Journal of Neurochemistry
Raven Press, New York

0022-3042/82/0102-0525/$02.75/0

Phospholipid Degradation and Cellular Edema Induced by Free Radicals in Brain Cortical Slices

Pak Hoo Chan, Mary Yurko, and Robert A. Fishman

Brain Edema Clinical Research Center, Department of Neurology, School of Medicine, University of California, San Francisco, California, U.S.A.

Abstract: Cellular edema and increased lactate production were induced in rat brain cortical slices by xanthine oxidase and xanthine, in the presence of ferric ions. Lipid peroxidation, as measured by thiobarbituric acid-reactive malondialdehyde, was increased 174%. Among the various subcellular fractions of brain cortex, xanthine oxidase-stimulated lipid peroxidation was highest in myelin, mitochondria, and synaptosomes, followed by microsomes and nuclei. Antioxidants, catalase, chlorpromazine, and butylated hydroxytoluene inhibited lipid peroxidation in both homogenates and synaptosomes, indicating H_2O_2 and radicals were involved. Further, several free fatty acids, especially oleic acid (18:1), arachidonic acid (20:4), and docosahexaenoic acid (22:6) were released from the phospholipid pool concomitant with the degradation of membrane phospholipids in xanthine oxidase-treated synaptosomes. These data suggest that lipases are activated by free radicals and lipid peroxides in the pathogenesis of cellular swelling. **Key Words:** Brain edema—Phospholipid degradation—Free radicals—Arachidonic acid—Polyunsaturated fatty acids. **Chan P. H. et al.** Phospholipid degradation and cellular edema induced by free radicals in brain cortical slices. *J. Neurochem.* **38**, 525–531 (1982).

Free radicals may participate in the induction of various pathological states in brain tissue. Free radicals, which possess an odd number of electrons and have extreme reactivity, can oxidize membrane lipids, forming lipid peroxides (Mead, 1976; Demopoulos et al., 1979). Lipid peroxidation has been shown to occur in cellular membranes of cerebral tissues *in vitro* (Barber, 1966; Rehncrona et al., 1980) and has been hypothesized to be associated with cellular damage (Kellogg and Fridovich, 1975; Tappel, 1980) and increased membrane permeability. Previously, we provided evidence that cellular edema in rat brain cortical slices can be induced by polyunstaurated fatty acids (PUFA) concomitantly with the formation of superoxide radicals ($O_2^{\cdot -}$) and lipid peroxides. The latter was measured by the formation of malondialdehyde (MDA) (Chan and Fishman, 1978, 1980). The swelling of cortical slices was characterized by a reduced inulin space, indicative of cellular (cytotoxic) edema (in an *in vitro* system that excluded the blood brain barrier). Although arachidonic acid associated with an increase in free radicals proved to be involved in brain swelling *in vitro*, the direct effect of free radicals on cellular volume and membrane integrity was not clearly established. It was shown earlier that xanthine oxidase, in the presence of ferric ions, produced $O_2^{\cdot -}$, H_2O_2, and singlet oxygen (Pederson and Aust, 1973; Kellogg and Fridovich, 1975). Using this free-radical-producing system, we investigated further the direct effects of free radicals on tissue swelling and membrane integrity. We studied cellular membranes to determine whether changes in the lipid peroxides, endogenous fatty acids, and/or the phospholipid pool characterize this model of cellular brain edema.

MATERIALS AND METHODS

Incubation media and reagents

The composition of Krebs-Ringer buffer was reported previously (Chan et al., 1979). Xanthine oxidase-iron medium consists of (in final concentration): xanthine

Received May 19, 1981; accepted August 20, 1981.
Address correspondence and reprint requests to Dr. Pak Hoo Chan, Department of Neurology, University of California, School of Medicine, San Francisco, California 94143, U.S.A.

Abbreviations used: DMSO, Dimethylsulfoxide; MDA, Malondialdehyde; $O_2^{\cdot -}$, Superoxide radical; PUFA, Polyunsaturated fatty acid; TBA, 2-Thiobarbituric acid.

oxidase (milk) 0.081 units/ml; xanthine, 0.1 mM; ADP, 0.1 mM; $FeCl_3$, 0.01 mM. Hypoxanthine (0.1 mM) and NADPH (0.1 mM) were used in other experiments. Superoxide dismutase (bovine blood, 2900 units/mg protein), xanthine oxidase (milk, 10.8 units/ml), catalase (bovine liver, 3900 units/mg protein), butylated hydroxytoluene, xanthine, hypoxanthine, ADP, NADPH, lactic acid, lactate dehydrogenase, aspirin, α-tocopherol, chlorpromazine, indomethacin, and thiobarbituric acid were purchased from Sigma, St. Louis, MO. 2,5-Dimethylfuran was obtained from Aldrich Chemical Co., Milwaukee, WI. Dimethylsulfoxide and $FeCl_3 \cdot 6H_2O$ were purchased from Baker, Phillipsburg, NJ. Fatty acid methyl ester phospholipid standards and silica gel H plates were obtained from Applied Sciences, State College, PA. Sodium thiopental was purchased from Abbott Laboratories, North Chicago, Il.

Tissue preparation and tissue swelling

Sprague-Dawley male rats (purchased from Simonsen, Gilroy, CA) weighing 100 ± 10 g were decapitated, and the brains rapidly removed and placed on ice. The preparation of single first cortical slices, weighing 40–50 mg, was reported previously (Harvey and McIlwain, 1969). The initial wet weight of each slice was measured with a Precision Balance (Federal Pacific Electric Co, Northboro, MA). Each slice was then incubated in 5 ml Krebs-Ringer buffer for 20 min at 37°C for reconstitution. Slices were then transferred to the Krebs-Ringer solution containing the experimental compounds and incubated at 37°C for 60 min in an Aquatherm water shaker. After incubation, the final wet weight of each slice was measured and the tissue swelling was calculated as follows (Chan and Fishman, 1980):

$$\text{Tissue swelling (\%)} = \frac{\text{Final wet weight} - \text{Initial wet weight}}{\text{Initial wet weight}} \times 100$$

Subcellular fractionation

Cortical slice homogenates were prepared by homogenizing the tissue slices with Krebs-Ringer buffer with 50 mg tissue/ml of buffer. Tissue homogenates were centrifuged twice at 1000 × *g* to obtain a pellet containing the nuclear fraction. The supernatant was then used as the starting material for the isolation of microsomes, synaptosomes, and mitochondria. The supernatant was centrifuged at 12,000 × *g* for 30 min to obtain the P_2 fraction (crude synaptosomes). The resulting supernatant was then centrifuged at 145,000 × *g* for 40 min to obtain the microsomal pellet. The preparation procedures of synatosomal membrane from the P_2 pellet was based on the method of Cotman and Matthews (1971). The P_2 pellet was resuspended in 0.32 M-sucrose and was layered on top of a discontinuous sucrose gradient containing 0.8 M- and 1.2 M-sucrose and was centrifuged at 65,000 × *g* for 2 h with an SW 41 rotor. The synaptosomal fraction was pipeted from the gradient layer between 0.8 M- and 1.2 M-sucrose. Myelin was pipeted from the upper layer. The synaptosomal fraction was treated by the addition of 5 vol. of hypotonic Tris buffer (6 mM, pH 8.1) for 1.5 h. After this hypotonic shock treatment, synaptosomal membrane was subjected to a second sucrose discontinuous gradient, containing 0.8 M- and 1.2 M-sucrose. The purified synaptosomal membrane was obtained from the interface of the gradient and was resuspended in 0.32 M-sucrose. The remaining myelin and synaptosomal trapped mitochondria were eliminated. Since sucrose interferes with the MDA assay, synaptosomes thus prepared were washed at least four times with Krebs-Ringer buffer before use. The mitochondrial fraction was obtained by centrifuging the nuclei-free supernatant (S1) at 9000 × *g* for 15 min. The resuspended pellet was then centrifuged twice with Ficoll (8% w/v) at 9000 × *g* as described by Marchbanks (1975).

Determination of lipid peroxidation

The determination of lipid peroxidation in tissue homogenates was based on the reaction of MDA, a product of lipid peroxidation, with 2-thiobarbituric acid (TBA) to form a pink-colored substance (Dahle et al., 1962). The procedure for the MDA assay using cortical slice homogenates was reported earlier (Chan and Fishman, 1980). The direct effect of xanthine oxidase-iron on lipid peroxidation of cortical slice homogenates was measured as follows: each rat brain cortical slice was homogenized in 1 ml Krebs-Ringer solution; 0.4 ml of homogenate was incubated with an equal volume of the experimental medium. After incubation, the entire reaction mixture was assayed for lipid peroxidation. In studies of the effects of several lipid-soluble free radical scavengers and antioxidants on lipid peroxidation, these agents were solubilized in 10% dimethylsulfoxide (DMSO). In these studies, the xanthine oxidase-iron system in 10% DMSO was used in the control experiments.

Determination and separation of phospholipid and free fatty acids

Determination of the total phospholipid phosphorus was based on the method of Ames (1966). The reduced phosphomolybdate complex was read at 820 nm. Standard curves obtained from both inorganic phosphate and dipalmitoyl phosphatidylcholine were used for calibration.

Separation of various phospholipids and free fatty acids from synaptosomes was based on the method of Kimelberg and Papahadjopoulos (1972). Synaptosomal membrane preparations were extracted with chloroform: methanol (2:1, v/v). The lipid extracts were applied to a 20 × 20 cm silica gel H plate (Applied Science, State College, PA) previously activated at 110°C for 30 min, and developed by two-dimensional chromatography with two solvent systems: (1) chloroform:methanol:15 M-ammonium hydroxide (130:55:10, by vol.), (2) chloroform: methanol:water:acetic acid:acetone (150:30:15:30:60, by vol.). The phospholipids, free fatty acids, and cholesterol were visualized by iodine vapor. The areas with free fatty acids from the unreacted plate were removed and subjected to methylation according to the method of Abood et al. (1978). Five milliliters of 5% HCl in methanol was added to the silica gel containing free fatty acid in a conical centrifuge tube and incubated at 98–100°C for 2 h. Then 10 ml of water was added to the incubation mixture and extracted with 4 ml of hexane three times. The upper hexane phases were pooled and evaporated to dryness under N_2. The fatty acid methyl ester was resuspended in hexane and 2 μl of the sample was injected into a gas chromatograph (Hewlett Packard 5830A) equipped with a column containing Gas Chrom Q with silar 5CP (3%). The injector temperature was 250°C, and the column temperature was 210°C. A known concentration of heptadecanoic

acid (17:0) was routinely added to the samples as an internal standard.

RESULTS

Free radical-induced swelling and lipid peroxidation of cortical slices: Effects of xanthine oxidase-xanthine

Xanthine oxidase plus xanthine or hypoxanthine stimulated tissue swelling and MDA formation (Table 1). The substrates alone were not effective. When ferric ions (0.01 mM) and ADP (0.1 mM) were added to the incubation medium containing xanthine oxidase and xanthine to augment free radical production, tissue swelling and MDA formation were significantly enhanced. Ferric ions and ADP alone did not have an effect on either brain swelling or lipid peroxidation. Further, the addition of NADPH caused a slight increase in both tissue swelling and lipid peroxidation, whereas NADPH alone was not effective.

Time course of free radical-induced cortical swelling, lactate production and lipid peroxidation

The swelling of rat brain cortical slices was increased from 7.8% after 10 min to 13% after 90 min incubation in Krebs-Ringer buffer as shown in Fig. 1a. The addition of the free-radical-generating system, i.e., xanthine oxidase, xanthine, ferric ions, and ADP, caused a substantial increase in tissue swelling. After 10, 30, 60, and 90 min incubation in the xanthine oxidase system, cortical swelling induced by free radicals was increased to 10%, 17.5%, 23.4%, and 27.4%, respectively. Thus, cortical swelling induced by free radicals was time-dependent. This time-dependent phenomenom was also observed in lactic acid content by rat brain slices. After 30, 60, and 90 min incubation with the free-radical-generating system, lactic acid was increased by 96%, 153%, and 221%, respectively. The increase in lactic acid content was also linear with time. However, there was no significant difference in lactate content after 10 min, whereas the tissue swelling was already slightly increased, indicating that the induction of tissue swelling was apparent before the increase in lactate content was detected.

The time course of lipid peroxidation in cortical slices induced by the xanthine oxidase-Fe^{3+} system is shown in Fig. 2. The formation of MDA in cortical slices increased linearly throughout the period of study following 10 min of incubation. MDA was increased 71%, 155%, and 231% after 30, 60, and 90 min of incubation, respectively.

Effects of antioxidants and radical scavengers on lipid peroxide formation

Among the subcellular fractions prepared from cortical slices, myelin, mitochondria, and synaptosomes were shown to be the most affected followed by microsomes, nuclei, and homogenates (Table 2). The effects of water-soluble or DMSO-soluble free radical scavengers and antioxidants on xanthine oxidase-Fe^{3+}-induced lipid peroxides in cortical homogenates and synaptosomes are shown in Table 3. Among the water-soluble drugs, chlorpromazine and catalase were highly effective against lipid peroxidation. DMSO (10%) was effective in homogenates, but failed to inhibit lipid peroxidation in synaptosomes. Superoxide dismutase (10 μg/ml) also failed to protect brain tissue from peroxidation. Both ethanol and aspirin were inactive. Butylated hydroxytoluene, a lipid-soluble antioxidant, inhibited MDA formation in both homogenate and synaptosomes by 56% and 70%, respectively. Among other lipid-soluble antioxidants, α-tocopherol and dimethylfuran were effective against lipid peroxidation in synaptosomes, whereas indomethacin and thiopental were inactive.

TABLE 1. *Effects of xanthine oxidase-Fe^{3+} system on tissue swelling and lipid peroxidation in rat brain cortical slices*

Incubation medium	Swelling (%)	MDA (nmol · mg protein^{-1})
Krebs-Ringer	11.3 ± 0.9 (26)	5.0 ± 0.3 (10)
Xanthine	11.6 ± 0.9 (19)	5.5 ± 0.9 (5)
Xanthine + xanthine oxidase	16.2 ± 1.3 (26)[a]	8.8 ± 0.6 (4)[a]
ADP-Fe^{3+}	10.3 ± 0.9 (4)	6.5 ± 0.8 (4)
Hypoxanthine	12.4 ± 1.5 (4)	4.2 ± 0.8 (4)
Hypoxanthine + xanthine oxidase	15.6 ± 2.2 (8)[a]	9.0 ± 0.5 (8)[a]
Xanthine + xanthine oxidase + ADP-Fe^{3+}	23.4 ± 0.9 (12)[a]	13.7 ± 0.8 (12)[a]
NADPH	10.2 ± 1.0 (4)	7.0 ± 1.5 (4)
Xanthine + xanthine oxidase + ADP-Fe^{3+} + NADPH	26.7 ± 1.4 (4)[a]	14.9 ± 2.7 (4)[a]

Results are expressed as means ± S.E.M. The concentration of the compounds used in these studies was presented in Materials and Methods. The number of cortical slices is indicated in parenthesis.

[a] $P < 0.001$, Student's t-test.

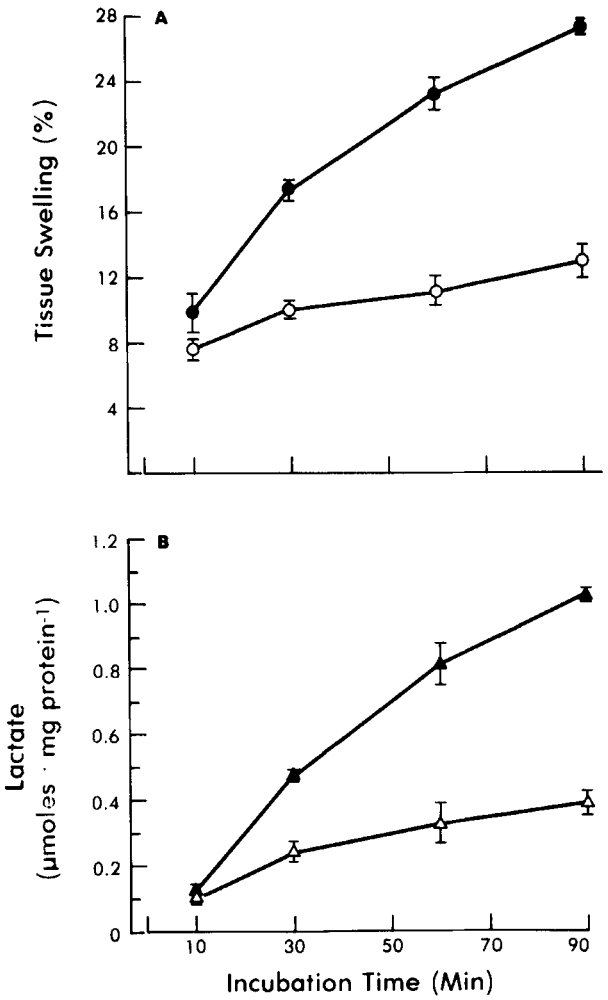

FIG. 1. Time course studies of xanthine oxidase-ferric ion-stimulated tissue swelling and lactate production in rat brain cortical slices. Each point represents an average value of at least four cortical slices (± S.E.M.). **(a)** (○), Krebs-Ringer; (●), xanthine oxidase system. **(b)** (△), Krebs-Ringer; (▲), xanthine oxidase system.

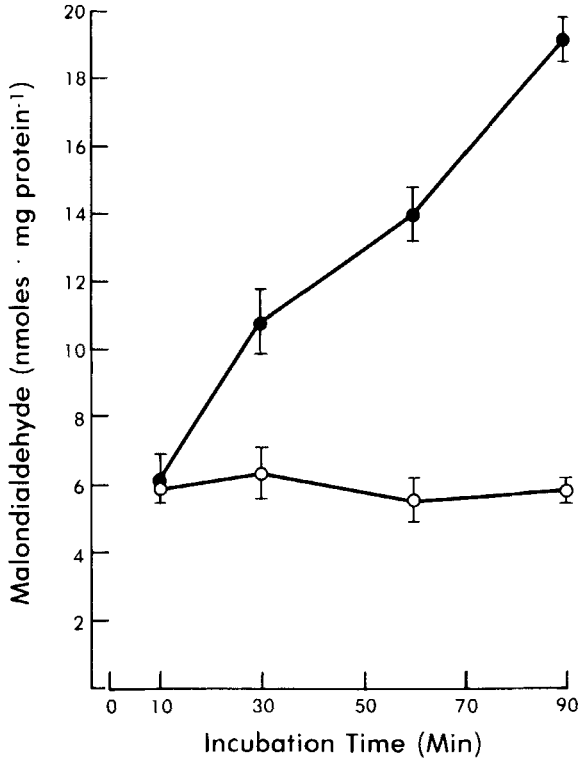

FIG. 2. Time course studies of xanthine oxidase-ferric ion-stimulated lipid peroxidation (measured by malondialdehyde formation) in rat brain cortical slices. Each point is a mean value of at least four cortical slices (± S.E.M.). (○), Krebs-Ringer; (●), xanthine oxidase system.

Effects of free radicals on the degradation of membrane phospholipid and the release of free fatty acids

Free radicals, generated from the xanthine oxidase-Fe^{3+} system, caused a decrease in total phospholipid content of synaptosomal membranes (Fig. 3). This phospholipid degradation process was seen after 30 min of incubation with xanthine oxidase-Fe^{3+}. It progressed further after a longer period of incubation. After 90 min of incubation, the membrane phospholipid content was decreased by approximately 50%.

After the incubation with either Krebs-Ringer buffer or the xanthine-oxidase-Fe^{3+} system for 60 min at 37°C, the separated free fatty acids obtained from synaptosomes were analyzed for fatty acid composition by gas chromatography. A significant increase was observed in the free fatty acids of the xanthine oxidase-Fe^{3+}-treated synaptosomes, especially arachidonic acid (20:4), oleic acid (18:1), linoleic acid (18:2), and docosahexaenoic acid (22:6). The levels of palmitic acid (16:0) and stearic acid (18:0) were not increased by free radicals (Table 4). The saturated, monounsaturated, and PUFA contents of control synaptosomes were 9.8,

TABLE 2. *Effects of xanthine oxidase-Fe^{3+} system on lipid peroxidation of subcellular fractions of rat brain cortical slices*

Subcellular fraction	MDA (nmol·mg protein^{-1}) Control	Xanthine oxidase-Fe^{3+}	% Increase
Mitochondria	1.6	4.1	155
Synaptosomes	3.0	6.9	133
Microsomes	3.1	5.4	73
Myelin	3.4	9.4	176
Nuclei	4.1	7.7	85

Subcellular fractions were incubated with xanthine-oxidase-Fe^{3+} at 37°C for 30 min. The incubation mixtures were then used for malondialdehyde assays. These data were obtained from a dose curve with various protein concentrations of the subcellular fractions. The increment of MDA content in both control and experimental subcellular fractions was linear at the range of 0.1–1.0 mg protein.

TABLE 3. *Effects of free radical scavengers and antioxidants on lipid peroxidation induced by xanthine oxidase-Fe^{3+} system*

	MDA content (% of control)	
	Homogenate	Synaptosomes
Xanthine oxidase-Fe^{3+} in Krebs-Ringer	100	100
+ Ethanol (50 mM)	110	113
+ Aspirin (0.1 mM)	105	105
+ Superoxide dismutase (10 μg/ml)	93	93
+ Catalase (0.8 mg/ml)	70	63
+ Dimethylsulfoxide (10%)	68	90
+ Chlorpromazine (1.0 mM)	37	27
Xanthine oxidase-Fe^{3+} in 10% DMSO	100	100
+ Indomethacin (0.1 mM)	118	93
+ Dimethylfuran (10 mM)	97	75
+ α-tocopherol (0.1 mM)	89	76
+ Thiopental (1.0 mM)	82	98
+ Butylated hydroxytoluene (0.1 mM)	44	30

The antioxidant or free radical scavenger was preincubated with homogenates or synaptosomes at 37°C for 30 min followed by the incubation of xanthine oxidase-Fe^{3+} at 37°C for an additional 30 min. The absolute values for tissue homogenates and synaptosomes were 13.7 ± 0.8 (12) nmol/mg protein and 6.9 ± 0.3 (6) nmol/mg protein, respectively.

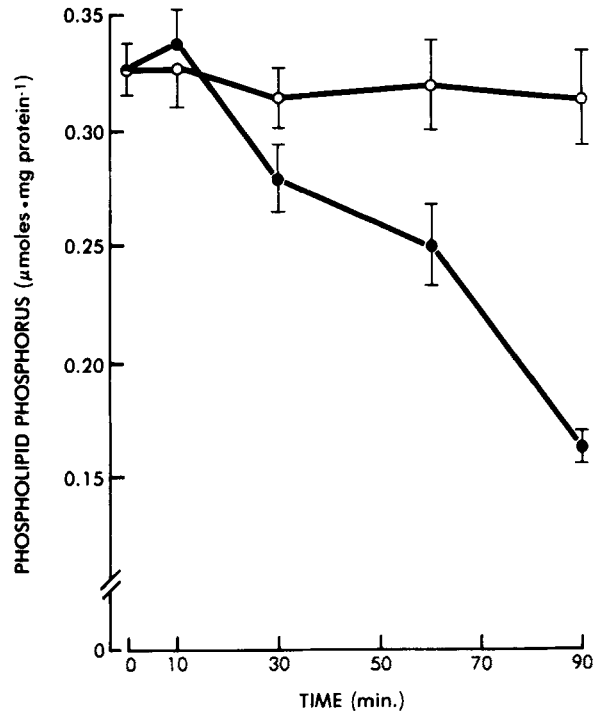

FIG. 3. Time course studies of membrane phospholipid degradation of synaptosomes induced by xanthine oxidase-Fe^{3+}. The purified synaptosomal membrane (0.1 mg protein) was incubated either with Krebs-Ringer solution (control) or with xanthine oxidase-Fe^{3+} for various times. The phospholipids were extracted by 3 ml chloroform and methanol 2:1, v/v) plus 3 ml of water. The organic phase was dried under N_2 and was used for the measurement of inorganic phosphorus. The reduced phosphomolybdate complex is measured at 820 nM. (○), control Krebs-Ringer; (●), xanthine oxidase-Fe^{3+}. Mean ± S.E.M.

2.7, and 2.5 μg/mg protein, respectively for control synaptosomes, and 11.5, 8.2, and 11.8 μg for xanthine oxidase-Fe^{3+}-treated synaptosomes. This represents a fourfold increase in the unsaturated fatty acid content of xanthine oxidase-Fe^{3+}-treated synaptosomes.

DISCUSSION

Using the rat brain single first cortical slice as the bioassay system for brain swelling *in vitro*, we demonstrated that xanthine oxidase in the presence of ferric ions, a free-radical-generating system, stimulated brain swelling. This cytotoxic brain edema is characterized by the increase of lactic acid and the disruption of cellular membranes, as judged by the

TABLE 4. *Effects of xanthine oxidase-Fe^{3+} system on free fatty acid composition of synaptosomal membrane of cortical slices*

Fatty acid	Control (μg fatty acid/mg protein)	Xanthine oxidase-Fe^{3+} (μg fatty acid/mg protein)
12:0	0.09 ± 0.02	0.08 ± 0.04
14:0	0.35 ± 0.09	0.48 ± 0.03
16:0	4.09 ± 0.55	5.06 ± 0.48
18:0	4.15 ± 0.45	4.80 ± 0.39
18:1	2.70 ± 0.85	8.19 ± 1.21[a]
18:2	0.27 ± 0.05	0.61 ± 0.12[a]
20:4	1.10 ± 0.28	4.58 ± 0.80[a]
20:5	0.06 ± 0.02	0.07 ± 0.03
22:6	Trace	0.69 ± 0.10[a]

Results are expressed as mean ± S.E.M. Data were obtained from three individual studies.
[a] $P < 0.01$, Student's *t*-test.

enhancement of membrane lipid peroxides, the release of unsaturated free fatty acids, and the decrease in overall phospholipid content.

The participation of various species of free radicals related to membrane lipid peroxidation was further examined by using scavengers and antioxidants. Our data indicate that catalase inhibited xanthine oxidase-induced lipid peroxidation in both homogenate and synaptosomes, suggesting the direct involvement of hydrogen peroxide. Superoxide radical was apparently not involved, as its specific scavenger, superoxide dismutase, was inactive. These findings differ somewhat from the recent observation of Fridovich and Porter, who found that cooxidation of arachidonic acid and xanthine oxidase in micelles required both $O_2^{\cdot -}$ and H_2O_2 (Fridovich and Porter, 1981). Furthermore, the involvement of other radical species including hydroxy radicals ($\cdot OH$) and singlet oxygen ($^1O_2$) cannot be excluded in our studies because the specificity of the other radical scavengers and antioxidants is not well defined. Our present studies also indicate that, besides catalase, chlorpromazine was the most effective water-soluble antioxidant against lipid peroxidation. The inhibitory effect of chlorpromazine on the xanthine oxidase system was similar to that observed on ischemia-induced lipid peroxidation (Smith et al., 1980). However, the lipid-soluble barbiturate thiopental was inactive in our free radical system, which differs from the studies of ischemia-induced lipid peroxidation (Smith et al., 1980). Present studies indicate that free-radical-induced lipid peroxidation may be associated with cellular injury of brain slices; however, the exact role of lipid peroxides on the induction of edema is not clear. Recently Stacey and Klaassen have proposed that lipid peroxidation is unlikely to be totally responsible for the loss of cell viability of incubated hepatocytes with toxic chemicals such as sodium iodoacetamide and diethyl maleate (Stacey and Klaassen, 1981). These observations warrant further studies of the injury of brain cells associated with lipid peroxidation.

The formation of lipid peroxides was stimulated by free radicals in mitochondria, synaptosomes, myelin, nuclei, and microsomes. Among these subcellular fractions, myelin, mitochondria, and synaptosomes were the most susceptible structures. The susceptibility of synaptosomes, mitochondria, and myelin might be related to the higher content of PUFA in their membrane phospholipids (O'Brien and Sampson, 1965; Sharma, 1977; Deutsch and Kelly, 1981). The specificity of free radical effects on neurons, glia, or capillaries was not studied in the present experiments. Further investigations of *in vivo* preparations and of cell cultures will clarify such effects. These are actively under study in our laboratory.

Of special interest is the fourfold increase in free unsaturated fatty acids concomitant with overall phospholipid degradation induced by free radicals in synaptosomal membranes. Arachidonic acid (20:4), oleic acid (18:1), and docosahexaenoic acid (22:6) were the major fatty acids released from phospholipids. Among these fatty acids, arachidonic acid was one of the major species released from phospholipids. A significant quantity of arachidonic acid was also released from membrane phospholipid when the brain cells were subjected to various pathological insults (Bazan, 1970; Marion and Wolfe, 1979; Gardiner et al., 1981). Arachidonic acid, once released from the cellular membrane, is rapidly converted either by cyclooxygenase to prostaglandins and radicals, or by lipoxygenase to form hydroxy fatty acids and slow-reacting substances (Samuelsson et al., 1979). Prostaglandins and intermediate radical species are known to be involved in the inflammatory process in various biological systems (Higgs et al., 1979; Kuehl et al., 1979). The precise role of prostaglandins in the development of brain edema is not clear at present. However, the cyclooxygenase inhibitors aspirin and indomethacin were not effective in ameliorating tissue swelling and lipid peroxide formation induced by PUFA and by free radicals (Chan and Fishman, 1978). Therefore, the prostaglandins derived from the cyclooxygenase pathway may not be involved. However, the role of lipoxygenase products could not be excluded. We reported earlier that free arachidonic acid, which was capable of inducing swelling of cortical slices (Chan and Fishman, 1978; Chan et al., 1980*a*) inhibited Na^+,K^+-ATPase activity in brain (Chan et al., 1980*b*). This inhibitory process may be mediated by superoxide and other free radicals (Hexum and Fried, 1979; Chan and Fishman, 1980). These data lead us to suggest a cascade or cyclic interaction between free radicals and arachidonic acid in neuronal tissue. Free radicals, hydrogen peroxide, and lipid peroxides, once produced by the xanthine oxidase-Fe^{3+} system, may activate the membrane phospholipase A_2, which causes the release of arachidonic acid and other PUFA at the C-2 positions of phospholipids. On the other hand, synaptosomal phospholipase C, which hydrolyzes phosphatidylinositol forming diacylglycols, may also be activated by free radicals and lipid peroxides. It was shown recently that the release of diacylglycerols and arachidonic acid from phosphatidylinositol require activities of both phospholipases C and A in synaptosomes (Der and Sun, 1981). However, the possible role of diacylglyceride lipase, which degraded platelet diacylglyceride to monoacylglyceride and arachidonic acid, could not be excluded (Bell et al., 1979). Thus, the effects of free radicals on the phospholipases require further investigation. We speculate that the activation of membrane phospholipase(s) by free radicals may initiate the release of arachidonic acid and the for-

mation of free radicals in neuronal tissue. These processes may underly the molecular mechanism of the formation of brain edema in various pathological conditions.

Acknowledgments: This work was supported by NIH Brain Edema Clinical Research Center grant NS-14543. We thank Bob McClary for editorial assistance.

REFERENCES

Abood L. G., Salem N., Macneil M., and Butler M. (1978) Phospholipid changes in synaptic membrane by lipolytic enzymes and subsequent restoration of opiate binding with phosphatidylserine. *Biochim. Biophys. Acta* **530**, 35–46.

Ames B. N. (1966) Assay of inorganic phosphate, total phosphate and phosphatases, In *Methods in Enzymology Vol. VIII* (Colowick S. P. and Kaplan N. O., eds), pp. 115–118. Academic Press, New York.

Barber A. A. (1966) Lipid peroxidation in rat tissue homogenates: Interaction of iron and ascorbic acid as the normal catalytic mechanism. *Lipids* **1**, 146–151.

Bazan N. G. (1970) Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. *Biochim. Biophys. Acta* **218**, 1–10.

Bell R. L., Kennerly D. A., Stanford N., and Majerus P. W. (1979) Diglyceride lipase: A pathway for arachidonate release from human platelets. *Proc. Natl. Acad. Sci. USA* **76**, 3238–3241.

Chan P. H. and Fishman R. A. (1978) Brain edema: induction in cortical slices by polyunsaturated fatty acids. *Science* **201**, 358–360.

Chan P. H. and Fishman R. A. (1980) Transient formation of superoxide radicals in polyunsaturated fatty acid-induced brain swelling. *J. Neurochem.* **35**, 1004–1007.

Chan P. H., Fishman R. A., Lee J. L., and Candelise L. (1979) Effects of excitatory neurotransmitter amino acids on swelling of rat brain cortical slices. *J. Neurochem.* **33**, 1309–1315.

Chan P. H., Fishman F. A., Lee J. L., and Quan S. C. (1980*a*) Arachidonic acid-induced swelling in incubated rat brain cortical slices: Effect of bovine serum albumin. *Neurochem. Res.* **5**, 629–640.

Chan P. H., Quan S. C., and Fishman R. A. (1980*b*) Inhibition of rat brain (Na^+ + K^+)-ATPase by polyunsaturated fatty acids. *Trans. Am. Soc. Neurochem.* **11**, 120.

Cotman C. W. and Matthews D. A. (1971) Synaptic plasma membranes from rat brain synaptosomes: Isolation and partial characterization. *Biochim. Biophys. Acta* **249**, 380–394.

Dahle L. K., Hill E. G., and Holman R. T. (1962) The thiobarbituric acid reaction and the autooxidations of polyunsaturated fatty acid methyl esters. *Arch. Biochem. Biophys.* **98**, 253–261.

Demopoulos H. B., Flamm E. S., Seligman M. L., Mitamauir J. A., and Ransohoff J. (1979) Membrane perturbations in central nervous system injury: Theoretical basis for free radical damage and a review of the experimental data, in *Neural Trauma* (Popp J., Bourke R. S., Nelson L. R., and Kimelberg H. K., eds), pp. 63–78. Raven Press, New York.

Der O. M. and Sun G. Y. (1981) Degradation of arachidonoyl-labeled phosphatidylinositols by brain synaptosomes. *J. Neurochem.* **36**, 355–362.

Deutsch J. W. and Kelly R. B. (1981) Lipids of synaptic vesicles: Relevance to the mechanism of membrane fusion. *Biochemistry* **20**, 378–385.

Fridovich S. E. and Porter N. A. (1981) Oxidation of arachidonic acid in micelles by superoxide and hydrogen peroxide. *J. Biol. Chem.* **256**, 260–265.

Gardiner M., Nilsson B., Rehncrona S., and Siesjö B. K. (1981) Free fatty acids in the rat brain in moderate and severe hypoxia. *J. Neurochem.* **36**, 1500–1505.

Harvey J. A. and McIlwain H. (1969) Electrical phenomena and isolated tissues from the brain, in *Handbook of Neurochemistry, Vol. 2* (Lajtha A., ed), pp. 115–136. Plenum Press, New York.

Hexum T. D. and Fried R. (1979) Effects of superoxide radicals on transport (Na + K) adenosine triphosphatase and protection by superoxide dismutase. *Neurochem. Res.* **4**, 73–82.

Higgs G. A., Moncada S., and Vane J. R. (1979) The role of arachidonic acid metabolites in inflammation. *Adv. Inflam. Res.* **1**, 419–430.

Kellogg E. W. and Fridovich I. (1975) Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. *J. Biol. Chem.* **250**, 8812–8817.

Kimelberg H. K. and Papahadjopoulos E. (1972) Phospholipid requirements for (Na^+ + K^+)-ATPase activity: Head-group specificity and fatty acid fluidity. *Biochim. Biophys. Acta* **282**, 277–292.

Kuehl F. A., Humes T. C., Torchiana M. L., Ham E. A., and Egan R. W. (1979) Oxygen-centered radicals in inflammatory process. *Adv. Inflam. Res.* **1**, 419–430.

Marchbanks R. M. (1975) Cell-free preparations and subcellular particles from neural tissues, in *Practical Neurochemistry*, (McIlwain H., ed), pp. 208–242. Churchill Livingstone, New York.

Marion J. and Wolfe L. S. (1979) Origin of the arachidonic acid released post-mortem in rat forebrain. *Biochim. Biophys. Acta* **574**, 25–32.

Mead J. F. (1976) Free radicals mechanisms of lipid damage and consequences for cellular membranes, in *Free Radicals in Biology Vol. 1* (Pryor W. A., ed), pp. 51–68. Academic Press, New York.

O'Brien J. S. and Sampson E. L. (1965) Fatty acid and fatty aldehyde composition of the major brain lipids in normal gray matter, white matter, and myelin. *J. Lipid Res.* **6**, 545–551.

Pederson T. C. and Aust S. D. (1973) The role of superoxide and singlet oxygen in lipid peroxidation promoted by xanthine oxidase. *Biochem. Biophys. Res. Commun.* **52**, 1071–1078.

Rehncrona S., Smith D. S., Åkesson B., Westerberg E., and Siesjö B. K. (1980) Peroxidative changes in brain cortical fatty acids and phospholipids, as characterized during Fe^{2+}-and ascorbic acid-stimulated lipid peroxidation *in vitro*. *J. Neurochem.* **34**, 1630–1638.

Samuelsson B., Hammarstrom S., and Borgeat P. (1979) Pathway of arachidonic acid metabolism. *Adv. Inflam. Res.* **1**, 405–411.

Sharma O. P. (1977) Peroxidation of rat brain mitochondrial lipids. *J. Neurochem.* **28**, 1377–1379.

Smith D. S., Rehncrona S., and Seisjö B. (1980) Inhibitory effect of different barbiturates on lipid peroxidation in brain tissue *in vitro:* Comparison with the effects of promethazine and chlorpromazine. *Anethesiology* **53**, 186–194.

Stacey N. H. and Klaassen C. D. (1981) Inhibition of lipid peroxidation without prevention of cellular injury in isolated rat hepatocytes. *Toxicol. Appl. Pharmacol.* **58**, 8–18.

Tappel A. L. (1980) Measurement of and protection from *in vivo* lipid peroxidation, in *Free Radicals in Biology Vol. 4* (Pryor W. A., ed), pp. 1–47. Academic Press, New York.