CHANGES IN ANTIOXIDATIVE ACTIVITY AND COMPOSITION OF BLOOD ERYTHROCYTE PHOSPHOLIPIDS INDUCED IN NEUROTIC PATIENTS BY PHENAZEPAM

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Exposure of experimental animals and man to various stressor influences gives rise to intensification of free-radical lipid peroxidation (LPO) of cell membranes [4, 9]. The intensity of LPO is interconnected with the physicochemical properties of membrane lipids [3] and with functional activity of proteins, including enzymes, receptors, and channel-forming units, dependent on them. Considering the importance of emotional stress in the genesis and development of neurotic disorders, it can be tentatively suggested that a definite role in their pathogenesis is played by changes in peroxidation processes.

The aim of this investigation was to study LPO activity and the composition of blood phospholipids (PL) in patients with neuroses and their changes during phenazepam therapy. Erythrocytes were chosen as the test object, in view of information [7, 13] that disturbances of the phospholipid composition of neuronal membranes are accompanied by consistent changes in erythrocyte membrane lipids.

EXPERIMENTAL METHOD

Altogether nine patients with neurotic disorders (three men and six women aged 25-48 years), manifested as emotional lability, increased irritability and anxiety, combined with sleep disturbances and vasovegetative disorders, were studied. Patients with concomitant somatic and neurologic pathology were excluded from the study. Phenazepam was given in a daily dose of 1 mg, divided into two halves. During treatment with phenazepam no other psychotropic drugs were given. The duration of administration of the drug was at least 14 days. LPO was studied before treatment and also on the 7th and 14th days of phenazepam therapy. A sample of 2-3 ml of blood was taken from the ulnar vein of all the patients in a fasting state. Erythrocytes were isolated by the method described in [12]. Lipids were extracted from the erythrocytes by the method of Bligh and Dyer [8]. The composition of PL and the content of their various fractions were determined by thin-layer chromatography on silica-gel [14]. The level of LPO was judged by the antioxidative activity (AOA) of the erythrocytes, determined by means of a methyl oleate model [1]. On the basis of the composition of PL, their oxidizability was calculated as the ratio of the total content of readily oxidized fractions of PL: phosphatidylethanolamine (PEA) + phosphatidylinositol (PI) + phosphatidyl-serine (PS) to the total quantity of not easily oxidized PL: phosphatidylcholine (PCh) + sphingomyelin (Sph), allowing for coefficients characterizing the ability of each fraction to undergo peroxide formation, which were calculated in [5].

In the control group, 10 healthy subjects aged 20-35 years were tested once.

EXPERIMENTAL RESULTS

The results given in Table 1 demonstrate a difference in content of the individual PL fractions in patients with neuroses compared with the healthy controls. In the group of

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TABLE 1. Content of PL Fractions (in % of total content) of Blood Erythrocyte Lipids from Neurotic Patients and Healthy Subjects (M ± m)

Fraction	Control	Patients with neuroses
Lysophosphatidylcholine	1,0±0,3	3,6±0,5*
SpH	30,0±2,9	23,2±0,6*
PCh	32,9±1,5	31,2±1,1
PS + PI	12,0±1,2	15,3±0,9*
DEA	21,8±1,4	25,2±0,3*
Phosphatidic acid	5,3±1,0	0,9±0,5*
Oxidizability (S)	14	19

Legend. *p < 0.005 compared with control.

TABLE 2. Dynamics of Content of PL Fractions (in % of total content) and AOA of Blood Erythrocyte Lipids of Neurotic Patients during Treatment with Phenazepam (M ± m)

Fraction	Before treatment	Day of treatment	
		7-th	14-th
PS + PI	15,3±0,9	13,3±0,2*	11,6±0,1*
PCh	31,2±1,1	28,8±3,7	40,6±4,6*
PEA	25,2±0,3	28,9±2,5*	19,3±2,6*
SpH	23,2±2,2	24,7±2,1	22,3±2,5
S	19	22	13
ADA, h·ml/h	—650±90	—175±50	—1200±100

Legend. *p < 0.05 compared with value before treatment.

patients with neurotic disorders, whereas the PCh level was virtually unchanged, the PEA and the combined level of PS + PI were increased, whereas Sph was reduced. The AOA of the blood erythrocyte lipids of patients with neuroses was significantly lower than normal. Whereas in the control group, erythrocyte lipids delayed oxidation of methyl oleate (AOA amounted to 4400 ± 300 conventional units), in the patients, on the other hand, they accelerated its oxidation (AOA had negative values, on average —650 ± 100 conventional units). The oxidizability of the erythrocyte lipids of patients with neuroses was 1.4 times higher than that of healthy subjects.

Thus during the development of neurotic disorders significant changes are found in the lipid component of the erythrocyte membrane, as shown by the lowered AOA and changes in the relative content and oxidizability of the PL fractions.

Phenazepam therapy had some effect on the erythrocyte lipids, as revealed by a change in all the parameters studied (Table 2). The clearest changes are found in PL fractions such as PS + PI and PEA. The combined PS + PI content fell toward the 7th day of treatment by comparison with the original value, and the PEA level increased a little. AOA of the lipids was significantly increased by the 7th day of treatment. Meanwhile a tendency was noted for the oxidzability of PL to increase.

By the 14th day of treatment a further decrease was observed in the combined PS + PI content. The PEA level was distinctly lower, and returned to values characteristic of healthy subjects. The Sph content remained lower than in the control, whereas PCh increased toward the 14th day of treatment. These changes in the composition of PL were observed at a time of a considerable decrease in AOA of the lipids. Oxidizability showed a decrease of more than one-third compared with its value on the 7th day of treatment.

Changes discovered in the erythrocyte lipids were accompanied by a decrease in the intensity of the psychopathological symptoms, in the form of reduction primarily of the affective disturbances: anxiety, emotional instability, and increased irritability. Mean-

while a tendency was noted for sleep to return to normal and for the vegetative disorders to decrease.

As was stated above, AOA of the erythrocyte lipids was depressed during the development of neuroses. This is in agreement with the view that intensification of LPO plays an important role in the pathogenesis of neurotic disorders. However, during treatment with phenazepam AOA underwent phasic changes: an increase on the 7th day of treatment and a fall below the initial level on the 14th day of treatment, although the therapeutic effect of phenazepam at these times was clearly distinguished. At first glance, this fact contradicts the suggestion made above that LPO plays a role in the pathogenesis of neuroses. Probably it was not intensification of LPO itself, but changes in lipids connected with it, that has definite importance.

It can now be regarded as established [3] that cell membranes contain a physicochemical regulatory system maintaining homeostasis of oxidative reactions and AOA in lipids. It has been shown [3, 6] that under normal conditions, the membrane responds to any decrease in AOA of the lipids by a change in their composition, leading to a decrease in oxidizability and, consequently, facilitating the return of AOA to its normal level. Our data are evidence that besides intensification of LPO processes in patients with neuroses, before the beginning of treatment this regulatory interconnection in the AOA-oxidizability stage is upset. As will be clear from Table 2, during a 14-day therapeutic course phasic changes in AOA coincided in direction with changes in oxidizability. This evidently reflects the ability of the tranquilizer to restore the initially disturbed system regulating the intensity of LPO processes in patients with neuroses.

Changes in the content of the PS + PI fractions in the erythrocytes must be noted as an important factor. The results are in agreement with those of experimental studies which revealed an increase in the content of these fractions in rat brain synaptosomes in the presence of predominance of excitatory processes and efficiency of the inhibitory GABA-ergic system. It has also been shown experimentally that reduction of the excitability of the CNS under the influence of pharmacologic agents maintaining a GABA-positive action is accompanied by a decrease in the content of the PS + PI fractions [11].

The results thus suggest that changes in the intensity of LPO can make an important contribution to the pathgenesis of neurotic disorders. Probably under these circumstances it is the change in the composition of PL arising due to changes in the intensity of LPO, which plays an important role, especially the increase in the content of PS + PI in the erythrocyte lipids and disturbance of the AOA-oxidizability connecting link.

The question arises, to what can be attributed the essential decrease in AOA of the erythrocyte lipids after 2 weeks of phenazepam therapy. This is probably a reflection of some kind of injurious action of the drug on membranes, exhibited when administered for a long time. Such a harmful action of phenazepam was demonstrated previously for hepatocytes in both experimental and clinical investigations [2, 6]. Incidentally, according to the test of a change in AOA of lipids, no such harmful action of phenazepam for the brain has been discovered [13].

The facts described above are evidence of the usefulness of administration of antioxidant LPO inhibitors in conjunction with tranquilizers of the benzodiazepine series, more especially because several antioxidants, tested under experimental conditions, have been found to possess psychotropic activity (including a tranquilizing action) [10, 11].

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EFFECT OF CHLORPROMAZINE AND TRIFLUPERAZINE ON SYNAPTOSOMAL MEMBRANES OF THE RAT CEREBRAL CORTEX

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The writers showed previously that psychotropic preparations belonging to various groups differ in their affinity for phosphatidylcholine liposomes [2]. The neuroleptics studied, unlike antidepressants and tranquilizers, penetrated deeper into the bilayer, into the zone where a hydrophobic fluorescent probe, 3-methoxybenzanthrone, was located. Penetration of phenothiazine neuroleptics into the central zone of the lipid bilayer has been demonstrated on erythrocyte membranes [9].

It was accordingly decided to study the effect of the neuroleptics chlorpromazine and trifluperazine and to compare it with that of the tricyclic antidepressant imipramine, on viscosity of the lipid bilayer of synaptosomal membranes of the rat cerebral cortex, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

A pure fraction of cerebral cortical synaptosomal membranes from male rats weighing 180-200 g was obtained by the usual methods of gradient centrifugation of a coarse synaptosomal fraction after cold and osmotic shock, as described previously [5]. As fluorescent probes we used 1, 6-diphenyl-1,3,5-hexatriene (DPHTE, from Fluka, West Germany) and pyrene (Sigma, USA). Luminescence of DPHTE was excited at 350 nm and recorded at 430 nm. The final concentration of the probe in the sample was 10^{-6} M and the insertion time 30 min at 37°C in darkness, with constant mixing. Luminescence of pyrene was excited at 334 nm and recorded at 373 and 480 nm; the excitation filter was 350 nm, the concentration of the probe in the sample $5 \cdot 10^{-6}$ M, and the insertion time 5 min at 37°C in darkness with constant mixing. Luminescence of tryptophan of the membranes and of L-tryptophan (Sigma) was excited at 286 nm and recorded at 330 nm. Fluorescence was recorded using corrected spectra on a Hitachi M-850 fluorescent spectrophotometer (Japan). Next, 25 µl of synaptosomal suspension (average 56 µg protein) was added to 1 ml of incubation medium containing 150 mM NaCl, 6 mM KCl, 0.5 mM ethylenediaminetetra-acetic acid, and 0.01 M Tris-HCl buffer, pH 7.4. Solutions of the probes (10 mM) in dimethylformamide were added to the membranes in the incubation medium from a microsyringe at the rate of 1 µl/min, with constant mixing. The viscosity of the lipid component of the synaptosomal membranes was judged from the ratio of the intensity of fluorescence of pyrene at 373 nm (monomer) and 480 nm (excimer), and also from the change in polarization of the fluorescence of DPHTE, calculated by the equation:

$$P = \frac{(F_{\parallel} - f_{\parallel}) - (F_{\perp} - f_{\perp})}{(F_{\parallel} - f_{\parallel}) + (F_{\perp} - f_{\perp})},$$

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