intracellular synthesis of the main product of the cyclo-oxygenase pathway of arachidonic acid metabolism in the blood vessels, namely $PGI_2$ which, as was shown above, possesses an antiatherosclerotic action. This increase was caused by a decrease in consumption of arachidonic acid as a result of inhibition of lipo-oxygenase activity. On the other hand, we know that leukotrienes can be formed in the lipo-oxygenase pathway of arachidonic acid metabolism in blood vessels [7]. Since the latter, like $TXA_2$, when they act on the cell, cause the calcium level to rise, and their effect is blocked by calcium antagonists, it can be tentatively suggested that leukotrienes also possess more or less distinct "atherogenic" properties. Consequently, the effect of lipo-oxygenase inhibitors on intimal cells described above may be due also to inhibition of leukotriene formation in them.

The cascade of arachidonic acid metabolites is thus one element in the endogenous regulation of atherogenesis, and the direction of their influence is largely determined by equilibrium between functionally heterogeneous groups of eicosanoids. Their action is evidently based on an effect on calcium homeostasis of the cells. This applies not only to $TXA_2$ and leukotrienes, but also to $PGI_2$, which may lead to reduction of the cytoplasmic calcium concentration on account of elimination of calcium from the cells via the sodium-calcium antiport [1]. The positive interaction with calcium antagonists, preventing the entry of calcium into the cytoplasm, may also be connected with this component of this mechanism.

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# EFFECT OF SUBCHRONIC ADMINISTRATION OF PHENAZEPAM AND SYNTHETIC ANTIOXIDANTS ON CEREBRAL CORTICAL SYNAPTIC MEMBRANE FUNCTION OF RATS EXPOSED TO LONG-TERM STRESS


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It is generally accepted that membrane lipids can actively influence the functional state of membrane-bound enzymes, such as Na, K-ATPase [10, 13], adenylate cyclase [9, 12], and monoamine oxidase (MAO) [14], which play an important role in synaptic transmission. Any modification to the lipid bilayer of biological membranes leads to a disturbance of one of the main functions of the lipids, which is to create a definite hydrophobic environment around the


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TABLE 1. Characteristics of Interaction of Fluorescent Probe MBA with Cerebral Cortical Synaptic Membranes of Rats after Administration of Preparations for 7 Days (M ± m)

| Experimental conditions | $K_b$ $M^{-1}$ ($\cdot 10^4$) | N, moles/mg protein ($\times 10^{-8}$) | $K_b \cdot N$ ($\cdot 10^2$) |
|---|---|---|---|
| Control | 7,45±1,66 | 2,38±0,20 | 1,77±0,20 |
| Placebo | 4,60±0,84 | 9,83±0,93* | 4,31±0,31* |
| Stress + dibunol | 4,45±0,66 | 7,46±0,49* | 3,26±0,28* |
| Stress + 3-HP | 1,50±0,13*,** | 9,35±0,85* | 1,37±0,02** |
| Stress + phenazepam | 1,69±0,19*,** | 6,90±0,51* | 1,13±0,03*,** |

Legend. *p < 0.05 compared with control, **the same, compared with placebo.

protein, without which the native conformation of the enzyme could not be maintained. One of the factors leading to a disturbance of function of the lipid bilayer is peroxidation (LPO), which is based on free-radical reactions [4, 6]. Activity of enzymatic reactions is known to be lowered in various models of extremal states, such as acute and chronic stress caused by painful electrical stimulation, sudden cooling, anoxia, and hyperoxia [1, 3, 7].

The aim of this investigation was to study the effect of subchronic administration of the psychotropic drug phenazepam, and of hydrophobic (dibunol; 4-methyl-2,6-di-tert-butylphenol) and hydrophilic (2-ethyl-6-methyl-3-hydroxypyridine, 3-HP) antioxidants on the state of cerebral cortical synaptic membranes of rats exposed for 15 days to psychogenic stress, and also on MAO activity.

## EXPERIMENTAL METHOD

Experiments were carried out on a noninbred male albino rats weighing 180-200 g. Chronic psychogenic stress was induced by a specially developed scheme with stochastic electronociceptive reinforcement [11]. The test substances were given perorally in the form of aqueous solutions by atraumatic tube for 7 days after the last exposure to stress, in accordance with the scheme, in the following doses: phenazepam 0.5 mg/kg, dibunol 30 mg/kg, and 3-HP 50 mg/kg. Dibunol and phenazepam were suspended beforehand in Tween-80. The animals were killed 24 h after the last dose of the test substances.

The coarse fraction of synaptosomes and a pure fraction of synaptic membranes were obtained by the usual centrifugation method. MAO activity was determined in brain homogenate by isothermic distillation of ammonia followed by nesslerization [2]. Malonic dialdehyde (MDA) was determined quantitatively by the reaction with 2-thiobarbituric acid [4]. The state of the lipid phase of the synaptic membranes was studied by means of a fluorescent probe (3-methoxybenzanthrone, MBA), which can penetrate into the lipid bilayer at the level of the 5th-8th carbon atom of the fatty acid tails of lipid molecules [8]. Parameters of binding of MBA with the membrane were determined on a Hitachi-850 fluorescent spectrophotometer (Japan). The results were processed on an HP 33E electronic calculator USA).

## EXPERIMENTAL RESULTS

It will be clear from Table 1 that administration of the placebo led to a decrease in the binding constant ($K_b$) of MBA with the membranes, but the change was not significant. The same result was also observed when dibunol was given. A considerable increase in the number of binding sites (N) of the probe, significant compared with the control, was found in all the experimental groups of animals. Estimation of the total affinity ($K_b \times N$) of the probe for synaptic membranes indicates a considerable change in the state of the lipid phase of the membranes in the placebo group. Administration of dibunol did not promote restoration of the physicochemical properties of the synaptic membranes, and after administration of phenazepam and 3-HP the total affinity of MBA for membranes fell to control values.

An important role in the disturbance of the state of biological membranes is ascribed to LPO processes, which may be activated in various types of stress [7]. During stress for 15 days an increase was observed in the basal level of MDA formation (Fig. 1). Addition of a pro-oxidant ($Fe^{++}$ + ascorbate) to the incubation mixture led to reduced formation of TBA-active (TBA = thiobarbituric acid) products compared with the control. Administration

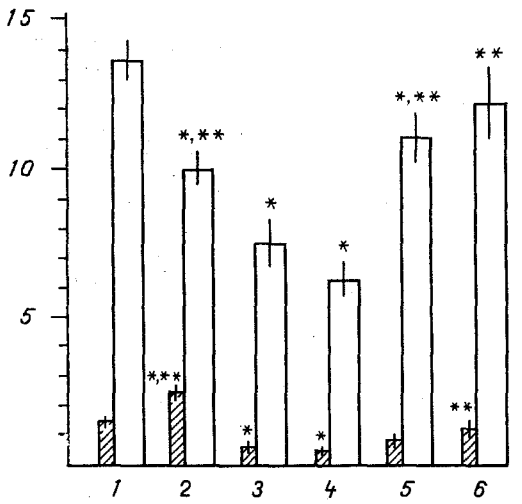


Fig. 1. Concentration of LPO products in cerebral cortical synaptosomes of rats after administration of preparations for 7 days in association with chronic stress. Ordinate) MDA level (in μmoles/g lipid). 1) Control, 2) chronic stress, 3) placebo, 4-6) administration of dibunol, 3-HP, and phenazepam respectively. Shaded columns) basal level of MDA; unshaded columns) level of MDA formation stimulated by $Fe^{++}$ + ascorbate. *p < 0.05 compared with control; **p < 0.05 compared with placebo.

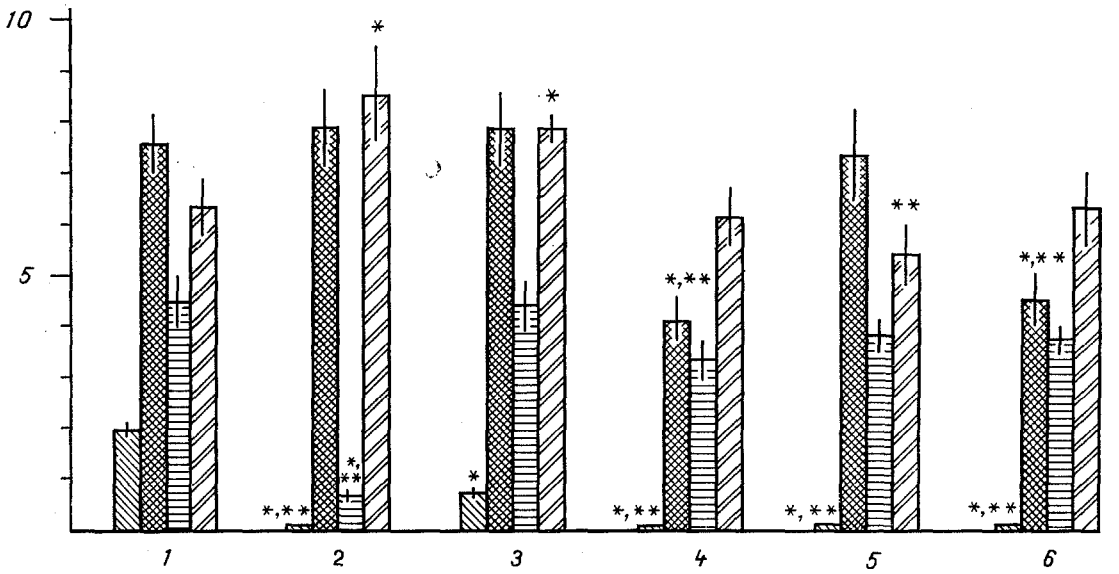


Fig. 2. MAO activity of rat brain after chronic exposure to psychogenic stress followed by administration of preparations for 7 days. Ordinate) MAO activity (in mmoles ammonia/mg protein/min); oblique shading) noradrenalin; cross-hatching) dopamine; horizontal shading) serotonin; double oblique shading) phenylethylamine. Remainder of legend the same as Fig. 1.

of the placebo for 7 days led to a marked fall of these LPO parameters. Dibunol had a similar effect. After administration of 3-HP, although both the basal and the $Fe^{++}$-ascorbate-stimulated levels of LPO differed significantly from the control, there was a tendency for these parameters to return to normal, in comparison with administration of the placebo and dibunol. Only when phenazepam was given was the normal level of LPO restored in the synaptosomes. Comparison of data reflecting the state of the membrane lipids in synaptic membranes (Table 1) with the degree of LPO activation in the synaptosomes, against the background of subchronic administration of the drugs chosen for testing, showed close correlation between these parameters. For instance, administration of dibuonol did not restore the physicochemical state

of the synaptic membranes or LPO processes in the synaptosomes, when disturbed during stress, and conversely, administration of phenazepam and 3-HP facilitated restoration of the characteristics studied.

Data on the effect of exposure for 15 min to psychogenic stress on rat brain MAO activity are given in Fig. 2. Chronic stress induced a complete blockade of noradrenalin-deaminase activity and a well marked reduction (by 85-90%) of the velocity of the serotonin-deaminase reaction in the rat brain. Conversely, deamination of type B MAO substrates was not reduced (dopamine) but actually increased somewhat (2-phenylethylamine) as a result of the procedure used. In various physiologically extremal states (sudden cooling, anoxia, hyperoxia), other workers also observed inhibition of type A MAO activity in the rat brain, but it was not so marked as in the case of subchronic stress [3]. Under these circumstances MAO was able to oxidize substrates which normally it does not oxidize: histamine, cadaverine, putrescine, GABA, aminosugars, and AMP [3, 5], possibly due to activation of LPO processes in the brain during stress. The data showing a sharp fall in type A MAO activity in chronic stress, given in Fig. 2, may reflect general rules governing the changes in monoamine metabolism in the CNS under various types of extremal conditions.

It follows from the data in Fig. 2 that 7 days after termination of exposure to stress the rate of deamination of all the substrates studied was virtually the same as in the control, the only exception being noradrenalin, deamination of which was still reduced by more than 50%. This slow normalization of noradrenalin oxidation in the brain evidently reflects the important role of the noradrenergic transmitter system in the development of the response to stress.

Administration of dibunol, 3-HP, and phenazepam for 7 days did not cause recovery of the noradrenalin-demainase activity of rat brain (but actually reduced it compared with the placebo). Serotonin-deaminase activity under the influence of all the preparations was almost identical with that in the control and during stress, but the rate of the 2-phenylethylamine-deaminase reaction was lowered to control values, which was not observed in the placebo group. The ability of rat brain MAO to oxidize dopamine, which was unchanged during stress and in the placebo group, was sharply reduced after a 7-day course of dibunol and phenazepam, but unchanged under the influence of 3-HP.

Of all the compounds tested, 3-HP thus restored the brain monoamine oxidase activity of stressed rats most effectively, a result which correlates with its ability to restore the physicochemical properties of synaptic membranes and the LPO processes in them, as well as behavioral parameters disturbed during stress.

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