K. Kogure, K.-A. Hossmann, B. K. Siesjö and F. A. Welsh (Eds.),
Progress in Brain Research, Vol. 63.

Cellular and molecular effects of polyunsaturated fatty acids in brain ischemia and injury

Pak Hoo Chan, Robert A. Fishman, Susan Longar, Sylvia Chen and Albert Yu

Brain Edema Clinical Research Center, Department of Neurology, University of California School of Medicine, San Francisco, CA 94143, USA

Introduction

It is generally recognized that the level of free polyenoic fatty acids (PUFAs) is very low in brain in situ. These PUFAs are usually localized at C-2 position of glycerol backbond in membrane phospholipids. It has been demonstrated that free PUFAs, especially arachidonic acid and docosahexaenoic acid are rapidly released following ischemia, electroconvulsive seizures and various pathological insults (Bazan, 1970, 1971; Bazan et al., 1971; Marion and Wolfe, 1979; Gardiner et al., 1981; Rehncrona et al., 1982; Tang and Sun, 1982; Yoshida et al., 1982; Chan et al., 1983a; Yoshida et al., 1983). The release of and accumulation of PUFAs arachidonic acid in particular is due the activation of phospholipase A_2 (Edgar et al., 1982; Au et al., 1985). It has been suggested that phosphatidylinositol-specific phospholipase C is also involved in the formation of diacylglycerols, the metabolic precursors of arachidonic acid (Sun et al., 1984). Other inositol phospholipids including phosphatidylinositol-4-phosphate and phosphatidyl-4,5-biphosphate may also be involved in the production of diacylglycerols (Nishizuka, 1984).

The role of polyunsaturated fatty acids in brain edema and injury

Free PUFAs, and arachidonic acid in particular, have both physiological and pathological effects on cellular systems. It has been demonstrated that free arachidonic acid readily intercalates into the membrane and produces significant changes in the packing of the lipid molecules (Usher et al., 1978; Klausner et al., 1980a, b). PUFA-induced membrane fluidity has been associated with the stimulation of chloride transport in corneal epithelium (Schaeffer and Zadunaisky, 1979) and it enhanced activities of both membrane-associated adenylate cyclase and guanylate cyclase (Wallach and Pastan, 1976; Anderson and Jaworski, 1977; Asakawa, 1978). Furthermore, free arachidonic acid has been postulated as a potential second messenger since it causes Ca^{2+} metabolization (Cheah, 1981) and activates Ca^{2+}-dependent protein kinase C (Mc Phail et al., 1984). These physiological functions of arachidonic acid are summarized in Fig. 1. Although the physiological effects of unsaturated fatty acids on various cellular systems are well documented, little is known about the pathological effects of high level PUFAs on CNS metabolism and function.

Using single cortical slices of the rat brain as a bioassay system in vitro, we have demonstrated that PUFAs, including linoleic acid (18:2), linolenic acid (18:3) and arachidonic acid (20:4) are potent inducers of cellular (cytotoxic) edema (Chan and Fishman, 1978; Chan et al., 1980). In these experiments, the edematous brain slices were characterized by increased sodium and decreased potassium contents. The [^{3}H]inulin space, an extracellular space marker, was also decreased significantly. Cel-

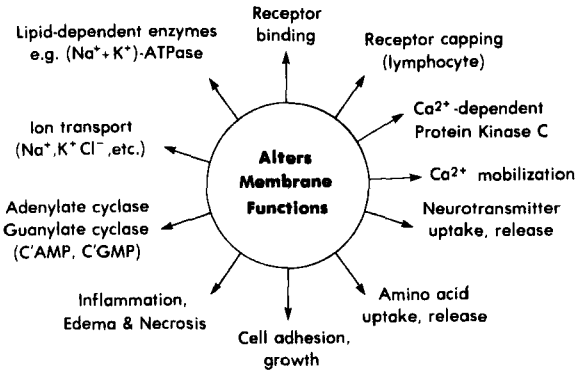

Fig. 1. Physiological and pathological functions of arachidonic acid in cellular systems.

lular metabolism was also affected: lactic acid levels were increased and high energy nucleotides were reduced. Both saturated fatty acids and monounsaturated fatty acids were not effective (Table 1).

From our in vitro studies we have concluded that high levels of PUFAs from damaged cell membrane are associated with the development of cellular brain edema. We have further studied the local effects of arachidonic acid on vasogenic edema in rats in vivo. Animals were infused with 0.05 mmol of saturated, monounsaturated or polyunsaturated fatty acids into thalamus, and the edema and cation levels were studied at 24 hours following the injection. Among the fatty acids, arachidonic acid was the most potent fatty acid in inducing cerebral edema concomitant with the increase in sodium and decrease in potassium contents (Table 2). A lesser degree of change in hemispheric water, sodium and potassium contents was seen after injection of linolenic acid (18:3). Oleic acid (18:1), palmitic acid (16:0) and nonanoic acid (9:0) were ineffective in inducing brain edema. Furthermore, arachidonic acid and linolenic acid caused 3-fold and 2-fold increases, respectively in [^{125}-I]albumin space at 24 hours in the injected hemisphere when compared with Krebs-Ringer (Table 2). Oleic acid, palmitic acid and nonanoic acid were not effective in altering blood-brain barrier permeability (Chan et al., 1983b).

Gross examination revealed that the hemispheres injected with PUFA were swollen, with evidence of compression of the ipsilateral ventricle. The edema dissected along the ipsilateral corpus callosum, expanding it two to three times its normal width. Microscopic examination of hemispheres injected with PUFA revealed a consistently abnormal area

TABLE 1

The role of polyunsaturated fatty acids in the development of cellular edema in brain slices

Fatty acid	Swelling (%)	Inulin space (%)	Lactic acid (mmol/kg dry wt)	Na^+ (mEq/kg dry wt)	K^+ (mEq/kg dry wt)
Control	11.3	46.11	236	599	393
Palmitic acid (16:0)	9.4	46.12	258	838[b]	387
Oleic acid (18:1)	11.0	41.07[b]	278[a]	694[a]	419
Linoleic acid (18:2)	32.5[b]	33.47[b]	519[b]	1340[b]	92[b]
Linolenic acid (18:3)	26.0[b]	42.88[b]	391[b]	949[b]	188[b]
Arachidonic acid (20:4)	35.2[b]	32.6[b]	527[b]	1239[b]	140[b]
Docosahexaenoic acid	33.5[b]	41.47[b]	543[b]	1215[b]	77[b]

Data obtained from Chan and Fishman, 1978.
[a] $p < 0.02$, [b] $p < 0.001$, Student's t test.

TABLE 2

The role of polyunsaturated fatty acids in the development of vasogenic brain edema

Fatty acid	Water content (%)	[^{125}I]BSA space (%)	Na^+ (mEq/kg dry wt)	K^+ (mEq/kg dry wt)
Krebs-Ringer (Control)	79.5 ± 0.2	2.04 ± 0.1	241 ± 1	556 ± 2
Nonanoate (9:0)	79.1 ± 0.07	21.7 ± 0.02	248 ± 1	554 ± 1
Palmitate (16:0)	79.3 ± 0.07	2.30 ± 0.13	255 ± 1[a]	546 ± 1[a]
Oleate (18:1)	79.1 ± 0.08	2.40 ± 0.01[a]	265 ± 1[a]	536 ± 2[a]
Linolenate (18:3)	79.8 ± 0.04	4.25 ± 0.02[a]	278 ± 1[a]	320 ± 1[a]
Arachidonate (20:4)	80.6 ± 0.2[a]	6.68 ± 0.29[a]	284 ± 1[a]	258 ± 2[a]

Data obtained from Chan et al., 1983b.
All values are expressed as means ± SE of the mean.
[a] $p < 0.01$, compared to control group, Student's t test.

of neuropil 3 to 4 mm in diameter and centered at the tip of the needle (Fig. 2). The neuropil in this region stained poorly and contained cystic spaces. Ventriculocisternal perfusion with arachidonic acid produced a characteristic acute lesion consisting of ependymal disruption, subependymal edema, and local blood-brain barrier disruption as evidenced by Evans blue leakage from periventricular capillaries. Ventriculocisternal perfusion with Krebs-Ringer, or nonanoic acid did not cause any apparent periventricular injury (Martin et al., 1982). These morphological data indicate that PUFAs are potent inducers of membrane damage and may cause development of vasogenic edema.

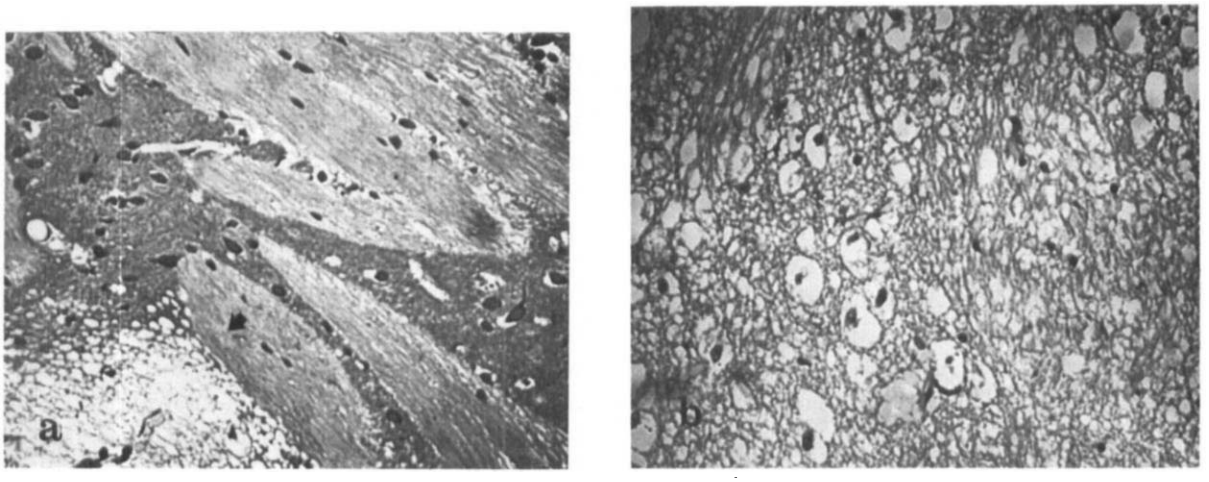

Fig. 2. Effects of arachidonic acid on microscopic morphology of corpus striatum at 24 hours. (a) Control animals injected with Krebs-Ringer buffer, arrow indicates area damaged by needle insertion; (b) animals injected with arachidonic acid (both × 160). (Data from Chan et al., 1983b.)

Molecular mechanisms of arachidonic acid induced brain edema

The molecular mechanisms of PUFA-induced cellular edema were studied further in our in vitro cortical slices system. The amphiphilic nature of the PUFA lead us to raise the question whether the development of brain edema is due to the alteration of membrane integrity by arachidonic acid. Our approach to this problem was to study neurotransmitter uptake and $(Na^+ + K^+)$-ATPase activity which are known to be associated with membrane functions. We have demonstrated that the addition of arachidonic acid (0.5 mM) caused a significant reduction of high affinity uptake of neurotransmitters GABA (γ-aminobutyric acid) and glutamate in brain slices and in synaptosomes (Chan et al., 1983c). However, the uptake of α-aminoisobutyric acid (AIBA), the non-metabolized amino acid, was not inhibited by arachidonic acid. The ID_{50} values for glutamic acid and GABA were 3.3×10^{-5} M and 4.1×10^{-5} M respectively, in synaptosomal preparations. This inhibitory effect of GABA uptake was partly counteracted by the membrane-stabilizing antioxidant, α-tocopherol (0.1 mM). These data suggest that 20:4-induced membrane perturbations play an important role in affecting GABA uptake. It has been shown by others that free arachidonic acid also stimulates the release of amino acids from synaptosomes (Rhoads et al., 1983a, 1983b). In order to address the question regarding the specificity of cellular vulnerability in various types of brain cells, we have recently developed primary cell cultures of both astrocytes and neurons from rats (Yu et al., 1985). Our preliminary studies have shown that arachidonic acid (0.01 mM) caused a significant reduction in GABA uptake in GABAergic neuronal cultures. The uptake of GABA in primary astrocytes was not affected. Furthermore, arachidonic acid also stimulates the release of prelabelled [^{3}H]GABA from primary neurons (Yu et al., 1985). These pilot experiments suggest that primary cell cultures are useful tools to study the functional vulnerability of various cell types in the central nervous system affected by arachidonic acid and other cellular factors.

Besides the uptake and release of neurotransmitter amino acids, we have also studied the effect of PUFAs on membrane-bound enzyme $(Na^+ + K^+)$-ATPase. Sodium arachidonate (20:4) and docosahexaenoate (22:6) were more effective in the reduction of $(Na^+ + K^+)$-ATPase in synaptosomes (Chan et al., 1983c). The inhibition of $(Na^+ + K^+)$-ATPase activity by arachidonic acid is temperature dependent. The transition temperature of 20°C and 25°C was obtained from the Arrhenius plot for control and 20:4-treated enzyme, respectively. These data indicate that the lipid fluidity of the membrane was affected by 20:4. The mechanism of the change is not clear since the incorporation of PUFA into membranes would increase membrane fluidity. These opposing findings suggest that the PUFA metabolites, especially oxygen radicals may be involved in causing the alteration of membrane integrity and fluidity.

Arachidonic acid as a precursor of oxygen radicals

Arachidonic acid, once released from membrane phospholipids, is readily metabolized to prostaglandins, thromboxanes via cyclooxygenase or to hydroxy, hydroperoxy fatty acids and leukotrienes (Gaudet and Levine, 1979; Ellis et al., 1983; Moskowitz et al., 1984). Prostaglandins and leukotrienes are involved in numerous physiological and pathological functions such as inflammation, membrane permeability, chemotaxis, microcirculating response, receptor binding, platelet aggregation and hormonal effects (Fig. 3) (Kuehl and Egan, 1980; Samuelsson, 1983). Furthermore, it has been proposed that a highly reactive oxygen radical species is formed during the conversion from PGG_2 to PGH_2 or from 5-hydroperoxyeicosatetroneic acid (5HPETE) to leukotriene A_4 (LTA_4) (Samuelsson et al., 1979; Kuehl and Egan, 1980). These active oxygen radical species further participate in radical propagation and membrane protein and lipid peroxidation, which are associated with degrading of membrane integrity (Mead, 1976; Freeman and Crapo, 1982). However, there was no direct

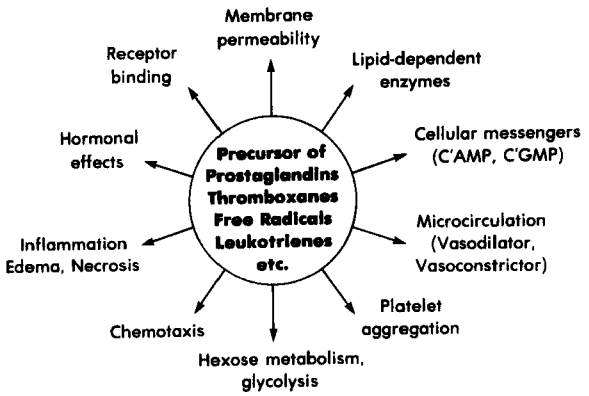

Fig. 3. Physiological and pathological functions of arachidonic acid metabolites in cellular systems.

experimental evidence regarding the existence of oxygen-free radicals associated with arachidonic acid metabolism in the central nervous system (CNS). Our experimental approach to this problem was to investigate the possible formation of oxygen radicals and lipid peroxidation in arachidonic acid incubated CNS tissues. We have demonstrated that linoleic acid, linolenic acid, arachidonic acid and docosahexaenoic acid caused both significant increases in superoxide ($O_2 \cdot -$) formation and lipid peroxidation as measured by thiobarbituric acid reactive malondialdehyde (Table 3) (Chan and Fishman, 1980). Palmitic acid and oleic acid were not effective.

TABLE 3

Superoxide radicals and lipid peroxidation in arachidonic acid incubated brain slices

Fatty acid	$O_2 \cdot -$ (nmol NBF/mg protein)	Malondialdehyde (nmol/mg protein)
Krebs Ringer (Control)	9.7 ± 0.6	5.3 ± 0.5
Palmitic acid (16:0)	10.1 ± 0.5	N.D.
Oleic acid (18:1)	9.0 ± 1.2	6.6 ± 0.9
Linoleic acid (18:2)	13.1 ± 1.3[a]	11.2 ± 0.6[a]
Linolenic acid (18:3)	14.4 ± 0.9[a]	N.D.
Arachidonic acid (20:4)	12.9 ± 0.7[a]	13.5 ± 0.7[a]
Docosahexaenoic acid (22:6)	N.D.	12.8 ± 1.7[a]

Data obtained from Chan and Fishman, 1980.
Mean ± SE. [a] $p < 0.001$, Students t test. N.D. not determined.

The role of oxygen radicals in membrane phospholipid degradation and brain edema

Lipid peroxidation has been shown to occur in cellular membranes of cerebral tissues in vitro (Kovachich and Mishra, 1980; Dirks and Faiman, 1982) and in vivo (Kogure et al., 1982; Watson et al., 1984). It has been hypothesized to be associated with membrane damage and increased endothelial permeability following ischemia and injury (Demopoulos et al., 1978, 1979, 1982; Willmore and Rubin, 1982; Yoshida et al., 1982). Little is known regarding the direct effect of oxygen radicals on cellular injury in CNS. Using an enzymatic free radical generating system including xanthine oxidase, hypoxanthine and ferric ions, we have studied the effects of oxygen radicals in cellular edema, lipid peroxidation, membrane phospholipid degradation and free fatty acid levels in rat brain slices. We have demonstrated that oxygen radicals produced by this enzymatic system stimulated both tissue swelling and malondialdehyde formation in incubated brain slices (Chan et al., 1982). Furthermore, membrane phospholipids were degraded concomitantly with the increases with PUFA in free fatty acid pool, as identified and measured by high performance liquid chromatography (HPLC) and capillary gas chromatography. The increased level of PUFA could be partially blocked by phospholipase A_2 inhibitors chloroquine and mepecrine, indicating that phospholipase A_2 was involved in free radical-mediated fatty acid release processes (Au et al., 1985). These in vitro data suggest a cyclic route for both arachidonic acid and oxygen radicals in cerebral tissue which may play a role in the delayed development

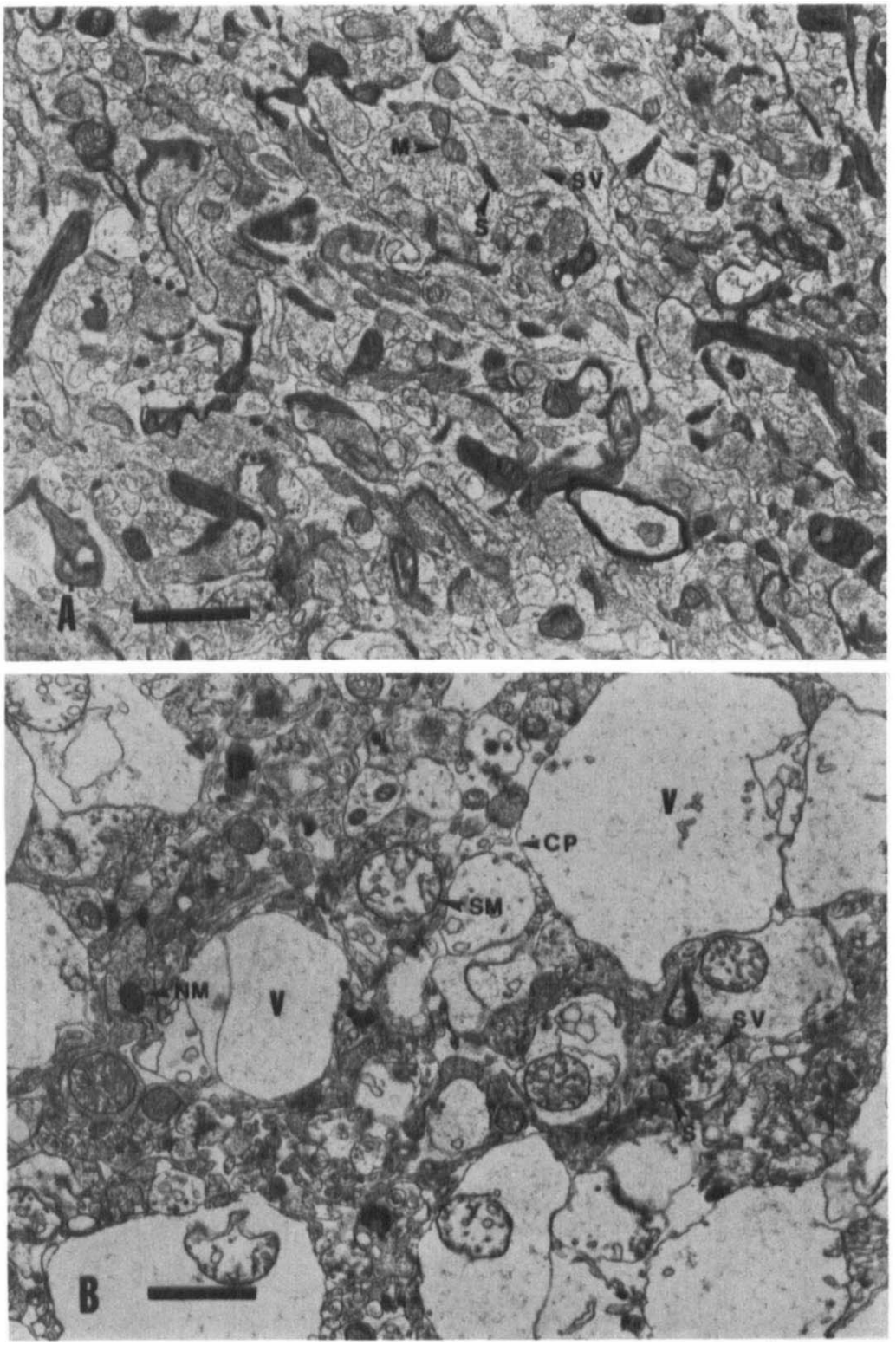

Fig. 4. Effects of oxygen radicals on ultrastructure of cortical slices. (A) Slice incubated in cortical medium for 60 min; M, normal mitochondria; S, synapse; SV, synaptic vesicles; scale bar, 5 μm. (B) Slice incubated in xanthine oxidase-Fe^{3+} system; CP, swollen cellular processes; NM, normal mitochondria; SM, swollen mitochondria; S, synapse; SV, synaptic vesicles; V, vacuole; scale bar, 5 μm. (Data from Chan et al., 1984a.)

of brain edema following in vivo injury. The ultrastructure of the molecular layer of slices exposed to the free radical generating system for 30 and 60 min was dominated by many obviously swollen cellular processes. These were generally empty, save for a few remnants of what appeared to be membranes and abnormal mitochondria. Most mitochondria were grossly swollen, with loss of normal electron density and fragmentation of cristae (Fig. 4).

The in vivo cerebral effects of oxygen radicals were further studied. Xanthine oxidase system was infused into caudate putamen followed by rapid in situ freezing (Pontén et al., 1973). Brain water and sodium content increased significantly concomitantly with decreased potassium content at 24 hours and 48 hours after the infusion. The degree of brain edema and injury depends on the dose of xanthine oxidase. Morpholgical studies further demonstrated that oxygen radicals damage both endothelial cells of blood-brain barrier as well as neurons and glia. The time course of cellular injury and edema was similar to that of the edema induced by cold injury (Chan et al., 1983) or by intracerebral infusion of arachidonic acid (Chan et al., 1983; Chan and Fishman, 1984). Staining of edematous region with fluorescent Evans blue was noted at 2 hours but little or no extravasation of Evans blue was observed at 24 hours or later, indicating that endothelial cell damage, perhaps mediated by free radicals, is an early event (Chan et al., 1984). Biochemical analysis of oxygen-injured caudate putamen further indicated the high level of free fatty acids, especially arachidonic acid and docosahexaenoic acid (Chan and Fishman, 1985a). These data thus support our in vitro studies that oxygen radicals stimulate the release of unsaturated fatty acids from membrane phospholipids. We have hypothesized that the arachidonic acid oxygen radical cycle may play an important role in cellular damage and the development of edema and necrosis following brain ischemia and injury (Chan and Fishman, 1985a) (Fig. 5).

Our studies have suggested that PUFAs, especially arachidonic acid, play a key role in membrane damage and the development of edema following ischemia and injury. The availability of residual oxygen during incomplete ischemia as well as the abundant oxygen during reperfusion will convert arachidonic acid to oxygen radicals (oxygen radicals could also be formed from disrupted mitochondria electron transport chain) which may then activate the membrane phospholipase A_2 (Chan and Fishman, 1985b). The latter step will generate more PUFAs (Fig. 5). However, this general hypothesis does not address the question of selective cellular vulnerability (e.g. neurons vs. glia, GABAergic neurons vs. glutamatergic neurons etc.) following ischemia and reperfusion. The use of various primary neuronal and glial cell cultures may be useful to address these questions.

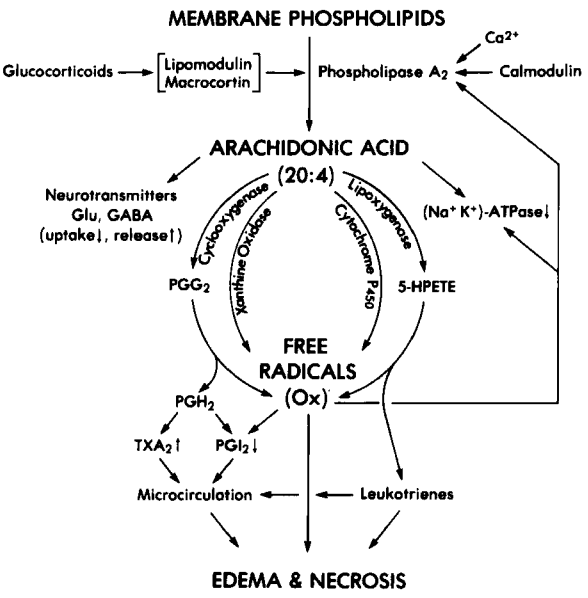

Fig. 5. Biochemistry of brain ischemia and injury.

Acknowledgements

This study was supported by USPHS Grant NS-14543. The assistance of Sheryl Colwell in the preparation of the manuscript is gratefully acknowledged.

References

Anderson, W.B. and Jaworski, C.J. (1977) Modulation of adenylate cyclase activity of fibroblasts by free fatty acids and phospholipids. *Arch. Biochem. Biophys.*, 180: 374–383.

Asakawa, T., Takenoshita, M., Uchida, S. and Tanaka, S. (1978) Activation of guanylate cyclase in synaptic plasma membranes of cerebral cortex by free fatty acids. *J. Neurochem.*, 30: 161–166.

Au, A.M., Chan, P.H. and Fishman, R.A. (1985) Stimulation of phospholipase A_2 activity by oxygen-derived free radicals in isolated brain capillaries. *J. Cell. Biochem.*, 27: 449–453.

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

Bazan, N.G. (1971) Changes in free fatty acids of the brain by drug-induced convulsions, electroshock and anesthesia. *J. Neurochem.*, 18: 1379–1385.

Bazan, N.G., Bazan, H.E.P., Kennedy, W.G. and Joel, C.D. (1971) Regional distribution and rate of production of free fatty acids in rat brain. *J. Neurochem.*, 18: 1387–1393.

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. and Fishman, R.A. (1982) Alterations of membrane integrity and cellular constituents by arachidonic acid in neuroblastoma and glioma cells. *Brain Res.*, 248: 151–157.

Chan, P.H. and Fishman, R.A. (1984) The role of arachidonic acid in vasogenic brain edema. *Fed. Proc.*, 43: 210–213.

Chan, P.H. and Fishman, R.A. (1985a) Free fatty acids, oxygen free radicals and membrane alterations in brain ischemia and injury. In: F. Plum and W. Pulsinelli (Eds.), *Cerebrovascular Diseases* (14th Research Princeton Conference). Raven Press, New York, pp. 161–169.

Chan, P.H. and Fishman, R.A. (1985b) In: A. Lajtha (Ed.), *Handbook of Neurochemistry*, Vol. 10. Plenum Publ. Corp., New York, pp. 153–174.

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

Chan, P.H., Yurko, M. and Fishman, R.A. (1982) Phospholipid degradation and cellular edema induced by free radicals in brain cortical slices. *J. Neurochem.*, 38: 525–531.

Chan, P.H., Longar, S. and Fishman, R.A. (1983a) Phospholipid degradation and edema development in cold-injured rat brain. *Brain Res.*, 277: 329–337.

Chan, P.H., Fishman, R.A., Caronna, J., Schmidley, J.W., Prioleau, G. and Lee, J. (1983b) Induction of brain edema following intracerebral injection of arachidonic acid. *Ann. Neurol.*, 13: 625–632.

Chan, P.H., Kerlan, R. and Fishman, R.A. (1983c) Reductions of γ-aminobutyric acid and glutamate uptake and $(Na^+ + K^+)$-ATPase activity in brain slices and synaptosomes by arachidonic acid. *J. Neurochem.*, 40: 309–316.

Chan, P.H., Fishman, R.A., Schmidley, J.W. and Chen, S.F. (1984a) Release of polyunsaturated fatty acids from phospholipids and alteration of brain membrane integrity by oxygen-derived free radicals. *J. Neurosci. Res.*, 12: 595–605.

Chan, P.H., Schmidley, J.W., Fishman, R.A. and Longar, S.M. (1984b) Brain injury, edema and vascular permeability changes induced by oxygen-derived free radicals. *Neurology*, 34: 315–320.

Cheah, A.M. (1981) Effect of long chain unsaturated fatty acids on the calcium transport of sarcoplasmic reticulum. *Biochim. Biophys. Acta*, 648: 113–119.

Demopoulous, H.B., Yoder, M., Gutman, E.G., Seligman, M.L., Flamm, E.S. and Ransohoff, J. (1978) The fine structure of endothelial surfaces in the microcirculation of experimentally injured feline spinal cords. *Scan. Electr. Microsc.*, 2: 667–682.

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

Demopoulos, H.B., Flamm, E.S., Seligman, M.L. and Pietronigro, D.D. (1982) Oxygen free radicals in central nervous system ischemia and trauma. In: A. P. Autor (Ed.), *Pathology of Oxygen*. Academic Press, London, pp. 127–155.

Dirks, R.C. and Faiman, M.D. (1982) Free radical formation and lipid peroxidation in rat and mouse cerebral cortex slices exposed to high oxygen pressure. *Brain Res.*, 248: 355–360.

Edgar, A.D., Strosznajder, J. and Horrocks, L.A. (1982) Activation of ethanolamine phospholipase A_2 in brain during ischemia. *J. Neurochem.*, 39: 1111–1116.

Ellis, E.F., Wright, K.F., Wei, E.P. and Kontos, H.A. (1983). Cyclooxygenase products of arachidonic acid metabolism in cat cerebral cortex after experimental concussive brain injury. *J. Neurochem.*, 37: 892–696.

Freeman, B.A. and Crapo, J.D. (1982) Biology of disease. Free radicals and tissue injury. *Lab. Invest.*, 47: 412–426.

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.

Gaudet, R.J. and Levine, L. (1979) Transient cerebral ischemia and brain prostaglandins. *Biochem. Biophys. Res. Commun.*, 86: 893–901.

Klausner, R.D., Bhalla, D.K., Dragsten, P., Hoover, R.L. and Karnovsky, M.J. (1980a) Model for capping derived from inhibition of surface receptor capping by free fatty acides. *Proc. Natl. Acad. Sci. USA*, 77: 437–441.

Klausner, R.D., Kleinfeld, A.M., Hoover, R.L. and Karnovsky, M.J. (1980b) Lipid domains in membranes. Evidence derived

from structural perturbations induced by free fatty acids and lifetime heterogeneity analysis. *J. Biol. Chem.*, 255: 1286–1296.

Kogure, K., Watson, B.D., Busto, R. and Abe, K. (1982) Potentiation of lipid peroxides by ischemia in rat brain. *Neurochem. Res.*, 7: 437–454.

Kovachich, G.B. and Mishra, O.P. (1980) Lipid peroxidation in rat brain cortical slices as measured by the thiobarbituric acid test. *J. Neurochem.*, 35: 1449–1452.

Kuehl, F.A. and Egan, R.N. (1980) Prostaglandins, arachidonic acid, and inflammation. *Science*, 210: 978–984.

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

Martin, N.A., Schmidley, J.W., Wissig, S.L., Fishman, R.A. and Chan, P.H. (1982) Cerebral injury and blood brain barrier disruption induced by arachidonic acid: histopathology. *Ann. Neurol.*, 12: 110.

Mc Phail, L.C., Clayton, C.C. and Snyderman, R. (1984) A potential second messenger role for unsaturated fatty acids: activation of Ca^{2+}-dependent protein kinase. *Science*, 224: 622–625.

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

Moskowitz, M.A., Kiwak, K.J., Herkimin, K. and Levine, L. (1984) Synthesis of compounds with properties of leukotrienes C_4 and D_4 in gerbil brains after ischemia and reperfusion. *Science*, 224: 886–889.

Nishizuka, Y. (1984) Turnover of inositol phospholipids and signal transduction. *Science*, 225: 1365–1370.

Pontèn, V., Ratcheson, R.A., Salford, L.G. and Siesjö, B.K. (1973) Optimal freezing conditions for cerebral metabolites in rats. *J. Neurochem.*, 21: 1127–1138.

Rehncrona, S., Westerberg, E., Åkesson, B. and Siesjö, B.K. (1982) Brain cortical fatty acids and phospholipids during and following complete and severe incomplete ischemia. *J. Neurochem.*, 38: 84–93.

Rhoads, D.E., Ockner, R.K., Peterson, N.A. and Raghupathy, E. (1983a) Modulation of membrane transport by free fatty acids: inhibition of synaptosomal sodium-dependent amino acid uptake. *Biochemistry*, 22: 1965–1970.

Rhoads, D.E., Osburn, L.D., Peterson, N.A. and Raghupathy, E. (1983b) Release of neurotransmitter amino acids from synaptosomes: enhancement of calcium-independent efflux by oleic and arachidonic acids. *J. Neurochem.*, 41: 531–537.

Samuelsson, B. (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science*, 220: 568–575.

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

Schaeffer, B.E. and Zadunaisky, J.A. (1979) Stimulation of chloride transport by fatty acids in corneal epithelium and relation to changes in membrane fluidity. *Biochim. Biophys. Acta*, 556: 131–143.

Sun, G.Y., Tang, W., Huang, S.F.-L. and Foudin, L. (1984) Is phosphatidylinositol involved in the release of free fatty acids in cerebral ischemia? In: J.E. Bleasdale, J. Eichberg and G. Hauser (Eds.), *Inositol and Phosphoinositides: Metabolism and Biological Regulation*. Humana Press, Clifton, New Jersey, in press.

Tang, W. and Sun, G.Y. (1982) Factors affecting the free fatty acids in rat brain cortex. *Neurochem. Int.*, 4: 269–273.

Usher, J.R., Epand, R.M. and Papahadjopoulos, D. (1978) The effect of free fatty acids on the thermotropic phase transition of dimyristoyl glycerophosphocholine. *Chem. Phys. Lipids*, 22: 245–253.

Wallach, D. and Pastan, I. (1976) Stimulation of guanylate cyclase of fibroblasts and free fatty acids. *J. Biol. Chem.*, 251: 5802–5809.

Watson, B.D., Busto, R., Goldberg, W.J., Santiso, M., Yoshida, S. and Ginsberg, M.D. (1984) Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. *J. Neurochem.*, 42: 268–275.

Willmore, L.J. and Rubin, J.J. (1982) Formation of malonaldehyde and focal brain edema induced by subpial of $FeCl_2$ into rat isocortex. *Brain Res.*, 246: 113–119.

Yoshida, S., Abe, K., Busto, R., Watson, B D., Kogure, K. and Ginsberg, M.D. (1982) Influence of transient ischemia on lipid-soluble antioxidants, free fatty acids and energy metabolites in rat brain. *Brain Res.*, 245: 307–316.

Yoshida, S., Inoh, S., Asano, T., Sano, K., Shimasaki, H. and Ueta, N. (1983) Brain free fatty acids, edema, and mortality in gerbils subjected to transient, bilateral ischemia, and effect of barbiturate anesthesia. *J. Neurochem.*, 40: 1278–1286.

Yu, A.C.H., Chan, P.H. and Fishman, R.A. (1985) Arachidonic acid effect on amino acid uptake in cultured brain cells. *Trans. Am. Soc. Neurochem.*, 16: 197.