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MOLECULAR MECHANISMS OF THE MEMBRANE-PROTECTIVE EFFECT OF LITHIUM NICOTINATE IN CHRONIC STRESS

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One of the leading trigger mechanisms in the development of stress-induced injuries is activation of free-radical oxidation (FRO) of lipids, which leads to changes in membrane structures, to disturbance of lipid–lipid and lipid–protein interactions, and modification of the properties of membrane enzymes [3, 9]. The organization of resistance of the body's adaptation to stress can be facilitated and accelerated with the aid of tranquilizers and antioxidants. One representative of these groups is lithium nicotinate, an atypical tranquilizer with a nootropic component of action, whose stress-protective properties are due to its membranotropic activity and its correcting influence on energy metabolism [5].

During this investigation several parameters of the morphological and physiological state of the cell membranes were studied under conditions of chronic stress and its prevention by lithium nicotinate. The total content of phospholipids (PL) and their fractions, total cholesterol (ChS), the ratio ChS/PL, and also the state of structural antioxidants and activity of enzymes detoxicating active forms of oxygen and lipid peroxides, were studied. The erythrocyte membrane was chosen as test object, for it allows the function of the membranes of the body as a whole to be judged [15].

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TABLE 1. State of Antiradical Protection System and Content of Principal Lipid Components in Erythrocyte Membranes during Chronic Stress against the Background of Preventive Administration of Lithium Nicotinate

Parameter studied	Group of animals					
	1-	2-	3-	p_{1-2}	p_{1-3}	p_{2-3}
SOD, conventional units/ mg protein	8,04±0,23	2,77±0,11	5,70±0,17	<0,001	<0,001	<0,001
Catalase, mmoles H_2O_2 /min·g protein	27,91±0,52	18,86±0,45	24,63±0,38	<0,001	<0,001	<0,001
TAOA of erythrocytes, μeq/min·ml packed erythrocytes	7,34±0,20	4,27±0,14	6,55±0,37	<0,001	>0,05	<0,001
PRE, % hemolysis	13,00±0,74	17,10±0,73	13,66±1,18	<0,01	>0,05	<0,05
Plasma TPA, conventional units/ml plasma	4,80±0,19	12,87±1,20	5,74±0,56	<0,001	>0,05	<0,001
Total PL, mg/100 ml packed erythrocytes	310,5±10,08	224,7±8,65	286,7±5,42	<0,001	>0,05	<0,001
PL fractions, %						
LPCh	6,52±0,57	14,91±0,67	7,65±0,35	<0,001	>0,05	<0,001
SPM	16,77±1,05	26,34±1,06	19,74±1,63	<0,001	>0,05	<0,01
PCh	49,08±1,69	37,78±1,20	48,19±2,03	<0,001	>0,05	<0,002
PEA	27,63±1,27	20,97±1,25	24,42±1,12	<0,01	>0,05	>0,05
Total ChS, m/100 ml packed erythrocytes	95,04±2,87	133,33±3,22	93,87±3,45	<0,001	>0,05	<0,001
ChS/PL, molar coefficient	0,62±0,03	1,19±0,04	0,66±0,03	<0,001	>0,05	<0,001

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar albino rats weighing 180-220 g. The animals were divided into three groups: 1) control, 2) animals with reproduced stress, 3) animals with chronic stress against the background of preventive administration of lithium nicotinate (30 mg/kg, subcutaneously, for 10 days). Chronic stress, corresponding to the stage of exhaustion, was produced by deprivation of sleep for 4 days. The rats were killed by decapitation and the blood stabilized with 4% sodium citrate in the ratio of 1:10. Erythrocytes were separated by centrifugation (3000 rpm, 10 min, 4°C, followed by washing 3 times with isotonic solution. Total peroxidase activity (TPA) was determined in the plasma [11]. Activity of superoxide dismutase (SOD) [16] and catalase [4], total antioxidative activity (TAOA) [13], and peroxide resistance of the erythrocytes (PRE) [2] were studied and the total ChS content was determined in the lipid extract [12]. Activity of the enzymes was calculated per milligram protein, determined by Lowry's method. Fractions of PL were obtained by thin-layer chromatography on silufol UV-254 plates (Czechoslovakia) and identified against known values of RF and reference substances. The quantity of individual PL fractions was determined from the content of lipid phosphorus and expressed in per cent. Total PL were judged by the sum of the separate fractions.

EXPERIMENTAL RESULTS

Chronic stress in the exhaustion stage is characterized by marked decompensation of the antiradical protection system of the cell. Activity of the key enzyme of antiradical defense, namely SOD, which controls enzymic dismutation of the superoxide anion-radical into less reactive H_2O_2 and triplet oxygen molecules, was reduced by almost two-thirds to 2.77 ± 0.11 compared with 8.04 ± 0.23 conventional unit/mg protein in the control ($p < 0.001$). The considerable disturbances of activity of these antioxidative enzymes are evidence of far advanced, uncontrollable processes of lipid peroxidation (LPO) with subsequent modification of SH-groups in the active centers of proteins [1], accumulation of active forms of oxygen, lipid hydroperoxides, and free fatty acids (FFA) in erythrocyte membranes, on which they have a damaging action [3]. As a result of activation of LPO not only the enzymic, but also the structural part of the antioxidant system of the cell is impaired: TAOA of the erythrocytes was reduced by 42% ($p < 0.001$), and the peroxide resistance, reflecting the availability of natural antioxidants to biomembranes and demonstrating their resistance to the damaging action of exogenous H_2O_2, also was considerably depressed. Whereas in intact animals the percentage of peroxide hemolysis was 13.00 ± 0.74, during stress it increased significantly to 17.10 ± 0.73 ($p < 0.01$). Preventive administration of lithium nitocinate largely prevented exhaustion of the antioxidant system of the body developing under conditions of stress. Under these circumstances SOD and catalase activity of the stressed animals was maintained at quite a high level (5.70 ± 0.17 conventional unit/mg protein and 24.63 ± 0.38 mmole H_2O_2/min · g protein, respectively). TAOA and PRE remained virtually within their original limits (Table 1).

The raised background level of peroxide formation during stress adversey affected the functional activity of the biological membrane and, in particular, the realization of membrane permeability. The increase of 168% ($p < 0.001$) in the plasma TPA during stress is an additional indicator of membrane destruction. An increase in the value of this parameter is evidence not only of increased permeability, but also of intensified intravascular hemolysis of erythrocytes. On the other hand, an increase in the

content of extraerythrocytic hemoglobin and of its iron-containing breakdown products is an additional factor potentiating lipid peroxidation [7]. During stress-induced destruction of leukocyte membranes the possibility of an increase in TPA due to the escape of the enzyme myeloperoxidase into the plasma cannot be ruled out. Lithium nicotinate restores normal membrane permeability, and the blood enzymes levels, which are high during stress, remain at virtually their initial level (Table 1).

An important place in the stabilization of structural and functional integrity of the biomembrane is occupied by PL, which are themselves the substrate for FRO. During stress the total PL content in the erythrocytes was significantly reduced (by 28%, $p < 0.001$), mainly due to the "flushing out" of the more readily oxidized fractions — phosphatidylcholine (PCh) and, in particular, phosphatidylethanolamine (PEA) (from 49.08 ± 1.69 in the control to 37.78 ± 1.20% during stress for PCh and from 27.63 ± 1.27 to 20.97 ± 1.25% for PEA). The possibility cannot be ruled out that under conditions of energy deficiency, characteristic of chronic stress in the exhaustion stage, readily oxidized PL play the role of additional oxidation substrate, and are utilized in compensatory and adaptive reactions. A significant increase in the relative content of the fraction readily oxidized — sphingomyelin (SPM) — was observed at the same time, and there was a more than twofold increase in the lysophosphatidylcholine (LPCh) level. According to some workers [3, 9], these changes in the phospholipid spectrum are evidence of activation of lipid peroxidation and of a disturbance of PL metabolism as a result of damage to the mechanisms of their acylation and reacylation. A special role in the development of stress-induced biomembrane destruction is ascribed to the LPCh fraction, a specific marker of phospholipase activity, with a marked membranolytic and cytotoxic action. Stress-induced activation of phospholipase hydrolysis, together with activation of LPO, is an additional factor in the modification of cell membranes. Oxidized PL are more easily attacked by endogenous phospholipases, and this enhances their membrane-destructive action [10]. Lithium nicotinate prevents the disturbance of phospholipid metabolism of erythrocytes in chronic stress. Their total level was 286.7 ± 5.42 compared with 224.7 ± 8.65 mg/100 ml packed erythrocytes during stress ($p < 0.001$). The quantitative ratio between individual phospholipid fractions also returned close to its original level (Table 1). Normalization of the LPCh content reflects a significant decrease in the intensity of LPO and also, evidently, activity of phospholipase A2. Under the influence of lithium nicotinate the PEA level flattened out significantly and the relative PCh content was virtually unchanged; it is this PL fraction that is most labile relative to phospholipase hydrolysis [8]. There is evidence that accumulation of the principal product of phospholipase hydrolysis (LPCh) leads to swelling of the mitochondrial membranes, disturbance of their function, inhibition of synthesis, of high-energy compounds, and disturbance of the ability of the mitochondria to accumulate Ca^{2+}. It has been suggested that LPCh is a powerful inhibitor of electron transport in the respiratory chain, and the effect of swelling of the mitochondria is linked with the action of lysophospholipids and peroxides of fatty acids as detergents [14]. Previous investigations [5] showed that lithium nicotinate prevents swelling of the mitochondria, stabilizes their membrane, reduces the FFA content, stimulates and coordinates tissue respiration and oxidative phosphorylation and, consequently, increases the concentration of high-energy compounds. If it is considered that changes in the basic parameters of phospholipid metabolism in erythrocytes and brain mitochondria are coordinated [15], it can be asserted that one of the leading mechanisms of the membrane-stabilizing action of lithium nicotinate is normalization of the LPCh content and, consequently, of phospholipase hydrolysis processes as a whole.

Another, no less important, structural component of biomembranes is cholesterol, which participates actively in the formation of the lipid bilayer, whose structural and functional activity is to some extent dependent on changes in the value of the ChS/PL ratio. Accumulation of ChS in the membranes increases the degree of packing of PL and the microviscosity of the lipid bilayer. The experimental results show that during chronic stress the total ChS content in the erythrocytes arises by 40% ($p < 0.001$) whereas the molar ratio ChS/PL is almost doubled (Table 1). Stress-induced hypercholesterolemia is evidently determined by disturbances of ChS turnover between high- and low-density lipoproteins, and also the erythrocyte membrane. Peroxide-induced damage to the principal enzyme of ChS catabolism in the liver, namely cholesterol 7a-hydroxylase, followed by accumulation of ChS in the blood cells, likewise cannot be ruled out [6]. Preventive administration of lithium nicotinate completely prevented the development of stress-induced hypercholesterolemia. The total ChS content and the ChS/PL ratio were virtually indistinguishable from their initial values.

The results described above thus demonstrate the effective stress-protective action of lithium nicotinate, realized in nonspecific protection of cell membrane structures. The membrane-protective action of lithium nicotinate is evidently determined by its antioxidative effects, and amounts to nonspecific defense and stimulation of the intrinsic antiradical defense system of the cell, with subsequent weakening of the intensity of phospholipase hydrolysis.

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