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BIOPHYSICS AND BIOCHEMISTRY

β-Adrenoreceptors of the Cerebral Cortex in Experimental Neurosis

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It is common knowledge that stress factors exert their effects on the brain by alterating the lipid component of cell membranes [1,2,5]. However, the interpretation of the adaptive, compensatory, and disadaptive changes of the lipid bilayer on the level of membranes, cells, parts of the brain, and the whole brain is not quite clear, especially in chronic stress, when contrasting processes are taking place. For example, the model of experimental neurosis reveals that after 1-3 weeks of combined stress, on the one hand, the capacity of the antiradical system is enhanced, leading to an inhibition of free-radical processes and to the depletion of cholesterol in the cell membrane, while, on the other hand, pathological changes in the cerebral membrane phospholipid pattern are observed, primarily as a result of a diminished proportion of readily oxidized lipids [2]. Thus, experimental neurosis, possessing incontestable features of free-radical pathology, can also be considered as an example of pathological adaptation (what has been termed adaptation with a high structural price [5]) of brain membranes to chronic stress. The revealed changes in membrane structure and composition point to probable functional alterations which result from the development of experimental neurosis. Nevertheless, the findings offer no direct information on the functional changes in membrane-associated processes. It should be very useful to analyze the β-adrenoreceptors (β-AR), which are the most widespread receptors in the brain. The indexes of β-AR activity are determined by the state of the lipid component of membranes.

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TABLE 1. Effect of Chronic Stress on β-Adrenoreceptors of Rat Cerebral Cortex

Experimental conditions	B_{max}	K_d
Control (100%)	17.3 fmol/mg protein	10.0 nmol
1st week of stress	219*	275*
2nd week of stress	206*	139
3rd week of stress	144	97

Note: here and in Table 2 the average data from three experimental series are listed (%). Asterisk: $p<0.05$ compared with the control.

The aim of this investigation was to study β-AR in rat cerebral cortex during the development of experimental neurosis in correlation with respective changes in brain lipid composition and free-radical oxidation of lipids (FROL).

MATERIALS AND METHODS

Experiments were carried out on 60 male rats weighing 150-200g. The animals were subjected to chronic stress using a routine model of experimental neurosis [1]. Every day during the three-week period experimental conditions of two types were applied: an asthenizing intermittent white noise of 80-90 dB for 7 hours per day and emotional-painful electrodermal stimulation. As a result of 3 weeks of combined emotional-painful stress (current and light, and then noise), the animals demonstrated a stable negative-emotional neurosislike state.

The rats of three experimental groups (1, 2, and 3 weeks under stress) and one control group were killed by decapitation; blood was collected, and the cerebral cortex was homogenized in a glass-teflon homogenizer. A method described in [7] was used to assess the products of FROL reacting with thiobarbituric acid (TBA). The lipids of the cerebral cortex homogenates were extracted after Folch [6] and analyzed using the method of thin-layer chromatography [8].

With the use of osmotic and cryogenic shock, followed by differential centrifugation, the fraction containing the plasma and synaptic membranes was separated. The state of β-AR was assayed according to the specific binding of the labeled β-AR antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) as described previously [4]. DL-propranolol was used as a competitive ligand. The results were processed statistically using the Wilcoxson-Mann-Whitney test.

RESULTS

The specific binding of ^{3}H-DHA by the membranes in Scatchard coordinates [9] showed an increase of the dissociation constant (K_d) toward the end of the first week of emotional-painful stress (Table 1). The simultaneous increase of maximal binding (B_{max}) reflects a rise of the number of binding sites. When stress is continued, the value of K_d drops to the level of the control, whereas B_{max} is 1.5 times greater than in the control. Neither index differs significantly from the control by the end of the third week under stress. Thus, it may be assumed that the relatively rapid compensatory processes (an increase of the number of binding sites in response to a decrease of their affinity) gradually give way to adaptation of the neuronal membranes to chronic stress.

A comparative assay of the accumulation of TBA-active products in the cerebral cortex and serum revealed an inhibition of FROL both in the brain and in the serum (the latter to a large extent reflects the changes of FROL in the whole organism) toward the end of the first week. The persistence of stress leads to a gradual increase of FROL, causing normalization of the TBA-active products in the cortex and their significant increase in the serum (Table 2). The inhibition of FROL and decrease of the cholesterol fraction in the membranes point to adaptive-compensatory changes of these indexes in the cortex [3], whereas the activation of FROL in the serum indicates probable impairment of certain adaptive mechanisms.

TABLE 2. Effect of Chronic Stress on Free-Radical Oxidation and Lipid Composition of Cell Membranes of Rat Brain

Experimental conditions	Brain		Blood serum
	TBA-active products	Cholesterol/phospholipids	TBA-active products
Control	100	100	100
1st week of stress	56*	60*	67*
2nd week of stress	83*	85*	123
3rd week of stress	104	114	160*

Thus, the structural and free-radical changes of the cerebral membranes under chronic stress suggest that adaptive alterations occur in which the membranes and, in particular, β-AR are involved. It may be assumed that the changes in lipid composition are probably disadaptive ("pathological") [2] and lead to a decrease of β-AR affinity. However, the adaptive capacities of the brain exceed those of the organism as a whole and the decrease of receptor affinity is compensated for by an increase in the number of binding sites.

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The Ability of Sodium Hypochlorite to Penetrate the Lipoid Phase

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Lipoproteins (LP) in human blood can be modified as a result of lipid peroxidation (LPO). It has been suggested that the accumulation of such modified LP in the blood can cause atherosclerosis [9,13]. LP in the blood are oxidized by $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and OCl^- formed during the activation of phagocytes [5]. In a recent study we have shown [6] that NaOCl can oxidize lipids that form part of the LP in human blood. This reaction takes place with the participation of free radicals and ions of transition metals [2,6].

It is known that LPO takes place at the site of unsaturated bonds in the fatty acid chain, i.e., in the hydrophobic hydrocarbon portion of the lipid phase of LP and in biological membranes [1]. But in that case it is not quite clear in what way NaOCl participates in LPO in lipoproteins, since NaOCl is readily soluble in water and is localized in all probability in the aqueous phase.

The present work is an attempt to ascertain whether sodium hypochlorite penetrates into the lipid phase of protein-lipid complexes. For this purpose NaOCl was introduced into an aqueous suspension of low-density lipoprotein (LDL) isolated from human blood, and the reaction of NaOCl with the electron donors localized in the lipid phase of LDL was investigated. Used as electron donors were nitroxide radicals which are derivatives of stearic acid with a paramagnetic center localized at various distances from the carboxyl group.

MATERIALS AND METHODS

LDL was isolated from human blood serum by ultracentrifugation of a NaBr solution of a certain density containing the serum [10]. The concentration of LDL was determined from the protein content in LDL by the Lowry method [11]. Sodium hypochlorite was prepared electrochemically with the aid of an EDO-3 setup by passing a direct current (1 amp) for 30 min through a 0.9% solution of NaCl. The concentration of NaOCl was determined at pH 12 from the absorption at 290 nm; the molar extinction coefficient of NaOCl was assumed to be 350 $mol^{-1}cm^{-1}$ [12].

The EPR spectra of the spin probes were recorded at 20°C with the aid of an ER-420 radio-

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