Lipid Peroxidation-induced Changes in Physical Properties of Annular Lipids in Rat Brain Synaptosomal Membranes

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Abstract. The effects of lipid peroxidation (LPO) on the physical state (fluidity) of the rat brain synaptosomal lipid bilayer matrix and the annular lipid domains were investigated using the fluorescent probe pyrene. The parameters of pyrene fluorescence intensity $\alpha = I_1/I_M$ were measured at excitation wavelengths 280 nm and 340 nm (α_{280} and α_{340}), reflecting fluidity of lipid bilayer matrix and annular lipids, respectively. LPO induction was shown to result in changes of fluidity of both the bilayer and annular lipids. Upon reducing formation of LPO products by carnosine, fluidity changes of both the lipid bilayer matrix and annular lipids were diminished. Conformational changes of the annular lipid domain by LPO may therefore be considered as a possible cause of the functional changes in the receptor mediated responses and of the inactivation of membrane-bound enzymes by oxidative stress.

Key words: Annular lipids — Lipid peroxidation — Oxidative stress — Pyrene — Synaptosomal membrane

Introduction

Under normal physiological conditions characterized by a balance between oxidants and antioxidants, the level of lipid peroxidation (LPO) is usually very low. However, LPO may be increased in a variety of pathological situations such as aging, hypoxia, ischemia, inflammation, drug toxicity, etc., in which oxygen free radicals are well known to increase (Sies 1985; Yagi 1982). High LPO levels may occur also in brain pathology, e.g. epilepsy, Parkinson's disease or schizophrenia (Clausen 1984; Prilipko et al. 1987).

One of the main consequences of LPO processes in nerve cells may be changes of physico-chemical properties of the membrane lipid bilayer and thus

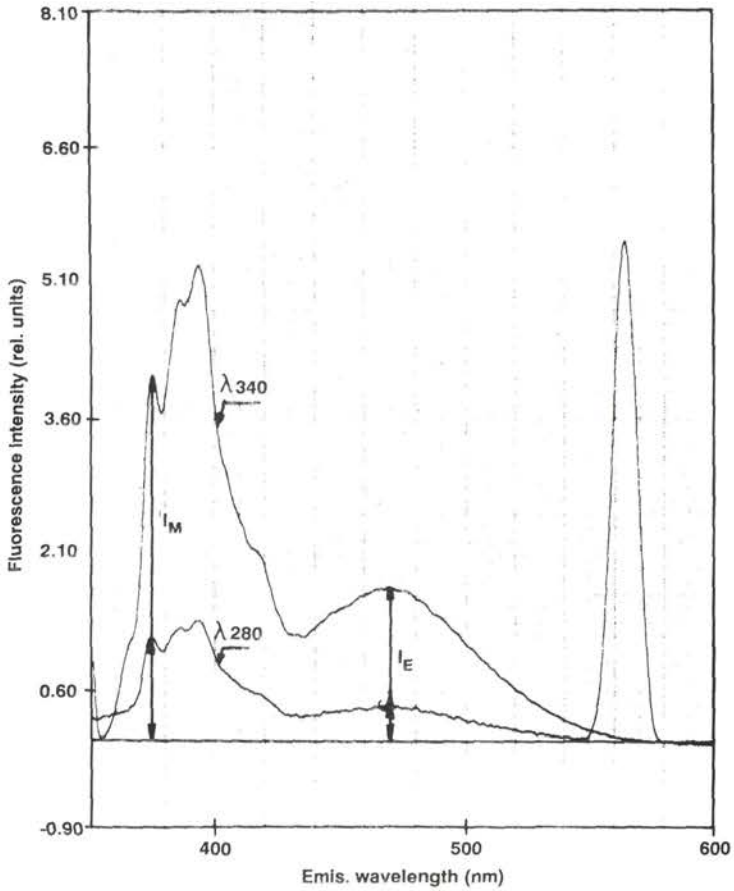

Fig. 1. The emission fluorescence spectrum of the pyrene fluorescent probe incorporated in the rat brain synaptosomal membrane. Excitation at 280 nm and 340 nm, temperature 25 °C. I_M = fluorescence intensity of pyrene molecule monomer, I_E = fluorescence intensity of pyrene molecule eximer.

altered membrane lipid-protein interactions. Therefore, LPO may affect the functional properties of membrane-bound receptors and enzymes (Raber et al. 1989; Yukawa et al. 1983).

LPO-induced changes of physico-chemical properties of the membrane lipid matrix as a whole are well-known fact. However, the pivotal role in the membrane lipid-protein interaction play the annular lipids. Their physical state in biomembranes, e.g. neuronal membranes, may be different from that of the lipid bilayer as a whole under normal conditions (Dergunov et al. 1982; Merevenskaya et al. 1986).

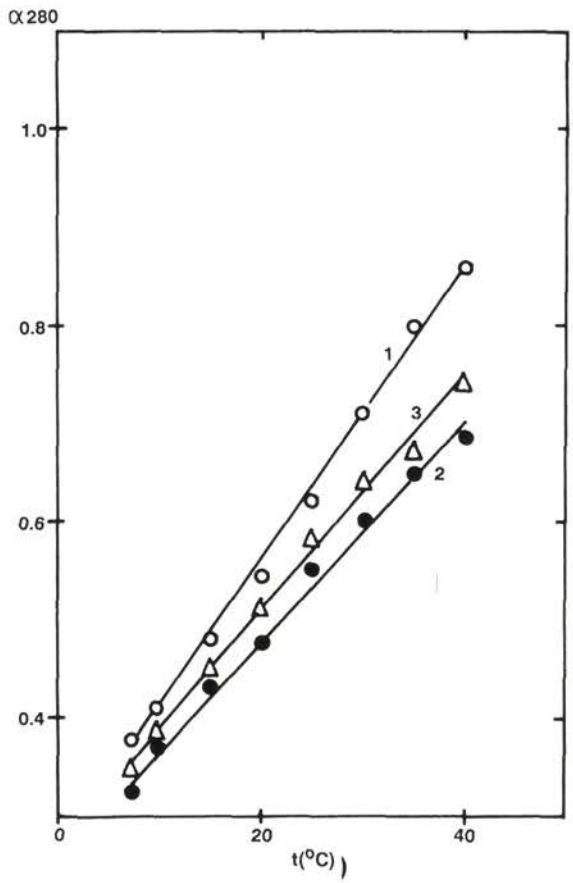

Fig. 2. The temperature dependence of the physical parameter $\alpha_{280} = I_{470}/I_{375}$ (excitation at 280 nm); rat brain synaptosomal membrane. 1 (○) control sample; 2 (●) after LPO activation by Fe^{2+} (10 μmol/l) and hydrogen peroxide (1 mmol/l); 3 (△) after LPO activation in the presence of carnosine (20 mmol/l).

Until recently, no evidence has been reported to show that the activation of LPO changes the physical properties of annular lipids in neuronal membranes; this would explain the membrane lipid-protein interaction disturbance observed as the result of oxidative stress-induced receptor injury and enzyme inactivation. This prompted us to study the effect of LPO activation on the physical properties of annular lipids in the rat brain synaptosomal membranes.

Table 1. The level formation of TBA-active products in the rat brain synaptosomes during LPO induction in the absence and in the presence of carnosine.

Sample	nmol MDA mg protein	LPO/Control
Control	2.0	
Fe^{2+} (10 μmol l) + H_2O_2 (1 mmol l)	23.6	11.8
Fe^{2+} (10 μmol l) + H_2O_2 (1 mmol l) + carnosine (20 mmol/l)	17.8	8.9

Materials and Methods

The synaptosomes were isolated from the gray matter of rat brain on a sucrose gradient according to Hajos (1975). Male Wistar rats (Velaz, Prague), aged 8 weeks, were used. The protein concentration was determined according to Bradford (1976). The suspension of isolated synaptosomes (final concentration 0.1 mg proteins per ml) was diluted by a medium of the following composition (in mmol/l): HEPES 20; NaCl 145; KCl 5; $NaHCO_3$ 5; $MgCl_2$ 1.3; glucose 10 (pH 7.4 at 37 °C). LPO was activated by Fe^{2+} (final concentration 10 μmol/l) and hydrogen peroxide (1 mmol l). In some samples LPO was inhibited by adding the water soluble antioxidant carnosine (20 mmol l) prior to LPO activation. The volume of any addition did not exceed 2 % of the total volume. All samples were incubated 30 min at 37 °C. Samples without LPO induction served as controls.

The level of LPO was determined according to Ohkawa et al (1979) by measuring of thiobarbituric acid (TBA) reactive compounds using a Shimadzu UV 2100 spectrophotometer (absorbance was measured at 532 nm). The obtained values were expressed as nmol malondialdehyde (MDA) per mg protein, using 1,2,3,3-tetraethoxypropane as a standard.

The physical state of total and annular lipids was determined according to Dergunov et al. (1982) using pyrene (20 μmol/l) as the fluorescent probe. The ratio of fluorescence intensities I_E / I_M (M = monomer, E = eximer of the probe) at the excitation wavelengths 280 nm and 340 nm respectively was determined as a parameter, reflecting the structural state of annular and total lipids.

Fluorescence measurements were performed in a thermostated cuvette (temperature range 5 — 40 °C) using a Shimadzu RF 5000 spectrofluorophotometer with the excitation and emission bandpass of 5 nm. The temperature accuracy in all measurements was ± 1 °C.

Chemicals: pyrene, HEPES, sucrose, glucose, L-carnosine, Coomasie Blue-G were from Serva, FRG; thiobarbituric acid, 1,1,3,3-tetraethoxypropane from Sigma, USA; all chemicals used were p.a. grade.

Results and Discussion

The physical state of the synaptosomal lipid bilayer as a whole and of the annular lipid domain was estimated using pyrene as a fluorescent probe which is spontaneously incorporated into the lipid region. Two forms of excited state

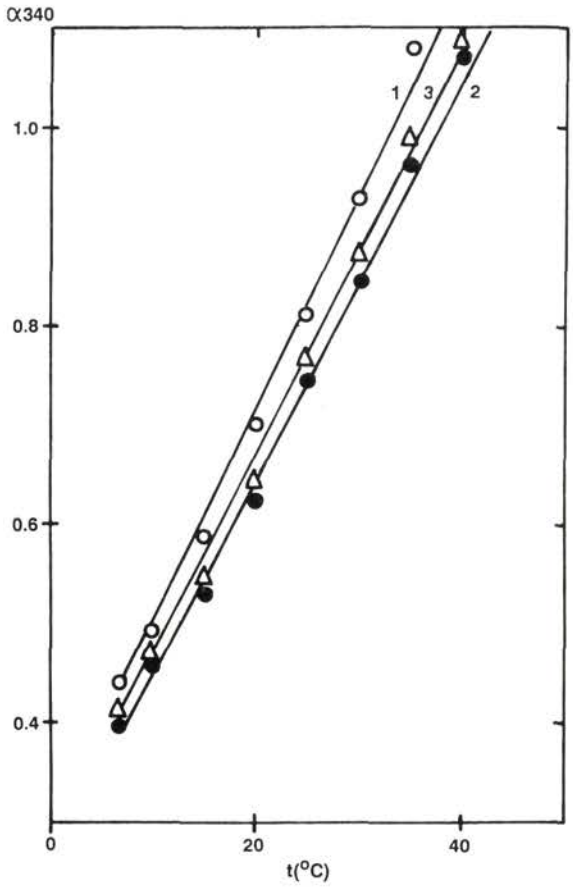

Fig. 3. The temperature dependence of the physical parameter $\alpha_{340} = I_{470}/I_{375}$ (excitation wavelength 340 nm); rat brain synaptosomal membrane. 1 (○) control sample; 2 (●) after LPO activation by Fe^{2+} (10 μmol/l) and hydrogen peroxide (1 mmol/l); 3 (△) after LPO activation in the presence of carnosine (20 mmol/l).

exist: a) monomer with a characteristic fluorescence emission spectrum of the multiplet state in the range of 340—400 nm; b) eximer, dimer of excited and nonexcited molecules with an emission maximum of 470 nm (Fig. 1). The eximer formation depends on the diffusion rate of pyrene molecules in the lipid bilayer, and thus it reflects the physical state (fluidity) of the membrane lipids. The parameter $\alpha = I_E/I_M = I_{470}/I_{375}$ was estimated as the ratio of fluorescence intensities of eximer at the emission wavelength 470 nm and that of monomer at 375 nm. At the excitation wavelength $\lambda_{ex} = 340$ nm, all pyrene molecules located

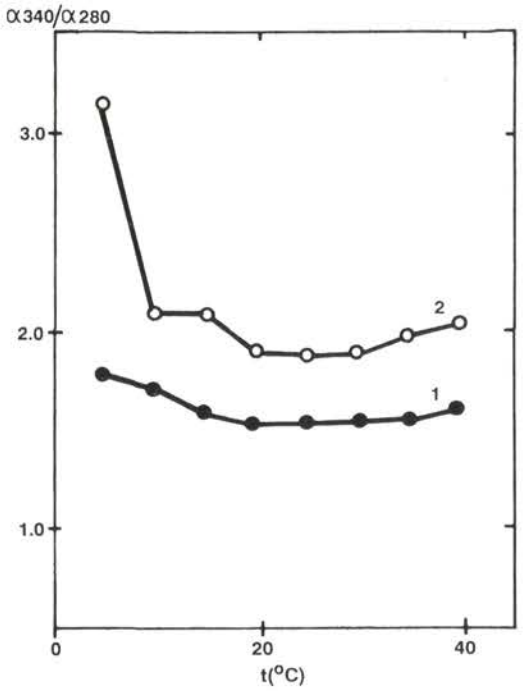

Fig. 4. The temperature dependences of the ratio $\alpha_{340}/\alpha_{280}$ 1 (●) control sample; 2 (○) after LPO activation (same as in Fig. 3).

in the membrane lipid region can be observed. Hence, α_{340} reflects total lipid fluidity. On the other hand, excitation at $\lambda_{ex} = 280$ nm induces fluorescence of pyrene molecules located at distances not exceeding the Forster radius of nonradiant energy transfer from a protein aromatic aminoacid residue to pyrene. As can be seen from Fig. 1, this concerns a minor part of all pyrene molecules located close the membrane bound-proteins. Thus, changes of parameter α_{280} reflect changes of the physical state (fluidity) of the annular lipids.

As can be seen from the temperature dependence of the parameters α_{280} and α_{340} (Fig. 2 and 3), within the given temperature range, i.e. 5—40 °C, the physical state of the lipid bilayer as a whole is different from that of the annular lipids. The activation of LPO (Table 1) in the synaptosomal membranes decreased the values of both parameters α_{280} and α_{340} within the entire temperature range tested. This decrease reflects the reduction of the lateral diffusion of pyrene molecules not only in the lipid bilayer matrix but also in the region of annular lipids.

To obtain convincing evidence that these changes in the physical state of total and annular lipids are due to LPO, the effect of LPO inhibition was studied. Considering that lipophilic antioxidants, such as α-tocopherol, retinol or butylhydroxytoluene, themselves affect the fluidity of membrane lipids, the water soluble compound carnosine was used as LPO inhibitor. At the conditions of our experiments, carnosine (20 mmol/l) inhibited LPO by approx. 25%. Simultaneously, changes of both parameters α_{280} and α_{340} could be observed over the entire temperature range tested (Table 1, Figs. 2 and 3).

The data obtained suggest that the activation of synaptosomal LPO causes changes of the physical state of both total and annular lipids. The question remains open which changes are more significant: these in the lipid bilayer matrix or those in the annular lipid domains. The ratio of parameters $\alpha_{340}/\alpha_{280}$ reflects the differences in changes of the physical state between total and annular lipids. The data shown in Fig. 4 indicate that LPO activation affects the physical state of annular lipids in a more significant manner than it does the lipid bilayer matrix. This fact could be interpreted in terms of ability of LPO products to form conjugated Schiff bases by crosslinking reaction between lipids and proteins (Donato 1981).

It may be concluded that the activation of LPO processes in the synaptosomal membranes affects the physical state of predominantly the annular lipids. This seems biologically important. The LPO-induced conformational changes of the annular lipid domain may be considered as a possible cause of the functional changes in the receptor mediated responses and of the inactivation of membrane-bound enzymes by oxidative stress. At the same time, since LPO activation may be blocked by antioxidants, the results obtained in the present work support the idea concerning the therapeutical effect of antioxidants in suppressing changes induced by oxidative stress (Šrám et al. 1988).

References

Bradford M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248—254

Clausen J. (1984): Demential syndromes and lipid metabolism. Acta Neurol. Scand. **70**, 345—355

Donato H. Jr. (1981): Lipid peroxidation, cross-linking reactions and aging. In: Age Pigments (Ed. R. S. Sohal), pp. 63—83, Elsevier, Amsterdam

Dergunov A. D., Kaprelyants A. S., Ostrovsky D. N. (1982): Structural state changes in annular lipids of bacterial membranes induced by membranotropic antibiotic gramicidin S. Biokchimiya **47**, 296—304, (in Russian)

Hajos F. (1975): An improved method for the preparation of synaptosomal fraction in high purity. Brain Res. **93**, 185—189

Merevenskaya N. V., Okun I. M., Volkovets T. M., Aksentsev S. L., Konev S. V. (1986): The

influence of arachidonic acid on physical properties of bilayer and annular lipids of synaptic membranes. Biofizika **31,** 523—534. (in Russian)

Ohkawa H., Ohishi N., Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. **95,** 353—358

Prilipko L. L., Erin A. N., Belyajev B. S., Pyatnicki A. N., Syunyakov S. A., Pantelyeyeva G. P., Cuculkovskaya M. J., Lideman R. R. (1987): Activation of lipid peroxidation in schizophrenia and manio-depressive patients. Zh. Neiropatol. Psichiat. **37,** 100—103. (in Russian)

Raber J., Bast A. (1989): Changes in receptor response by the effect of disease on membrane fluidity. Med. Hypotheses **28,** 169—171

Sies H. (1985): Oxidative Stress. Academic Press. London

Šrám R. J., Topinka J., Binková B., Kočišová J., Kotěšovec F. (1988): Unscheduled DNA synthesis and lipid peroxidation in a senescent population. Čas. Lék. Čes. **127,** 1365—69. (in Czech)

Yagi K. (1982): Lipid Peroxides in Biology and Medicine. Academic Press Inc., New York

Yukawa O., Nagatsuka S., Nakazawa T. (1983): Reconstitution studies on the involvement of radiation-induced lipid peroxidation in damage to membrane enzymes. Int. J. Radiat. Biol. **43,** 391—398

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