Neurochemical Research, Vol. 20, No. 6, 1995, pp. 689–695

Effect of Endothelin-1 Induced Ischemia on Peroxidative Damage and Membrane Properties in Rat Striatum Synaptosomes

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(Accepted January 30, 1995)

Synaptosomes obtained from rat striata lesioned by central injection of endothelin-1 (ET-1) were analyzed for the levels of lipid peroxidation products, the susceptibility to lipid peroxidation, the phospholipid and free fatty acid composition and the activity of Na^+,K^+-ATPase one hour after ET-1 treatment. The intrastriatal injection of ET-1 promoted an increase of endogenous thiobarbituric reactive substances (TBARS), as index of free radical mediated lipid damage, and a greater susceptibility to iron/ascorbate-induced lipid peroxidation. The pattern of free fatty acids showed a significant decrease of arachidonic and docosahexaenoic acid consequent to ET-1 treatment. The analysis of lipid composition showed a significant loss of phospholipids: among phospholipid species, sphingomyelin and phosphatidylethanolamine plasmalogen were particularly reduced by ET-1 treatment. The activity of membrane-bound Na^+,K^+-ATPase was also significantly reduced in synaptosomes obtained from ET-1 lesioned striata. Taken together these results indicate a significant modification of synaptosomal membrane of ET-1 treated rat striata, possibly due to a free radical mediated damage.

KEY WORDS: Ischemia; lipid peroxidation; endothelin-1.

INTRODUCTION

Reduction in the blood flow to the brain leads to a rapid decrease in tissue oxygen supply (pO_2) which in turn causes disturbance in the energy balance and important modifications in the structural and functional properties of plasma membranes (1). In particular a decrease in tissue energy stores and an altered ionic homeostasis can increase the metabolism and reduce the synthesis of lipids leading to modifications of the membrane lipid composition. Among the events that can induce plasma membrane damage during ischemia, particular attention has been given to the generation of oxygen derived free radicals. Evidence on free radical production not only during reperfusion but also during the ischemic (2-4) or hypoxic (5) insult has been recently obtained by direct Electron Paramagnetic Resonance (EPR) measurements. Possible mechanisms leading to free radical generation in the ischemic period include the leakage of free radicals from normal mitochondrial electron transport chain, the metabolism of polyunsaturated FFA to eicosanoids via intermediates possessing a free radical character and the decrease in ascorbic acid (4,6), α-tocopherol (7) and gluthatione (8) brain levels during ischemia. On the other hand contradictory findings have

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0364-3190/95/0600-0689$07.50/0

been reported about the brain levels of the antioxidant enzymes superoxide dismutase (SOD), gluthatione-peroxidase and catalase during ischemia (9,10).

It was recently demonstrated that centrally administered Endothelin-1 (ET-1) causes ischemia-like lesions in rat brain (11). ET-1 injection into neostriatum produces a reduction of intrastriatal blood flow, a marked increase in extracellular levels of lactate and dopamine but not aspartate and glutamate. ET-1 is an endothelium-derived 21 aminoacid peptide that appears to be the most potent vasoconstrictor identified so far (12). Cerebral microvessels show marked sensitivity to ET-1 (13). In view of the potent and long lasting vasoconstrictor action of this peptide it is possible that an activation of ET-1 mechanisms in the brain may play a role in the pathogenesis of ischemic neuronal damage (14).

In this study we have investigated the modifications of striatal synaptosomal membranes 1 hour after ET-1 intrastriatal injection in one hemisphere using the controlateral striatum as non-lesioned control. In particular we evaluated the endogenous levels of thiobarbituric acid reactive substances (TBARS) as an index of free radical-mediated lipid damage induced by ET-1 treatment and the time course of iron/ascorbate induced lipid peroxidation as a measure of altered membrane resistance to an oxidative stress. We also investigated the possible ET-1 induced modifications of structural and functional properties of striatal synaptosomal membranes by evaluating membrane fluidity, lipid composition and the activity of Na^+,K^+,-ATPase in control and ET-1 treated hemispheres.

EXPERIMENTAL PROCEDURE

Materials. Analytical grade chemicals, distilled solvents and doubly distilled water were used. Ouabain, 1,6-diphenylhexatriene (DPH) were purchased from Sigma Chemical Co. ATP and the Test-Combination Kit for cholesterol determination were purchased from Boehringer.

Animal Model. Adult male Sprague Dawley rats (200-250 g) kept in standard housing conditions were used. The animals were anesthesized by halothane inhalation at induction and maintenance doses of 5% and 1% respectively. The head was fixed in a stereotaxic frame and a thin cannula (27 G) was implanted into right striatum to a depth of 4.3 mm from dura mater, 0.9 mm anterior and 2.5 lateral to the bregma. ET-1 (porcine, Peninsula Lab. UK), 0.8 μg dissolved in 0.8 μl of saline, was injected over a 4 min period at a rate of 0.2 μl/min using a microiniection pump (CMA/100, Carnegie Medicin). Anesthesia was then discontinued and the rats quickly recovered from anesthesia. 1 hour after ET-1 injection animals were sacrificed by decapitation, the brains removed and the right, lesioned, and left, non lesioned, striata dissected.

Membrane Preparation. Striatal synaptosomes were prepared by using the procedure of Whittaker and Barker (15) with minor modifications (16). The procedure was carried out on striata obtained from a pool of five rats. The basic steps for this procedure are (a) separation of the crude mitochondrial fraction from the sucrose homogenate of striatum by differential centrifugation and (b) separation of the crude mitochondrial fraction into myelin, synaptosomes and mitochondria by density gradient centrifugation applying a discontinuous sucrose gradient of three steps i.e. 0.32/0.8/1.2 M. Fractions obtained from the sucrose gradient were diluted to isotonicity, centrifuged, resuspended in a known volume of 0.32 M sucrose, and stored at −80°C until use.

Protein Determination. The protein content was determined by the method of Lowry et al. (17), using bovine serum albumin as standard.

Determination of Na^+,K^+-ATPase Activity. Ouabain-inhibited Na^+,K^+-ATPase (E.C. 3.6.1.3) was assayed according to Morgan et al. (18); briefly 50 μg of synaptosomal proteins were incubated in 1 ml 192 mM Tris HCl, 150 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, pH 7.4, 3.6 mM ATP, in presence or not of 1 mM ouabain for 20 min at 37°C. At the end of incubation 0.5 ml trichloroacetic acid 18% (w/v) was added. Samples were then centrifuged at 2000 *g* for 15 min; an appropriate amount of supernatant was used to determine hydrolized phosphorus according to Bartlett (19). Ouabain sensitive Na^+,K^+-ATPase activity was calculated subtracting the activity obtained in presence of ouabain from that obtained in absence of the inhibitor.

Peroxidation of Synaptosomes. The peroxidation of synaptosomes was induced by adding $FeSO_4$ (50 μM) and ascorbic acid (250 μM) to synaptosomal proteins resuspended in 1 mM Tris HCl, 154 mM NaCl pH 7.4. At different times, 0.5 ml of the mixture was withdrawn and the peroxidative reaction stopped by adding 0.1 ml of 5 mM EDTA. Peroxidation was evaluated by measuring the formation of thiobarbituric acid-reactive substances (TBARS), according to the method of Buege and Aust (20) and using malondialdehyde (MDA) standard obtained by periodic acid oxidation of deoxy-D-ribose according to Varavdekar and Saslaw (21). A molar extinction coefficient (E_{535}) of $1.54 \times 10^5 mol^{-1}cm^{-1}$ was used.

Measurements of the Fluorescence Anisotropy. Control and ET-1 treated membranes were labelled with DPH (16) by adding 2 μl of a 10^{-3} M stock solution of DPH in tetrahydrofuran to 150 μg of synaptosomal proteins resuspended in 2 ml of 1 mM Tris HCl, 154 mM NaCl, 0.1 M EDTA, pH 7.4. The mixtures were incubated 20 min at 37°C in order to obtain a uniform probe incorporation in the membrane. The r_s was calculated by simultaneously measuring the intensities of parallel and perpendicular polarized light, according to a method previously described (16). Fluorescence measurements were carried out in a Jasco 770 spectrofluorimeter equipped with a cuvette holder, the temperature being maintained by a Haake GD3 thermostatic circulating bath. The rate of temperature increase was 0.5 °C/min. The temperature was monitored with a Subline PT-100 digital thermometer.

Extraction and Lipid Analysis. Synaptosomal lipids were extracted by adding 4 volumes of chloroform/methanol 2:1 (v:v) to a synaptosomal aqueous suspension. The aqueous phase, obtained after centrifugation at 5000 RPM for 15 min, was further extracted with 2 volumes of chloroform/methanol/HCl 4:1:0.013 (by vol); the organic phase, obtained by centrifugation as described above, was neutralized with NH_4OH. The combined organic phases were taken to dryness and resuspended in a known volume of chloroform/methanol 2:1 (v:v). The total phospholipid phosphorus was determined according to Bartlett (19), and the total cholesterol content was analyzed applying the Test-Combination Kit for cholesterol (Chol-PAP).

Individual phospholipids were separated by two dimensional thin layer chromatography, applying a known amount of phospholipid on a 10×10 cm HPTLC Silica Gel 60 plates. Plates were eluted with

chloroform/methanol/acetic acid/ water 30:20:2:1 (by vol) in the first dimension, dried in a stream of air and exposed to 12 N HCl fumes (22). Following hydrolysis, fumes were blown by a stream of air and plates were eluted in the second dimension with chloroform/ methanol/ acetone/ acetic acid/ water 10:2:4:2:1 (by vol). Following chromatography the different classes of phospholipids were visualized by spraying the plates with a phosphorus specific mixture prepared according to Vaskovsky and Kostetsky (23). Plates were scanned with an IBM computer assisted CAMAG densitometer and the phospholipid classes evaluated on the basis of phospholipids spotted on the plate.

Free fatty acids in the lipid extract were methylated with ethereal diazomethane in the presence of 10% methanol at room temperature (24). After evaporation under nitrogen, 600 mg of silicic acid and 3 ml of a mixture of hexane and diethyl ether 20:1 (v:v) were added and the tube vortexed to extract the methylated free fatty acids. The precipitate, removed by centrifugation, was washed again with the same volume of the hexane diethylether mixture. The combined extracts were evaporated under nitrogen and resuspended in hexane. A DANI 3900 gas chromatograph (flame ionization detector) connected with a 3390 Hewlett-Packard integrator was used. The analysis was performed by injecting 1 µl of the sample in a 25 m SE 30 capillary column with a temperature protocol of 10 °C/min from 180 to 280°C.

Statistics. Results were statistically evaluated by Student's *t*-test.

RESULTS

The time course of lipid peroxidation in striatal synaptosomes induced by the Fe^{2+} (50 µM)/ Ascorbate (250 µM) system is shown in Fig. 1. Synaptosomes obtained from ET-1 injected striata showed a small but significant increase in the initial amounts of TBARS, 17.2 ± 2 nmol/mg protein, compared with synaptosomes obtained from control striata, 14 ± 2 nmol/mg protein ($p \leq 0.02$). When synaptosomes were incubated in the presence of the peroxidation couple (Fe/Ascorbate) and O_2, an "in vitro" condition which can simulate "in vivo" reperfusion and therefore introduction of oxidative conditions, a higher (compared with controls) accumulation of lipid peroxidation products was observed in membranes obtained from ET-1 injected striata (Fig. 1). Beside the differences observed in TBARS levels, the rate of peroxidation of ET-1 treated synaptosomes is greater than that of controls.

During this study the different synaptosomes were incubated for up to 30 min at 25 °C. Thus it was of special concern to examine whether MDA a final product of lipid peroxidation, is stable and totally recoverable under the experimental conditions in all the samples examined. For this purpose two different amounts (1.5 and 3 nmol) of standard MDA were added to synaptosomes and the concentration determined at zero time and following a 30 min of incubation at 25 °C. Full recovery of exogenous MDA was always obtained under our experimental conditions (data not shown).

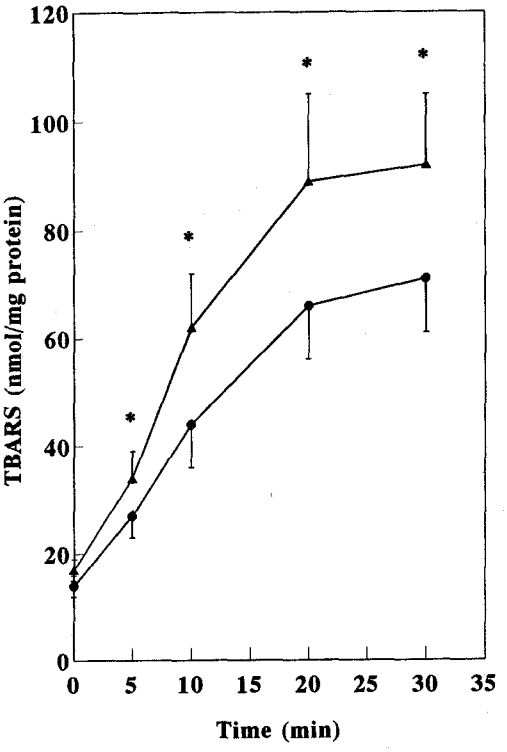

Fig. 1. Time course of iron/ascorbate-induced lipid peroxidation (measured as TBARS formed) in striatal synaptosomes of control (●) and ET-1 treated (▲) rats. Data are given as means (n = 6) ± SD. * $P \leq 0.02$ vs controls.

Synaptosomal membranes can be damaged as a consequence of the ET-1 treatment; membrane damage can include changes in membrane fluidity, permeability, lipid composition as well as modifications in the shape and function of intrinsic membrane proteins. Thus the fluorescence anisotropy of DPH, at different temperatures, was determined in control and ET-1 treated synaptosomes (Fig. 2). Values of r_s are similar in ischemic and control synaptosomes: at 37°C r_s values were 0.213 ± 0.016 and 0.203 ± 0.015 respectively. Although the r_s values were not significantly modified by the ischemic stress, it should be noted that the slope of the r_s vs temperature straight line observed in control synaptosomes 0.0029 ± 0.0006, was significantly ($p \leq 0.004$) greater than that observed in lesioned striatal membranes 0.00179 ± 0.0004; this indicates a greater constraint to the probe rotation in ischemic membranes possibly due to an increased "microviscosity" of the hydrophobic core of the bilayer.

The analysis of lipid composition (Fig. 3) demonstrated a small but significant ($p \leq 0.02$) loss of phospholipid in synaptosomes derived from ET-1 injected striata. The evaluation of single phospholipid species

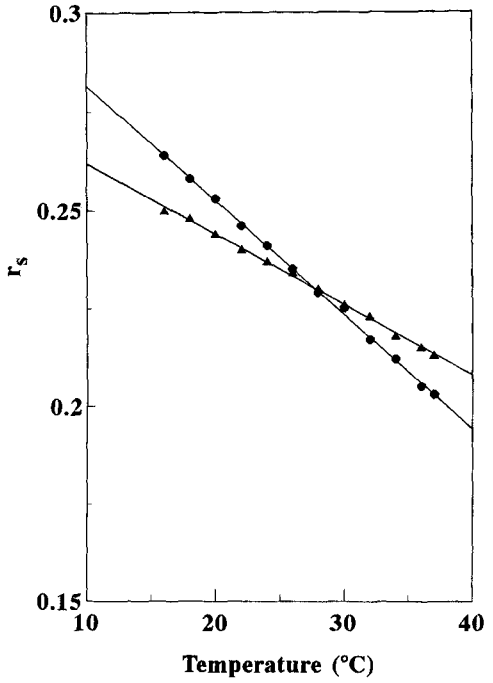

Fig. 2. Effect of ET-1 treatment on the r_s changes at different temperatures of the probe DPH incorporated into the control (●) and ET-1 treated (▲) synaptosomes. Each point is the mean of six synaptosomal preparations.

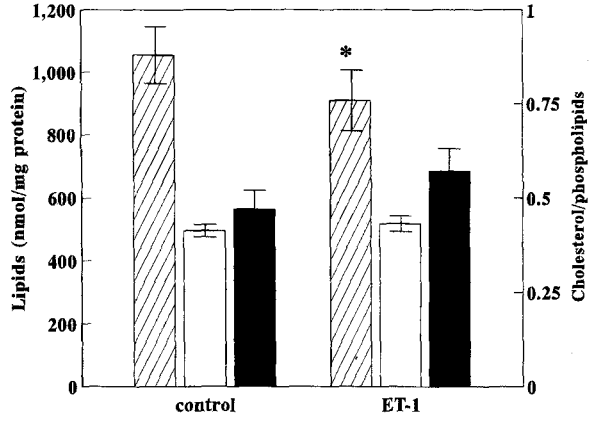

Fig. 3. Effect of ET-1 treatment on phospholipid (hatched bars) cholesterol (open bars) and C/PL mole ratio (filled bars) of synaptosomes derived from control and ET-1 treated rats. Data are given as means (n = 6) ± SD. * P≤0.02 vs controls.

(Fig. 4) demonstrated a significant decrease in sphingomyelin (p≤0.015) and ethanolamine plasmalogen (p≤0.015) concentrations in membranes obtained from ET-1 injected striata. Instead, the levels of membrane cholesterol were not modified by the ET-1 treatment (Fig. 3). Thus, the increase in cholesterol/phospholipid

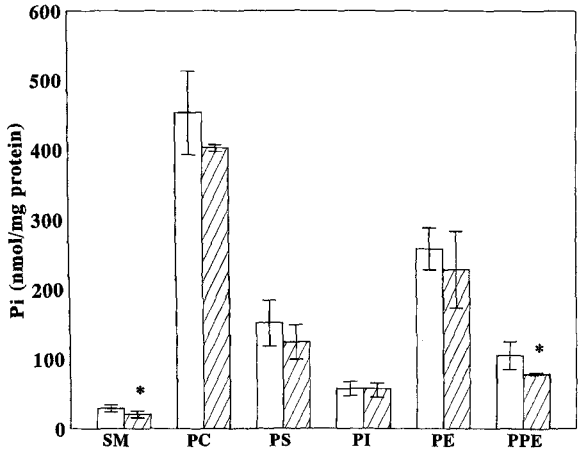

Fig. 4. Effect of ET-1 treatment on phospholipid molecular species of synaptosomes derived from control (open bars) and ET-1 treated (hatched bars) rats. Data are given as means (n = 6) ± SD. * P≤0.015 vs controls.

Table I. Effect of ET-1 Injection on the Molar Percent of Different Free Fatty Acids Associated with Striatal Synaptosomes

Fatty acid	Control	ET-1
16:0	23.9 ± 3.0	21.1 ± 2.5
18:0	24.7 ± 2.5	28.0 ± 3.0
18:1	12.9 ± 1.2	15.9 ± 1.3***
18:2	1.1 ± 0.1	1.3 ± 0.11
18:3	1.2 ± 0.4	2.1 ± 0.3**
20:4	13.0 ± 1.2	11.2 ± 1.5*
22:6	22.5 ± 1.5	20.0 ± 2.0*

Data are given as means (n = 6) ± SD.
*P ≤ 0.05; **P ≤ 0.02 ***P ≤ 0.002 vs controls.

molar ratio (C/PL) observed in ischemized striata (Fig. 3) is primarily due to the decrease in membrane phospholipid content.

The gas chromatographic profile of free fatty acids associated to synaptosomal membranes after ET-1 treatment (Table I) demonstrates an increase of oleic and linolenic acid molar fraction and a small but significant reduction of the molar fraction of polyunsatured free fatty acids (20:4 and 22:6) associated with ET-1 treated membranes. At last, membrane bound synaptosomal Na^+,K^+-ATPase was also affected by the intrastriatal injection of ET-1, as the specific activity was 240 ± 30 and 178 ± 30 nmol/min/mg protein ($p \leq 0.005$) in control and ET-1 injected striatal synaptosomes respectively.

DISCUSSION

ET-1 induced local lesion can be seen as a model of focal ischemia (11) sharing some neurohistochemical

features with striatal damage seen after transient forebrain ischemia. Striatal lesions caused by ET-1 local injection seem to develop rapidly within a day (11, 25) peaking 24 hours after ET-1 administration, and appear to be more closed to the site of injection rather than to striatal subregions of selective vulnerability (26). Interestingly, lactate was shown to increase hugly 45 min after ET-1 injection (11), as indirect sign of tissue hypoxia. Conversely, SOD activity started to increase significantly 60 min after ET-1 injection (25) suggesting the presence of oxidative processes probably linked to the maintenance of a residual blood flow (11). The presence of residual oxygen supply, can in fact favor mechanisms leading to the formation of highly active radical species, that have been proposed to occur during cerebral ischemia (4,5,27,28) *i.e* the metabolism of accumulated FFA (29) to eicosanoids via intermediates possessing a free radical character (30,31). This indirect evidence of derangement of tissue metabolism following ET-1 intrastriatal injection, find a further support in results obtained in the present experiment. Reduced phospholipid levels were found in ET-1 treated animals one hour after the injection. This result can be related to an increase of phospholipase activity and/or a decrease of phospholipid reacylating processes, as suggested in other models of brain ischemia (28,29,32,33). In addition the formation of lipid peroxidation products, demonstrated by the increase in endogenous TBARS levels, and the decrease in polyunsatured fatty acids, can contribute to the phospholipid loss on the basis of the inhibitory effect that low levels of hydroperoxides can exert on the reacylating reactions of synaptic membranes (34,35) and neuronal membranes (36). Among phospholipid species, sphingomyelin and phosphatidylethanolamine plasmalogen were particularly reduced. A decrease in sphingomyelin has also been reported in other models of cerebral ischemia (33,37). The observed greater reduction of phosphatidylethanolamine plasmalogen could be ascribed to a particular sensitivity of this molecule to the early stages of oxidative damage as demonstrated by Morand et al. (38). In addition an increase in extracellular concentrations of phosphoethanolamine has been reported to occur during transient focal ischemia in rabbit brain (39) and more specifcally in rat striatum (40). The extracellular appearance of phosphoethanolamine has been associated with the breakdown of the corresponding phospholipids and thus is indicated as a sensitive marker of plasma membrane disorders. Furthermore it has been demonstrated that extracellular levels of phosphoethanolamine also increase in other conditions involving cell membrane damage such as hypoglycemia (41) status epilepticus (42) and exocitotoxic drug administration (43).

As mentioned above, the presence of residual blood supply in the tissue, could increase the possibility of peroxidative processes, in contrast with the situation where the oxygen supply is completely abolished. In fact in ET-1 treated striata, we determined a small but significant increase in lipid peroxidation products, measured as endogenous TBARS; accordingly, differences observed in the molar ratio of polyunsatured to satured FFA and ET-1 treatment, may be the consequence of a greater intensity of peroxidative processes during the ischemic insult.

In vitro peroxidation was used to show whether ET-1 induced ischemia could induce changes in susceptibility to lipid peroxidation of striatal synaptosomes. Membranes obtained from ET-1 treated striata are much more sensitive to lipid peroxidation than those derived from control striata as shown by both the levels and the rate of TBARS production. These differences could reflect a depletion of the endogenous antioxidant protection during the ischemic insult (4,6-8). Also an altered lipid organization in the membrane could contribute to these differences: in fact the increase C/PL ratio, the modification of phospholipid pattern together with the increase in FFA characteristics of the ischemic stress (29), could determine a modified lipid distribution in the membrane, thus promoting the formation of free fatty acid enriched domains, as well as the presence of defect points in the membrane. This could facilitate the iron attack to PUFA and increase the rate of radical collisions responsible for propagation of peroxidative damage. The prevailing loss of phospholipids containing ethanolamine could also contribute to the increase in FFA peroxidation; this observation is made on the basis of our previous studies (44) in which we demonstrated that in model systems phosphatidylethanolamine strongly inhibits the iron induced peroxidation of arachidonic acid.

Finally ET-1 induced ischemia produces a significant reduction in membrane bound Na^+,K^+-ATPase activity determined in presence of exogenous ATP. The influence of the lipid environment and membrane fluidity on this enzyme is well known: in fact it has been demonstrated that the structural properties (45) and lipid composition (46) of synaptosomal membranes are essential for the activity of this enzyme; in addition low concentrations of free fatty acids have been shown to inhibit the Na^+,K^+-ATPase activity of rat brain (47,48). These observations together with the documented increase in FFA in transient focal ischemia may explain the decreased Na^+,K^+-ATPase activity observed in this study.

REFERENCES

1. Siesjo, B. K., and Wieloch, T. 1985. Molecular mechanisms of ischemic brain damage: Ca^{++} related events in cerebrovascular diseases. Pages 187–200 *in* Plum, F. and Pulsinelli, W. A. (eds), Cerebrovascular diseases. Raven Press N.Y.
2. Arroyo, C. M., Kramer, J. H., Leiboff, R. H., Hergner, G. W., Dickens, B. F., and Weglicki, W. B. 1987. Spin trapping of oxygen and carbon centered free radicals in ischemic canine myocardium. Free Rad. Med. Biol. 5:313–316.
3. Zini, I., Tomasi, A., Grimaldi, R., Vannini, V. and Agnati, L. F. 1992. Detection of free radicals during brain ischemia and reperfusion by spin trapping microdialysis. Neurosci. Lett. 138:279–282.
4. Flamm, E. S., Demopoulos, H. B., Seligman, M. L., Poser, R. G. and Ransohoff, J. 1978. Free radical in cerebral ischemia. Stroke 9:445–447.
5. Minyailenko, T. D., Pozharov, V. P. and Seredenko, M. M. 1990. Severe hypoxia activates lipid peroxidation in the rat brain. Chem. Phys. Lipids 55:25–28.
6. Pietronigro, D. D., Hovsepian, M., Demopoulos, H. B. and Flamm, E. S. 1983. Loss of ascorbic acid from injured feline spinal cord. J. Neurochem. 41:1072–1076.
7. 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.
8. Rehncrona, S., Folbergrova, J., Smith, D., and Siesjo, B. K. 1980. Influence of complete and pronounced incomplete cerebral ischemia and subsequent recirculation on cortical concentrations of oxidized and reduced glutathione in the rat. J. Neurochem. 34:477–486.
9. Mahadik, S. P., Makar, T. P., Murthy, J. N., Ortiz A., Wakade, C. G. and Karpiak, S. E. 1993. Temporal changes in superoxide dismutase, glutathione peroxidase and catalase levels in primary and peri ischemic tissue. Mol. and Chem. Neuropathol. 18:1–14.
10. Tokuda, Y., Uozumi, T. and Kawasaki, T. 1993. The superoxide dismutase activities of cerebral tissues, assayed by the chemioluminescence method, in the gerbil focal ischemia/reperfusion and global ischemia models. Neurochem. Int. 23:107–114.
11. Fuxe, K., Kurosawa, M., Cintra, A., Hallstrom, A., Goiny, M., Rosen, L., Agnati, F. L. and Ungerstedt, U. 1992. Involvement of local ischemia in Endothelin-1 induced lesions of the neostriatum of the anaesthesized rat. Exp. Brain Res. 88:131–139.
12. Yanagisawa, M., Kurihara, S., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Goto, K. and Masaki, T. 1988. A novel potent vasoconstrictor peptide produced by vascular endothelium. Nature 332:411–412.
13. Inoue, A., Yanagisawa, M., Kimura, S., Kasuya H., Miiyauchi T., Goto, K. and Masaki, T. 1989. The human endothelin family:three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. USA 86:2863–2867.
14. Cosentino, F., and Katusic S. Z. 1994. Does Endothelin-1 play a role in pathogenesis of cerebral vasospasm? Stroke 25:904–908.
15. Whittaker, V. P., and Barker, L. A. 1972. The subcellular fractionation of brain tissue with special reference to the preparation of synaptosomes and their component organelles. Pages 1–52 *in* Fried R.M. (ed) Methods of Neurochemistry. vol 2 Dekker New York.
16. Viani, P., Cervato, G., Fiorilli, A. and Cestaro, B. 1991. Age related differences in synaptosomal peroxidative damage and membrane properties. J. Neurochem. 56:253–258.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
18. Morgan, I. G., Wolfe, L. S., Mandel, S. and Gombos G. 1971. Isolation of plasma membranes from rat brain. Biochim. Biophys. Acta 241:737–751.
19. Bartlett, G. R. 1959. Phosphorus assay in column chromatography J. Biol. Chem. 234:466–488.
20. Buege, J. A. and Aust, S. D. 1978. Microsomal lipid peroxidation pages 302–351 *in* Fleischer S. and Packers L (eds.) Methods in Enzymology vol 52 Academic Press New York.
21. Varavdekar, V. S. and Saslaw, L. D. 1959. A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malondialdehyde thiobarbituric acid reaction J. Biol. Chem. 234:1945–1950.
22. Horrocks, L. A. 1968. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two dimensional thin layer chromatography. L. Lip. Res 9:469–472.
23. Vaskovsky, V. E. and Kostetsky, E. Y. 1968. Modified spray for the detection of phospholipids on thin layer chromatograms. J. Lip. Res. 396–398.
24. Yoshida, S., Inoh, S., Asano, T., Sano, K., Shimasaki, H. and Ueta, N. 1983. Brain fatty acids, edema, and mortality in gerbils subjected to transient, bilateral ischemia, and effect of barbiturate anesthesia. J. Neurochem. 40:1278–1286.
25. Biagini, G., Sala, D. and Zini, I. 1995. Diethyldithiocarbamate, a superoxide dismutase inhibitor couteracts the maturation of ischemic-like lesions caused by endothelin-1 intrastriatal injection. Neurosci Lett. in press.
26. Pulsinelli, W. A., Brierly, J. and Plum, F. 1982. Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann. Neurol. 11:491–498.
27. 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.
28. Braughler, J. M. and Hall, E. D. 1989. Central nervous system trauma and stroke. Free Rad. Biol. Med. 6:289–301.
29. Kubota, M., Kitahara, S., Narita, K., Tamura, A., Sano, K., Shimasaki, H. and Ueta, N. 1990. Changes in cerebral fatty acids and triacylglycerols in focal cerebral ischemia Int. J. Neurochem. 22: 1269–1272.
30. Siesjo, B. K., and Wieloch, T. 1983. Fatty acid metabolism and the mechanisms of ischemic brain damage. Pages 251–268 *in* Reivich M. and Hurtich, H.L. (eds.) Raven Press New York.
31. Chen, S. T., Hsu, C. Y., Hogan, E. L., Halushka, P. V., Linet, O. I., and Yatsu, F. M. 1986. Thromboxane, prostacyclin and leukotrienes in cerebral ischemia. Neurology 36:466–470.
32. Yoshida, S., Inoh, S., Asano, T., Sano, K., Kubota, M., Shimasaki, H. and Ueta, N. 1980. Effect of transient ischemia on free fatty acids and phospholipids in gerbil brain. Lipid peroxidation as possible cause of postischemic injury. J. Neurosurg. 53:323–331.
33. Enseleit, W. M., Domer, F. R., Jarrott, D. M., and Baricos, W. H. 1984. Cerebral Phospholipid content and Na^+,K^+-ATPase activity during ischemia and postischemic reperfusion in the Mongolian gerbil J. Neurochem. 43:320–327.
34. Zaleska, M. H. and Wilson, D. F. 1989. Lipid hydroperoxides inhibit reacylation of phospholipids in neuronal membranes. J. Neurochem 52:255–260.
35. Zaleska, M. H. and Wilson, D. F. 1992. Effect of hydroperoxy fatty acids on acylation and deacylation on arachidonoyl groups in synaptic phospholipids J. Neurochem. 58:107–115.
36. Alberghina, M., Lupo G. and Anfuso, C. D. 1993. Lipid peroxidation inhibits oleoyl-coA:1-acyl *sn* glycero-3-phosphocholine θ-acyltransferase in rats CNS axolemma enriched fractions. Neurochem. In. 23:229–237.
37. Yatsu, F. M. and Moss, S. A. 1971. Brain lipid changes following hypoxia. Stroke 2:587–293.
38. Morand, O., Zoeller, R. A., and Raetz, C. R. H. 1988. Disappearance of plasmalogens from membrane of animal cells subjected to photosensitized oxidation. J. Biol. Chem 263:11597–11606.
39. Hagberg, H., Lehmann, A., Sandberg, M., Nystrom, B., Jacobson, I. and Hamberger, A. 1985. Ischemia induced shift of inhibitory and excitatory amino acids from intra- to extracellular compartments. J. Cereb. Blood Flow Metabol. 5:413–419.

40. Uchiyama-Tsuyuki, Y., Araki, H., Yae, T. and Otomo, S. 1994. Changes in the extracellular concentrations of amino acids in the rat striatum during transient focal cerebral ischemia. J. Neurochem. 62:1074–1078.
41. Sandberg, M., Butcher, S.P. and Hagberg, H. 1986. Extracellular overflow of neuroactive amino acids during severe insulin induced hypoglycemia: in vivo dialysis of the rat hyppocampus. J. Neurochem. 47:178–184.
42. Lehmann, A., Hagberg, H., Jacobson, I. and Hamberger, A. 1985. Effects of status epilepticus on extracellular amino acids in the hyppocampus. Brain Res. 448:231–241.
43. Lehmann, A., Isacsson, H. and Hamberger, A. 1983. Effects of in vivo administration of kainic acid on the extracellular amino acid pool in the rabbit hyppocampus. J. Neurochem. 40:1314–1320.
44. Cervato, G., Viani, P., Masserini, M., Di Iorio, C., and Cestaro, B. 1988. Studies on peroxidation of arachidonic acid in different liposomes below and above phase transition temperature. Chem. Phys. Lipids 4:135–139.
45. Chong, P. L. G., Fortes, P. A. G., and Jameson D. M. 1985. Mechanisms of inhibition of Na^+,K^+-ATPase by hydrostatic pressure studied with fluorescent probes J. Biol. Chem. 260:14480–14490.
46. Sandermann, H. 1978. Regulation of membrane enzymes by lipids. Biochim. Biophys. Acta 515:209–237.
47. Chan, P. H., Kerlan, R. and Fishman, R. A. 1983. Reduction of γ-aminobutirric acid and glutamate and Na^+,K^+-ATPase activity in brain slices and synaptosomes by arachidonic acid. J. Neurochem. 40:309–316.
48. Swarts, H. G. P., Schuurmans Stekhoven, F. M. A. H. and De Pont J. J. H. H. M. 1990. Binding of unsatured fatty acids to Na^+,K^+-ATPase leading to inhibition and inactivation. Biochim. Biophys. Acta 1024:32–40.