^{3}H-SEROTONIN AND ^{3}H-DIAZEPAM BINDING AND LIPID PEROXIDATION IN BRAIN CELL MEMBRANES

M. L. Libe, E. D. Bogdanova, A. E. Rozenberg, L. L. Prilipko, V. E. Kagan, and Yu. P. Kozlov

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The action of extracellular signals (hormones, mediators) on target cells is effected through the binding of the ligand substances with receptors on the outer surface of the plasma membrane, leading to activation or inhibition of the effector system (enzyme, ionophore, etc.) [5]. An essential role in this interaction between ligands and membrane receptors is played by the lipid microenvironment, which provides the necessary protein conformation for binding the ligand [6]. A productive approach to the study of the role of lipids in maintenance of the functional activity of membrane receptors is the chemical modification of the lipid bilayer [7]. A physiologically important process in chemical modification of lipids is lipid peroxidation (LPO), which *in vivo* is activated by factors such as hyperoxia, stress, avitaminosis E, etc. [3].

The object of this investigation was to study changes in serotonin and diazepam binding with membranes of the microsomal fraction of rat brain, by inducing LPO processes in them *in vivo* (hyperbaric oxygenation) and *in vitro* (Fe^{++}, ascorbate-dependent systems of LPO induction).

EXPERIMENTAL METHOD

The cerebral cortex of Wistar rats was used. Hyperbaric oxygenation of the rats was carried out in an airtight chamber with oxygen under a pressure of 3.5 atm for 2 h. Rat brain microsomes (105,000 g) were obtained by the method described in [4]. LPO was induced *in vitro* by means of an Fe^{++}–ascorbate (10 μM; 0.5 mM) system. LPO was stopped by the addition of ionol ($5 \cdot 10^{-5}$ M) to the medium. The membranes were washed and radioligand analysis of binding carried out in a volume of 0.5 ml for ^{3}H-serotonin and 1 ml for ^{3}H-diazepam. Samples with ^{3}H-serotonin (25 nM) were incubated for 20 min at 37°C, samples with ^{3}H-diazepam (2 nM) for 1 h at 0°C; the protein content in the samples was 0.5 mg/ml. After the end of the incubation period the samples were filtered through GF/B filters. Activity of the preparations was counted with the aid of Bray's scintillator with a counting efficiency of 30%. Specific binding was determined as the difference between binding of the ligand in the absence and in the presence of an excess (1 μM) of the displacing agent – the nonradioactive ligand. LPO products were determined with thiobarbituric acid and determination of fluorescence of Schiff bases [2]. To measure the microviscosity of the lipids the fluorescent probe diphenylhexatriene (DPHT) was used in a final concentration of 1 μM. Fluroescence was measured on an Aminco-Bowman spectrofluorometer. Before each series of measurements the instrument was calibrated against a standard solution of quinine sulfate (1 μg/ml in 0.1 N H_2SO_4). The microviscosity was calculated by the method in [8].

EXPERIMENTAL RESULTS

Keeping the rat in an atmosphere of oxygen led to a significant increase in concentration of LPO products in the membrane structures of the brain *in vivo* (Table 1). Preliminary administration of the synthetic antioxidant 4-methyl-2,6-tert-butylphenol to the animals prevented this increase. A significant decrease in ^{3}H-serotonin binding with the microsomal membranes was recorded at the same time, although in animals receiving the antioxidant it did not differ significantly from the control. It can be concluded from a comparison of these results that induction of LPO *in vivo* causes a decrease in the ability of the microsomal membranes to bind ^{3}H-serotonin, and that administration of the LPO inhibitor abolishes this effect.

In an *in vitro* model of this effect of LPO (Fig. 1) incubation of brain microsomes in the presence of a Fe^{++}–ascorbate system also led to a fall in the level of ^{3}H-serotonin binding. Addition of the antioxidant to the system in these experiments also completely inhibited the accumulation of LPO products and, correspondingly, abolished the effect of a reduction in ^{3}H-serotonin binding by the microsomes. Similar results were obtained previously with respect to stereospecific binding of opiates with guinea pig brain homogenate *in vitro* [6].

Institute of Psychiatry, Academy of Medical Sciences of the USSR, Moscow. M. V. Lomonosov Moscow University.
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TABLE 1. Effect of Antioxidants on Hyperoxia-Induced Changes in ^{3}H-Serotonin Binding and on Content of LPO Products in Rat Cerebral Cortical Microsomes (M ± m)

Procedure (n = 9)	Intensity of fluorescence of Schiff bases, realtive units	Serotonin binding, %
Control	7,8±0,9	100
Hyperoxia	22,8±2,6	47±15
Ionol	8,0±1,0	94±17
Ionol + hyperoxia	7,0±3,7	78±13

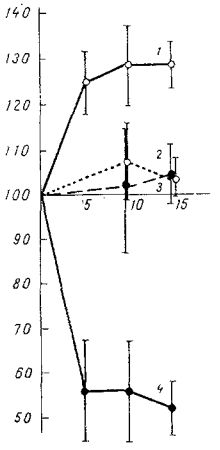

Fig. 1. Effect of LPO on serotonin and diazepam binding with rat cerebral cortical microsomes. Abscissa, time of incubation of membranes (in min): 1, 4) in medium not containing antioxidant (0.5 mM ascorbate, 10 μM $FeSO_4$, 0.1 M NaCl + 0.05 M Tris-HCl, pH 7.4), 2, 3) in medium with antioxidant (ionol, 5 X 10^{-5} M); ordinate, binding of labeled ligand (in % of control). Binding of: 1, 3) ^{3}H-diazepam, 2, 4) ^{3}H-serotonin.

Two facts must be noted when the results are analyzed. First, despite steady accumulation of LPO products during incubation for 15 min, the ability of the membranes to bind ^{3}H-serotonin decreased as early as during the first 5 min, after which it remained at the same level. Second, induction of LPO both *in vivo* and *in vitro* caused a decrease in ^{3}H-serotonin binding by 60-65%. This suggests that there are two types of serotonin binding sites in membranes of the microsomal fraction: sensitive and insensitive to the modifying action of LPO. Similar heterogeneity of binding sites, relative to sensitivity to LPO, also was found for a different type of ligand, ^{3}H-diazepam, for which induction of LPO increased binding by 30%. Analysis by Scatchard's method shows that this was due to an increase in the maximal number of binding sites for diazepam from 632 to 821 moles/mg protein; the dissociation constant was unchanged at 4.7 nM.

The modifying action of LPO caused opposite changes in ability to bind the ligands tested: a decrease in ^{3}H-serotonin binding and an increase in ^{3}H-diazepam binding, indicating different properties of the lipid microenvironments of receptors for these ligands. This means that LPO may be a specific modifier of different types of receptors, despite the fact that the effect of LPO itself on membranes is based on a common mechanism of a change in the physical properties of the lipid matrix (hydrophilic–hydrophobic balance, viscosity, charge). Analysis of changes in the degree of rotational relaxation of the fluorescent probe (DPHT) characterizing the microviscosity of the lipids, in fact revealed an increase in this parameter by 1.6 – 0.1 times in the brain microsomes during induction of LPO from 0.5 nmole malonic dialdehyde/mg protein in the control to 16 nmoles malonic dialdehyde/mg protein in the experiment. This result is in good agreement with data obtained previously on membranes of a different type, when an increase in viscosity of the lipid bilayer was demonstrated during accumulation of LPO products [1].

It can be concluded from these results that changes in the physical properties of the lipid matrix of nerve cell membrances induced *in vivo* and *in vitro* by LPO may have a significant effect on receptor–ligand interaction. The importance of the facts discovered is determined by the fact that endogenous LPO products may accumulate *in vivo* as a result of various physiological influences and also during the development of different forms of pathological states.

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TYPES OF TRIGLYCERIDASES IN THE PIG AORTA

G. I. Yusupova

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The triglyceridase activity of aortic tissue of different animals and man has been determined frequently [3, 7, 9, 10, 11]. It has usually been suggested that at least some of this activity is due to lipoprotein lipase. Nevertheless, data on the presence of lipoprotein lipase in the aorta are inadequate, and reliable evidence in support of this view has been obtained only in extracts of bovine aortas [1, 2].

Since the obtaining information on this topic is important, it was decided to study types of triglyceridases in the pig aorta.

EXPERIMENTAL METHOD

Pig aortas were obtained from an abattoir immediately after slaughter of the animals. All subsequent procedures were carried out at 4°C. Connective tissue and fat were removed from the aorta, which was washed with water and then cut into pieces with scissors. To 5 g of the tissue treated in this way 20 ml of 0.5M Tris-HCl, pH 8.0, was added, and the mixture was homogenized for 1 min in a Politron homogenizer. After extraction for 15-20 m in the homogenate was centrifuged for 20 min at 15,000g, and the cytoplasmic fraction was filtered through several layers of gauze.

The substrate emulsion for determination of triglyceridase activity was prepared by the method described in [8]. For this purpose 0.88 mg of glycerol-3-[1-^{14}C]-oleate with 0.1 ml of a 10% solution of ovolecithin was dispersed in 5.3 ml glycerol. The components of the substrate mixture were homogenized in a glass homogenizer.

To determine triglyceridase activity 0.1 ml of substrate was added to 0.1 ml of enzyme solution and incubated at 37°C for 1-3 h. Each sample contained 2.65 μg triolein, 3.3 $\times$ 10^5 cpm of glycerol-3-[1-^{14}C]-oleate (49 mCi/mmole), 0.06 mg ovolecithin, and 0.05 mg bovine serum albumin in 0.2 M Tris-HCl, pH 8.0. Fatty acids liberated during the reaction were extracted with a methanol–chloroform–heptane (1.41:1.25:1) mixture. Radioactivity was measured in a Mark 2 (Nuclear Chicago) liquid scintillation counter. When the effect of NaCl, protamine sulfate, or serum apoproteins on triglyceridase activity was studied, samples without the addition of these substances served as the control. Protein in the aortic extract was determined by Lowry's method [7]. The fraction of total apoproteins of the high-density lipoproteins was isolated from human blood serum by the method described in [4].

Laboratory of Physical Chemistry, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow.

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