CHANGES IN SYNAPTOSOMAL MEMBRANES IN ANIMALS DEVELOPING EXPERIMENTAL NEUROSES STUDIED BY THE USE OF DONOR–ACCEPTOR LUMINOPHORE PAIRS

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The writers showed previously that chronic exposure to psychological stress by a modified [12] Hecht's method [3], leading to the development of a neurotic state in animals, is accompanied by changes in the surface of the synaptosomal membranes of the cerebral cortex of rats and disturbance of activity of membrane-linked processes [1, 12].

For a more detailed analysis of the consequences of development of chronic neurosis at the neuronal membrane level, it was decided to study the trend of structural fluctuations in the membranes and changes in activity of an integral protein, namely the enzyme Na,K-ATPase. To evaluate structural changes in synaptosomal membranes we used endogenous and exogenous luminophores, and also the phenomenon of inductive-resonance transfer of energy.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g. Each experimental group contained 7-10 animals. They were exposed to stress as described previously [12]. The animals were decapitated 24 h after the last exposure to stress by means of a guillotine and the fraction of synaptosomal membranes of their cerebral cortex was obtained as described in [7]. To study structural changes in the membranes, endogenous tryptophan and two fluorescent probes with different localizations, namely pyrene, located in the center of the lipid bilayer of the membranes, and 1-anilinonaphthalene-8-sulfonate (ANS), bound with the membrane surface [2], were used as luminophores. The fluorescence measurements were made in 10-ml quartz cuvettes at 37°C, with constant mixing, on a "Hitachi M-850" fluorescent spectrophotometer, working on corrected spectra mode. In the course of the experiment synaptosomal membranes (on average 0.05 mg protein in 1 ml buffer) were added to 1 ml of buffer solution containing 150 mM NaCl, 6 mM KCl, 0.5 mM EDTA, and 0.01 M Tris-HCl buffer, pH 7.4, and fluorescence of endogenous tryptophan was recorded (width of slit: at excitation 3 nm, at recording 1.5 nm, wavelength of excitation (EX) 286 nm, filter 290 nm). Next, 1 μl of a solution of pyrene in dimethylformamide (10^{-6} M) was added to the cuvette at the rate of 1 μl/min with constant mixing, and 3 min later the change in fluorescence of endogenous tryptophan was recorded. The efficiency of energy transfer from tryptophan to pyrene was calculated by the formula: $(I_T - I_{TP})/I_T$, where I_T denotes the fluorescence of endogenous tryptophan and I_{TP} the same, after addition of pyrene. The fluorescence spectrum of pyrene (EX = 334 nm, wavelength of fluorescence EM = 350-500 nm, filter 350 nm) was recorded and the microviscosity of the membrane was estimated by the ratio of the intensity of fluorescence of the excimer (I_E = 480 nm) and the monomer (I_M = 373 nm) of the probe (I_E/I_M). Later, $5 \cdot 10^{-5}$ M ANS was added to the same cuvette and the change in the intensity of fluorescence of the pyrene monomer was recorded. The efficiency of energy transfer from pyrene to ANS was calculated by an equation similar to that given above. Na,K-ATPase activity was determined as described in [13]. The protein concentration in the samples was determined by the method in [11].

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TABLE 1. Effect of Chronic Exposure to Psychological Stress on Structural Parameters of Synaptosomal Membranes in the Rat Cerebral Cortex ($M \pm m$)

Day of experiment	$(I_T - I_{TP})$ (characteristic of clusterization of proteins)	I_E/I_M (characteristic of microviscosity)	$(I_P - I_{P\text{-}ANS})/I_P$ (characteristic of thickness of lipid bilayer)
3rd			
control	0,7490±0,207	2,1800±0,4010	0,5799±0,0150
stress	0,7231±0,0084*	2,0855±0,1873	0,56434±0,0191
6th			
control	0,7200±0,0051	1,9305±0,0820	0,6003±0,0330
stress	0,6975±0,0144*	1,6676±0,1754	0,5728±0,0127*
10th			
control	0,6866±0,0001	1,7407±0,3200	0,5556±0,0340
stress	0,7250±0,0413	1,9282±0,1910	0,5703±0,0279
15th			
control	0,7445±0,0190	2,2356±0,3300	0,5748±0,0140
stress	0,7275±0,0125	1,8892±0,1392*	0,5858±0,0132

Legend. I_p) Intensity of effect of pyrene monomer, $I_{p\text{-}ANS}$) intensity of fluorescence of pyrene monomer after addition of ANS. *$p < 0.05$ compared with control.

TABLE 2. Effect of Chronic Psychogenic Stress on Na,K-ATPase Activity in at Cerebral Cortex ($M \pm m$)

Day of experiment	V_{max}, μmoles P_i/mg protein/h	$K_M \cdot 10^{-4}$ M
3rd		
control	46,3±2,2	5,9±1,2
stress	39,6±4,6	5,8±1,5
6th		
control	90,3±10,4	15,3±3,2
stress	56,3±6,1*	5,4±2,3*
10th		
control	49,1±4,8	5,7±0,1
stress	74,7±8,9*	11,6±3,8
15th		
control	68,7±4,9	14,4±2,4
stress	66,0±3,3	5,3±1,4*

Legend. *$p = 0.05$ compared with control.

EXPERIMENTAL RESULTS

The data given in Tables 1 and 2 are evidence of significant changes in the structural parameters of the membrane and changes in activity of the membrane-bound enzyme linked with them.

When choosing a method of studying structural changes in the biomembranes we assumed that donor–acceptor pairs of fluorescent probes can be successfully used to evaluate the structural parameters of model and biological objects [2, 4, 5], for transfer of energy from donor to acceptor is possible only within the limits of a strictly fixed distance, which can be determined for a given concrete pair of fluorophores by Förster's radius [7]. Thus on the basis of the efficiency of inductive-resonance transfer of energy (IRTE) between pairs of fluorophores whose localization in the membrane is known, it is possible to estimate with adequate accuracy structural parameters of the membrane such as its thickness, the area of protein and lipids on the surface of the membrane, the mutual arrangement of the proteins, and so on [2]. The efficiency of energy transfer from tryptophan to pyrene is inversely proportional to the degree of clusterization of membrane-bound integral proteins [4, 5], but the efficiency of IRTE from pyrene to ANS is inversely proportional to the thickness of the lipid bilayer of the membrane [2]. Analysis of the data in Table 1 reveals a definite dynamics of the adaptive structural changes: an increase in the degree of protein clusterization on the 3rd and 6th days, thickening of the membrane on the 6th day, and condensation of the lipid bilayer on the 15th day. On the 10th day the membranes do not differ in structure from the native forms, possibly due to complete replacement of the proteins and lipids in these membranes in the previous period [6].

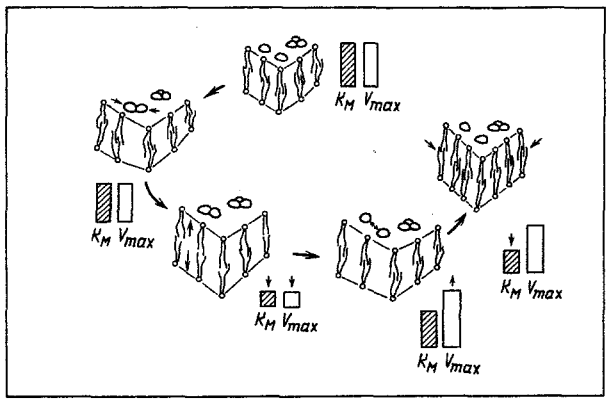

Fig. 1. Effect of chronic psychogenic stress on structures of synaptosomal membranes of rat cerebral cortex and on Na,K-ATPase activity.

The experimental data were analyzed with consideration paid to dependence of the efficiency of IRTE in tryptophan – pyrene and pyrene – ANS donor–acceptor pairs on the mutual arrangement of the integral proteins in the membranes and in the thickness of the lipid bilayer. The degree of excimerization of pyrene also is known to depend on the microviscosity of the lipid bilayer of the membrane [2]. significant changes in the degree of excimerization of the probe were observed only on the 15th day of the experiment, evidence of a change in the microviscosity of the lipid bilayer (condensation). For convenience of comparison of the trend of structural changes in the membranes and functional activity of the integral protein Na,K-ATPase, the basic trend of the changes is indicated in Fig. 1. Na,K-ATPase is known to be a globule, passing completely through the lipid bilayer, and about equal in diameter to the thickness of the membrane [13]. Na,K-ATPase activity is modulated by the liquid of the lipid bilayer [9] and for that reason this enzyme can react to changes in thickness of the lipid bilayer of membranes. Examination of the scheme reveals definite correlation between the structural and functional changes observed. For instance, the increase in protein clusterization on the 3rd day of stress is not reflected in activity of the marker enzyme, and the increase in thickness of the lipid bilayer of the membrane on the 6th day may lead to screening of individual centers of the enzyme, which is reflected in a decrease in V_{max} and a compensatory increase in affinity of the enzyme (a fall in K_m). By the 10th day the structure of the membrane reverts to its initial form (as was stated above, this time interval corresponds approximately to that of complete replacement of proteins and lipids in neuronal membranes) which is accompanied by a slightly increased content of enzyme protein globules, possibly as a result of activated outflow of the enzyme into the membrane in response to a decrease in their number on the 6th day. Significant changes in "flowability" of the lipid bilayer of the membranes were observed only on the 15th day of stress. There was a parallel increase in enzyme activity due to increased affinity for the substrate without any change in the number of Na,K-ATPase molecules. "Liquefaction" of the lipid bilayer leads to a fall in Na,K-ATPase activity [9, 13]. Our own data show that condensation of membrane lipids causes an increase in enzyme activity on account of an increase in its affinity. Consequently, this pathway of regulation of the work of the enzyme (through a change in microviscosity of the lipid bilayer of the membrane) also is effective both for increasing and for reducing its activity.

The investigations thus lead to the conclusion that chronic psychogenic stress causes changes in the structure of the biomembranes investigated and in activity of the membrane-bound enzyme. The technique of evaluating structural changes in biomembranes which we have developed is effective, highly informative, and a relatively nontraumatic approach. The data obtained by it provide additional valuable information on the trend of the change in functional activity of membrane-bound systems during exposure to different factors.

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EFFECT OF DALARGIN ON BRAIN TISSUE XANTHINE OXIDASE ACTIVITY DURING MYOPLEGIA

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The hexapeptide dalargin, a Soviet analog of endogenous Leu-enkephalin, whose effects are realized by interaction with peripheral μ- and Δ-opiate receptors, in doses of up to 500 μg/kg does not in fact pass through the blood-brain barrier and does not act on central targets [3]. The possibility of using dalargin as a basic agent for anesthesiologic protection, as a component of multicomponent general analgesia has been investigated in the Department of Anesthesia and Resuscitation of the A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR [6-8]. The facts described above will serve as evidence of the importance of the study of the connection between the antinociceptive action of dalargin, when used in therapeutic doses, with another component of general anesthesia, namely myoplegia. The antioxidative effect of dalargin on the liver [5] and pancreas [2] has been demonstrated experimentally, and it has therefore been used for the prevention of postoperative pancreatitis.

The aim of this investigation was to study activity of an intracellular enzyme, xanthine oxidase (EC 1.2.3.2), catalyzing the formation of hydrogen peroxide and the superoxide anion-radical, one component of the lipid peroxidation (LPO) system, in the brain, which can serve as an important metabolic test of the efficacy of action of the opioid peptide analog dalargin.

EXPERIMENTAL METHOD

Experiments were carried out on 70 noninbred albino rats weighing on average 200 g, anesthetized with ether. Tracheotomy was performed on all the animals, and the trachea was intubated with a PVC catheter, which was followed by mechanical artificial ventilation of the lungs (AVL) under moderate hyperventilation conditions: respiration rate 70 cycles/min, volume 2.1-2.3 ml. To make the conditions of the model as close as possible to those in clinical medicine, all the preparations were injected into a central vein (the right jugular), into which 18 g/1.2 mm OD (Sweden) venous cannulas had been introduced. The animals were divided into four groups: groups 1 (control) consisted of 15 rats without myoplegia, receiving an injection of

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