2. Time course of necrotic brain injury in four regions induced by diisopropylfluorophosphate poisoning (n=8). Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 10 mg/kg ($2LD_{50}$) diisopropylfluorophosphate. At the indicated time, whole brain was removed. Paraffin-embedded sections were stained using TUNEL technique, and necrotic brain injury (TUNEL-negative, green staining) was scored according to the grading system described in Methods section. ●, hippocampus; ■, thalamus; ▲, amygdala; ▼, piriform cortex.

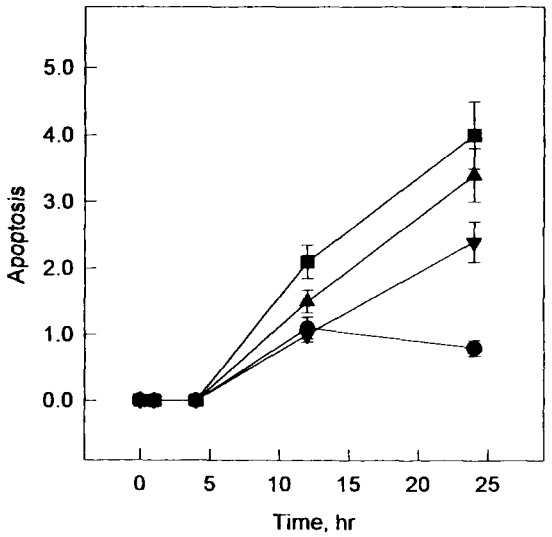

Fig. 3. Time course of apoptotic brain injury in four regions induced by diisopropylfluorophosphate poisoning (n=8). Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 10 mg/kg ($2LD_{50}$) diisopropylfluorophosphate. At the indicated time, whole brain was removed. Paraffin-embedded sections were stained using TUNEL technique, and apoptotic brain injury (TUNEL-positive, brown staining) was scored according to the grading system described in Methods section. ●, hippocampus; ■, thalamus; ▲, amygdala; ▼, piriform cortex.

similar pattern of apoptotic change was observed in amygdala, though wherein mixed type of injury was shown. On the other hand, no remarkable apoptosis was found in hippocampus, exhibiting predominently necrosis. Also, relatively-low incidence of apoptosis was seen in piriform cortex, although it increased in a time-dependent manner. For further analyses of the relationship between seizure intensity and features of brain injuries, necrosis in hippocampus and apoptosis in thalamus, rats were intoxicated with various doses (0, 4, 6, 8 or 10 mg/kg) of diisopropylfluorophosphate. As a result, necrotic brain injury was seen in rats showing seizure intensity higher than 2.0, displaying a good relationship with seizure intensity (Fig. 4). Interestingly, apoptosis was induced in rats exhibiting relatively low seizure intensity, reaching a peak level at 4.0 of seizure intensity. However, apoptosis was rather reduced in rats showing extremely severe seizures (seizure intensity>4.0). In contrast to seizure-related necrosis, delayed apoptosis was somewhat in parallel with

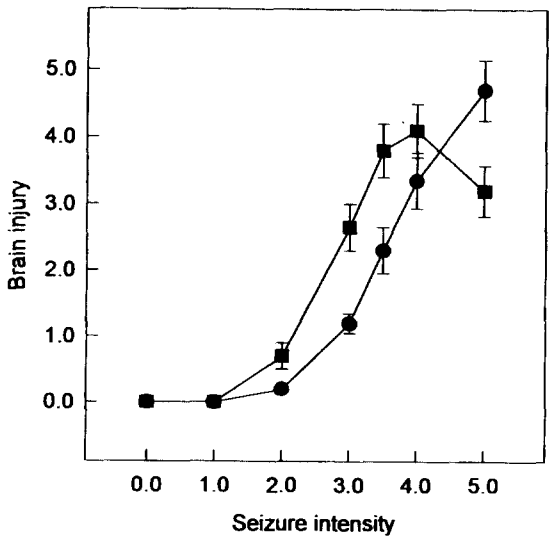

Fig. 4. Seizure-related changes in hippocampal necrosis (●) and thalamic apoptosis (■) induced by diisopropylfluorophosphate poisoning (n=8). Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 0-10 mg/kg diisopropylfluorophosphate. Twenty-four hours after diisopropylfluorophosphate poisoning, whole brain was removed. Paraffin-embedded sections were stained using TUNEL technique as described in Methods section.

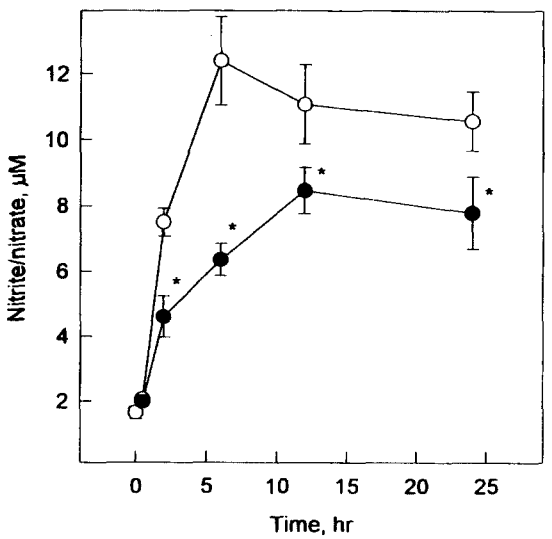

Fig. 5. Effect of $_{L}$-N^{G}-nitroarginine on the nitrite/nitrate content in cerebrospinal fluid of rats intoxicated with diisopropylfluorophosphate. Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 10 mg/kg (2LD$_{50}$) diisopropylfluorophosphate (n=10). $_{L}$-N^{G}-Nitroarginine (●) or vehicle (○) was i.p. administered 30 min prior to diisopropylfluorophosphate poisoning. Blood-free cerebrospinal fluid (0.1 ml) was collected 0.5-24 hr after diisopropylfluorophosphate poisoning, and incubated with NADPH (40 μM) and nitrate reductase (28 mU/0.1 ml) for 10 min, and with an equal volume of Griess reagent further 10 min at room temperature for the determination of nitrite/nitrate content. * Significantly different from vehicle control (P<0.05).

the increase in nitric oxide production (Fig. 5), although there is a lag time for the process of apoptosis. Nitric oxide concentration in cerebrospinal fluid significantly increased at 2 hr, the time of cessation of early severe tonic-clonic seizures, reaching a maximal level at 6 hr. $_{L}$-N^{G}-Nitroarginine (30 mg/kg), an inhibitor of nitric oxide synthase, reduced nitric oxide content to the half level of rats treated with diisopropylfluorophosphate alone up to 6 hr. Interestingly, $_{L}$-N^{G}-nitroarginine significantly attenuated only the apoptotic injury in all four brain regions without influencing seizures and necrotic injury (Fig. 6), confirming the seizure-related necrosis and the nitric oxide-mediated apoptosis.

The injury types of neural cells exposed to excitatory amino acids or their receptor agonists (excitotoxins) are controversial (Ankarcrona *et al.*, 1995; Bonfoco *et al.*, 1995; Dessi *et al.*, 1993; Ignatowicz *et al.*, 1991; Kure *et al.*, 1991; Pollard *et al.*, 1994). Nitric oxide was proposed as a mediator of neural cell injury induced by excitotoxins including N-methyl-$_{D}$-aspartate (Bolanos *et al.*, 1997; Bonfoco *et al.*, 1995; Dawson *et al.*, 1991 and 1993). It has been reported that excitotoxins can induce necrosis or apoptosis depending on the experimental conditions (Ankarcrona *et al.*, 1995; Bonfoco *et al.*, 1995), which may result from differences in the concentration of excitotoxins or reactive radicals, nitric oxide and oxygen radicals, produced following N-methyl-$_{D}$-aspartate receptor stimulation (Gunasekar *et al.*, 1995; Lafon-Cazai *et al.*, 1993). Reaction of nitric oxide with superoxide anion forms highly reactive peroxynitrite and hydroxyl radicals, the ultimately cytotoxic molecules (Beckman *et al.*, 1990; Xia *et al.*, 1996). It is of interest to note that immunohistochemical localization of nitric oxide synthases was identified in various brain regions including thalamus, entorhinal cortex, hippocampus and amygdala (Southam and Garthwaite, 1993; Vincent and Kimura, 1992). Furthermore, postsynaptic nitric oxide production, following activation of N-methyl-$_{D}$-aspartate receptors, results in cGMP accumula-

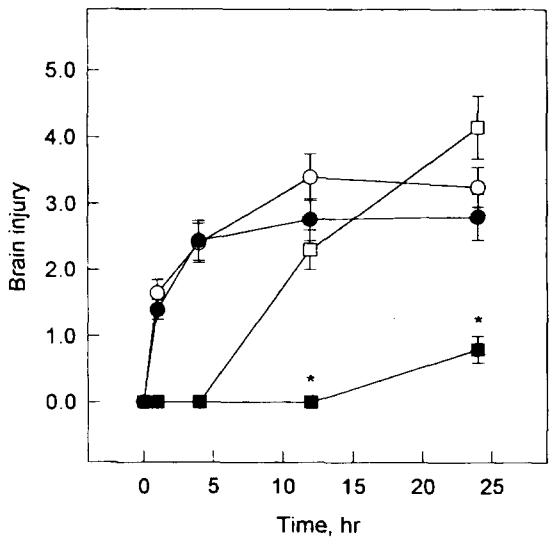

Fig. 6. Selective inhibition by $_{L}$-N^{G}-nitroarginine of delayed apoptotic injury in thalamus of rats intoxicated with diisopropylfluorophosphate. Pyridostigmine bromide (0.1 mg/kg) and atropine methylnitrate (20 mg/kg) were i.m. injected 30 min and 10 min, respectively, before i.p. challenge with 10 mg/kg ($2LD_{50}$) diisopropylfluorophosphate (n=8). $_{L}$-N^{G}-Nitroarginine (black) or vehicle (white) was i.p. administered 30 min prior to diisopropylfluorophosphate poisoning. At the indicated time, whole brain was removed. Paraffin-embedded sections were stained using TUNEL technique as described in Methods section. ○ and ●, necrosis; □ and ■, apoptosis. * Significantly different from vehicle control ($P<0.05$).

tion in several regions in vivo, including cerebral cortex, thalamus and hippocampus (Southam and Garthwaite, 1993), indicating the involvement of nitric oxide in brain injuries. However, the increase in nitric oxide content may come form different isoforms of nitric oxide synthase; calcium-dependent constitutive enzymes and cytokine-inducible one. Enough concentration (>10 μM) of nitric oxide for the induction of apoptotic injury could be produced by inducible nitric oxide synthase, which may be supported by the observation of selective potentiation of N-methyl-$_{D}$-aspartate-induced neuronal injury following induction of astrocytic nitric oxide synthase with cytokines (Hewett *et al.*, 1994). Cerebral insults trigger toxic activation of N-methyl-$_{D}$-aspartate receptors and also activate microglia to produce diverse cytokines, some of which induce nitric oxide synthase in astrocytes (Taupin *et al.*, 1993). It is of interest to note that soman poisoning induced early astrocytic swelling and later astrocytosis in hippocampus, thalamus and amygdala (Ballough *et al.*, 1995; Tryphonas and Clement, 1995), and excitotoxic or ischemic brain insults led to the intense nitric oxide synthase immunostaining in astrocytes (Endoh *et al.*, 1993).

In spite of controversial results on the role of nitric oxide in cholinergic models of seizures and brain injury (Baggeta *et al.*, 1992; Kim *et al.*, 1997a; Lallement *et al.*, 1996), the present data clearly show the roles of nitric oxide as a cytotoxic mediator during seizures induced by diisopropylfluorophosphate poisoning. Taken together, early necrotic and delayed apoptotic brain injuries induced by diisopropylfluorophosphate poisoning in rats may be related to seizure intensity and nitric oxide production, respectively.

REFERENCES

Ankarcrona, M., Dypbukt, J.M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S.A. and Nicotera, P. (1995) : Glutamate-induced neuronal death : a succession of necrosis and apoptosis depending on mitochondrial function, *Neuron*, **15**, 961-973.

Bagetta, G., Iannone, M., Scorsa, A.M. and Nistico, G. (1992) : Tacrine-induced seizure and brain damage in LiCl-treated rats can be prevented by N$^{\omega}$-nitro-L-arginine methyl ester, *Eur. J. Pharmacol.*, **213**, 301-304.

Ballough, G.P.H., Martin, L.J., Cann, F.J., Graham, J. S., Smith, C.D., Kling, C.E., Forster, J.S., Phann, S. and Filbert, M.G. (1995) : Microtubule-associated protein 2 (MAP-2) : a sensitive marker of seizure-related brain damage, *J. Neurosci. Meth.*, **61**, 23-32.

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P. A. and Freeman, B.A. (1990) : Apparent hydroxyl radical production by peroxynitrite : implications for endothelial injury from nitric oxide and superoxide, *Proc. Natl. Acad. Sci. USA*, **87**, 1620-1624.

Blanco, F.J., Ochs, R.L., Schwartz, H. and Lotz, M. (1995) : Chondrocyte apoptosis induced by nitric oxide, *Am. J. Pathol.*, **146**, 75-85.

Bolanos, J.P., Almeida, A., Stewart, V., Peuchen, S., Land, J.M., Clark, J.B. and Heales, S.J.R. (1997) : Nitric oxide-mediated mitochondrial damage in the brain : mechanisms and implications for neurodegenerative diseases, *J. Neurochem.*, **68**, 2227-2240.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P.

and Lipton, S.A. (1995) : Apoptosis and necrosis : two distinct events induced, respectively, by mild and intense insults with N-methyl-$_D$-aspartate or nitric oxide/superoxide in cortical cultures, *Proc. Natl. Acad. Sci. USA*, **92**, 7162-7166.

Charriaut-Marlangue, C., Margaill, I., Represa, A., Popovici, T., Plotkine, M. and Ben-Ari, Y. (1996) : Apoptosis and necrosis after reversible focal ischemia : an in situ DNA fragmentation analysis, *J. Cereb. Blood Flow Metab.*, **16**, 186-194.

Choi, D.W., Maulucci-Gedde, M. and Kriegstein, A.R. (1987) : Glutamate neurotoxicity in cortical cell culture, *J. Neurosci.*, **7**, 357-368.

Connor, J.R., Manning, P.T., Settle, S.L., Moore, W.M., Jerome, G.M., Webber, R.K., Tjoeng, F.S. and Currie, M.G. (1995) : Suppression of adjuvant-induced arthritis by selective inhibition of inducible nitric oxide synthase, *Eur. J. Pharmacol.*, **273**, 15-24.

Dawson, V.L., Dawson, T.M., Bartley, D.A., Uhl, G.R. and Snyder, S.H. (1993) : Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures, *J. Neurosci.*, **13**, 2651-2661.

Dawson, V.L., Dawson, T.M., London, E.D. and Bredt, D.S. (1991) : Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures, *Proc. Natl. Acad. Sci. USA*, **88**, 6368-6371.

De Sarro, G., Di Paola, E.D., De Sarro, A. and Vidal, M. J. (1993) : $_L$-Arginine potentiates excitatory amino acid-induced seizures elicited in the deep prepiriform cortex, *Eur. J. Pharmacol.*, **230**, 151-158.

Dessi, F., Charriaut-Marlangue, C., Khrestchatisky, M. and Ben-Ari, Y. (1993) : Glutamateinduced neuronal death is not a programmed cell death in cerebellar culture, *J. Neurochem.*, **60**, 1953-1955.

Endoh, M., Maiese, K., Pulsinelli, W.A. and Wagner, J. A. (1993) : Reactive astrocytes express NADPH-diaphorase in vivo after transient ischemia, *Neurosci. Lett.*, **154**, 125-128.

Fehsel, K., Kroncke, K.-D., Meyer, K.L., Huber, H., Wahn, V. and Kolb-Bachofen, V. (1995) : Nitric oxide induces apoptosis in mouse thymocytes, *J. Immunol.*, **155**, 2858-2865.

Garthwaite, G. and Garthwaite, J. (1989) : Neurotoxicity of excitatory amino acid receptor agonists in young rat hippocampal slices, *J. Neurosci. Meth.*, **29**, 33-42.

Gunasekar, P.G., Kanthasamy, A.G., Borowitz, J.L. and Isom, G.E. (1995) : NMDA receptor activation produces concurrent generation of nitric oxide and reactive oxygen species : implication for cell death, *J. Neurochem.*, **65**, 2016-2021.

Hewett, S.J., Csernansky, C.A. and Choi, D.W. (1994) : Selective potentiation of NMDA-induced neuronal injury following induction of astrocytic iNOS, *Neuron*, **13**, 487-494.

Ignatowicz, E., Vezzani, A.-M., Rizzi, M. and D'Incalci, M. (1991) : Nerve cell death induced *in vivo* by kainic acid and quinolinic acid does not involve apoptosis, *Neuroreport*, **2**, 651-654.

Illera, V.A., Perandones, C.E., Stunz, L.L., Mower, D.A., Jr. and Ashman, R.F. (1993) : Apoptosis in splenic B lymphocytes, *J. Immunol.*, **151**, 2965-2973.

Kim, Y.-B., Hur, K.-H., Lee, Y.-S., Han, B.-G. and Shin, S. (1997a) : A role of nitric oxide in organophosphate-induced convulsions, *Environ. Toxicol. Pharmacol.*, **3**, 53-56.

Kim, Y.-B., Hur, K.-H., Shin, S., Kang, J.-K. and Sok, D.-E. (1997b) : Organophosphate- induced brain injuries : delayed apoptosis mediated by nitric oxide, *Neuroscience*, submitted.

Kure, S., Tominaga, T., Yoshimoto, T., Tada, K. and Narisawa, K. (1991) : Glutamate triggers internucleosomal DNA cleavage in neuronal cells, *Biochem. Biophys. Res. Comm.*, **179**, 39-45.

Lafon-Cazal, M., Pietri, S., Culcasi, M. and Bockaert, J. (1993) : NMDA-dependent superoxide production and neurotoxicity, *Nature*, **363**, 535-537.

Lallement, G., Carpentier, P., Collet, A., Pernot-Marino, I., Baubichon, D. and Blanchet, G. (1991) : Effects of soman-induced seizures on different extracellular amino acid levels and glutamate uptake in rat hippocampus, *Brain Res.*, **563**, 234-240.

Lallement, G., Shih, T.-M., Pernot-Marino, I., Baubichon, D., Foquin, A. and McDonough, J.H., Jr. (1996) : The role of nitric oxide in soman-induced seizures, neuropathology, and lethality, *Pharmacol. Biochem. Behav.*, **54**, 731-737.

Lancaster, J.R., Jr. (1992) : Nitric oxide in cells, *Am. Scientist*, **80**, 248-259.

McDonough, J.H., Jr., Dochterman, L.W., Smith, C.D. and Shih, T.-M. (1995) : Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment, *Neurotoxicology*, **15**, 123-132.

McDnough, J.H., Jr., McLeod, C.G., Jr. and Nipwoda, M.T. (1987) : Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology, *Brain Res.*, **435**, 123-137.

Montague, P.R., Gancayco, C.D., Winn, M.J., Marchase, R.B. and Fieldlander, M.J. (1994) : Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex, *Science*, **263**, 973-977.

Okamoto, M., Matsumoto, M., Chtsuki, T., Taguchi, A., Mikoshiba, K., Yanagihara, T. and Kamada, T. (1993) : Internucleosomal DNA cleavage involved in

ischemia-induced neuronal death, Biochem. *Biophys. Res. Comm.*, **196**, 1356-1362.

Olney, J.W., de Gubareff, T. and Labruyere, J. (1983) : Seizure-related brain damage induced by cholinergic agents, *Nature*, **301**, 520-522.

Pollard, H., Charriaut-Marlangue, C., Cantagrel, S., Represa, A., Robain, O., Moreau, J. and Ben-Ari, Y. (1994) : Kainate-induced apoptotic cell death in hippocampal neurons, *Neuroscience*, **63**, 7-18.

Rink, A. Fung, K.-M., Trojanowski, J.Q., Lee, V.M.-Y., Neugebauer, E. and McIntosh, T.K. (1995) : Evidence of apoptotic cell death after experimental traumatic brain injury in the rat, *Am. J. Pathol.*, **147**, 1575-1583.

Sarih, M., Souvannavong, V. and Adam, A. (1993) : Nitric oxide synthase induces macrophage death by apoptosis, *Biochem. Biophys. Res. Comm.*, **191**, 503-508.

Shih, T.-M., Koviak, T.A. and Capricio, B.R. (1991) : Anticonvulsants for poisoning by the organophosphorus compound soman : pharmacological mechanisms, *Neurosci. Biobehav. Rev.*, **15**, 349-362.

Southam, E. and Garthwaite, J. (1993) : The nitric oxide-cyclic GMP signalling pathway in rat brain, *Neuropharmacology*, **32**, 1267-1277.

Sparenborg, S., Brennecke, L.H. Jaax, N.K. and Braitman, D.J. (1992) : Dizocilpine (MK-801) arrests status epilepticus and prevents brain damage induced by soman, *Neuropharmacology*, **31**, 357-368.

Starr, M.S. and Starr, B.S. (1993) : Paradoxical facilitation of pilocarpine-induced seizures in the mouse by MK-801 and the nitric oxide inhibitor $_{L}$-NAME, *Pharmacol. Biochem. Behav.*, **45**, 321-325.

Taupin, V., Toulmond, S., Serrano, A., Benevides, J. and Zavala, F. (1993) : Increase in IL-6, IL-1 and TNF levels in rat brain following traumatic lesion, *J. Neuroimmunol.*, **42**, 177-186.

Thompson, C.B. (1995) : Apoptosis in the pathogenesis and treatment of disease, *Science*, **267**, 1456-1462.

Tryphonas, L. and Clement, J.G. (1995) : Histomorphogenesis of soman-induced encephalocardiomyopathy in Sprague-Dawley rats, *Toxicol. Pathol.*, **23**, 393-409.

Vincent, S.R. and Kimura, H. (1992) : Histochemical mapping of nitric oxide synthase in the rat brain, *Neuroscience*, **46**, 755-784.

Xia, Y., Dawson, V.L., Dawson, T.M., Snyder, S.H. and Zweier, J.L. (1996) : Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury, *Proc. Natl. Acad. Sci. USA*, **93**, 6770-6774.