MOLECULAR-BIOCHEMICAL MECHANISMS OF THE ACTION OF NOOTROPIC DRUGS

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The search for drugs with an antistressor action among derivatives of GABA, which occupies a leading place in the development of adaptation to extremal factors, A topic of particular interest is the nootropic drugs, which improve energy metabolism in the tissues, stimulate RNA and protein synthesis, accelerate the turnover of mediators and important amino acids, and improve the microcirculation [10]. This suggests that nootropic drugs exhibit activity relative to nerve and other tissues equally. Considering that one of the leading mechanisms of formation of the stress syndrome is systemic labilization of cell membranes [9], it is interesting to study the structure and functional activity of biomembranes of brain mitochondria and erythrocytes during the prevention of stress by nootropic drugs. Since the pharmacological mechanisms of the action of nootropic drugs have not been adequately studied, and since there are only isolated and contradictory references in the literature to the membranotropic activity of these compounds, the aim of the present investigation was to study the presence of a membrane-protective effect in a number of nootropic agents and the possibility of realization of that effect at the level of membrane structures not only of the brain, but also of other tissues.

EXPERIMENTAL METHOD

The state of the enzyme antiradical protection (ARP) system, the level of lipid peroxidation (LPO) products, the qualitative and quantitative ratios between individual fractions of phospholipids (PL), and activity of marker enzymes of the plasma membranes, were studied in experiments on 100 male Wistar rats weighing 180-220 g, with a view to obtaining objective characteristics of the state of the membranes of the body as a whole. The animals were divided into five groups: 1) control, 2) animals with a model of stress, 3, 4, and 5) animals with chronic stress against the background of preventive injections of piracetam (500 mg/kg), picamilon (10 mg/kg), and the new GABA derivative B-44 (30 mg/kg) respectively. (Picamilon is the sodium salt of N-nicotinoyl-γ-aminobutyric acid). Chronic stress in the stage of exhaustion was created by Jouvet's method in the course of 4 days. The brain was removed, cooled, and homogenized with an ice-cold solution of 0.25 M sucrose-Tris (pH 7.4) and the mitochondria were isolated by differential centrifugation [8]. The blood was 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. Catalase [6] and superoxide dismutase (SOD) [5] activity, the malonic dialdehyde (MDA) concentration [12], and Na^+, K^+-ATPase and 5′-nucleotidase (5′-NC) [11] were determined in the erythrocytes and in brain mitochondria, disintegrated beforehand with 0.2% Triton X-100 solution. Considering that Na^+,K^+ -ATPase and 5′-NC are marker enzymes specific for plasma membranes, their activity was studied in brain homogenate. Concentrations of total PL and their fractions — lysophosphatidylcholine (LPC), sphingomyelin (SPM), phosphatidylcholine (PC), and phosphatidylethanolamine (PEA) — were determined in the lipid extract of the erythrocytes by thin-layer chromatography, and the compounds were identified from their known *Rf* values and by comparison with reference substances. The quantity of each PL fraction was determined as the content of lipid phosphorus.

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TABLE 1. State of the Antiradical Protection System, Activity of Membrane-Bound Enzymes, and Content of Individual Phospholipid Fractions in Membrane Structures of Brain and Erythrocytes during Chronic Stress and Its Prevention by Nootropic Drugs

Parameter studied	Control	Stress	Stress + piracetam	Stress + picamilon	Stress + B-44
MDA, $\frac{\text{nmoles/mg protein}}{\text{nmoles/ml erythrocytes}}$	0,114±0,005 / 352,6±15,5	0,200±0,007 / 600,4±11,1	0,159±0,009* / 456,6±10,7*	0,138±0,003* / 381,6±10,8*	0,134±0,007* / 431,0±9,3*
Catalase, $\frac{\text{mmoles } H_2O_2}{\text{min·g protein}}$	1,748±0,071 / 27,91±0,52	1,301±0,027 / 18,86±0,44	1,447±0,001* / 25,18±0,43*	1,608±0,029* / 25,98±0,46*	1,510±0,055* / 24,56±0,51*
SOD, conventional units/mg protein	16,05±0,53 / 8,04±0,23	8,37±0,35 / 2,77±0,11	11,32±0,43* / 6,85±0,14*	15,00±0,31* / 7,70±0,28*	11,79±0,34* / 7,99±0,75*
Na^+,K^+-ATPase, $\frac{\text{nmoles } P_i}{\text{min·mg protein}}$	63,45±1,55 / 0,135±0,005	47,04±1,52 / 0,075±0,007	58,66±1,60* / 0,114±0,008*	65,16±0,75* / 0,123±0,01*	73,74±0,96* / 0,121±0,009*
5'-NC, $\frac{\text{nmoles } P_i}{\text{min·mg protein}}$	32,49±0,45 / 0,155±0,005	37,21±0,54 / 0,198±0,008	33,92±1,11* / 0,150±0,007*	33,88±0,82* / 0,157±0,005*	29,68±1,28* / 0,111±0,009*
Total PL, mg/100 ml erythrocytes	310,5±10,1	224,7±8,6	304,3±5,1*	297,0±7,4*	272,2±2,5*
PL fractions: LPC	20,37±2,14	33,34±1,27	36,42±2,56	24,38±2,72*	29,32±1,30
SPM	52,16±4,04	59,26±3,58	60,19±5,25	54,62±2,89	53,70±1,59
PC	151,8±4,66	85,19±5,24	129,9±6,21*	136,9±6,58*	117,9±4,08*
PEA	86,11±5,77	46,91±2,51	77,78±3,73*	81,10±4,21*	71,30±1,57*

Legend. Numerator — brain, denominator — erythrocytes; asterisk indicates difference significant compared with stress.

EXPERIMENTAL RESULTS

Chronic stress in the exhaustion stage is characterized by profound destructive disturbances of morphology and function of the cell membranes of the body. The content of the end product of LPO (MDA) in brain mitochondria and erythrocytes is increased by more than 70% in stress, whereas activity of the key enzymes of ARP, namely catalase and SOD, regulating catabolism of hydrogen peroxide and of the superoxide anion-radical, and thereby preventing lipid peroxidation at its initiation stage, is significantly depressed. Reduction of catalase activity in the brain and erythrocytes by 26 and 33% respectively ($p < 0.001$) leads to the accumulation of H_2O_2, a natural SOD inhibitor, in these structures, and the possibility therefore is not ruled out that enzyme SH-groups may undergo peroxide damage. As a result, SOD activity in the brain fell from 16.05 ± 0.53 conventional unit/mg protein in the control to 8.37 ± 0.355 conventional unit/mg protein during stress, whereas in the erythrocytes it fell by almost two-thirds (Table 1). Disturbance of the mechanisms regulating the formation and utilization of active forms of oxygen leads to degradation of lipid—lipid and lipid—protein interactions and to opposite changes in activity of the lipid-dependent marker enzymes of the plasma membranes. Na^+,K^+-ATPase activity in the brain and erythrocytes during stress was regularly depressed by 26 and 45% ($p < 0.001$), and that of 5'-NC was increased by 15 and 27% respectively ($p < 0.001$). Incidentally, whereas the functional activity of Na^+, K^+-ATPase has been studied in sufficient detail during the damaging action of stress [3, 7], activity of the other membrane marker (5'-NC) has virtually not been studied under similar conditions. Yet 5'-NC is a leading component of the regulatory system of nucleic acid metabolism and of synthesis and transmembrane transport of adenosine. The degree of its activity is determined by the state of the membranes, a character of the bond with the structural elements and, in particular, with PL, and it is inversely related to the intensity of cell metabolism, which suggests that 5'-NC may be called a "degradation enzyme." The strength of the bond of the enzyme with the membrane determines the degree of depression of its activity [1]. Activation of 5'-NC during stress is evidence of destruction of the cell membranes. This process also is facilitated by a fall of the ATP and creatine phosphate levels, for these are natural inhibitors of 5'-NC [7].

Preventive administration of nootropic agents largely prevented exhaustion of the antioxidant system, developing under conditions of stress. SOD and catalase activity in the stressed animals, in both mitochondria and erythrocytes, was maintained at a sufficiently high level, which effectively protected the membrane against accumulation of LPO products. Under these circumstances new GABA derivatives — picamilon and B-44 — not only are not inferior to piracetam in the efficacy of their membrane-protective action, but with respect to some parameters they actually exceed it (Table 1). The effect of picamilon was so strong that the parameters studied during stress were virtually identical with levels in the intact group. Normalization of the working of the Na^+,K^+-pump and of 5'-NC activity by nootropic agents during stress was evidently due both to their antioxidative effects and to metabolic restructuring of the cell membranes, followed by stabilization of the disturbed lipid—lipid and

lipid–protein complexes. In this regard the GABA derivative B-44, prophylactic injection of which increased Na^+,K^+-ATPase activity and correspondingly depressed 5′-NC activity, not only by comparison with the stressed, but also in the intact group, performed particularly well. The marked increase in the intensity of protein and nucleic acid synthesis characteristic of GABA derivatives [4] under conditions of stress stabilizes the protein component of the membrane, including Na^+,K^+-ATPase and 5′-NC, which are integral protein enzymes. The increased turnover of ATP and PL, which is a feature of the nootropic agents studied [13], also had a beneficial effect on the functional stability of the membrane markers.

Normalization of the relative quantitative proportions of the individual PL fractions confirmed the marked stabilizing effect of the nootropic drugs against the damaging action of chronic stress. PL are not only very important structural components of biomembranes and the substrate for free-radical oxidation, but they are also allosteric effectors of membrane-bound enzymes, protecting the protein component of the membrane against pathological hydrolysis [5, 14]. Piracetamand picamilon under stress conditions prevented degradation of the phospholipid basis of the cell virtually completely, but the qualitative ratio between individual PL fractions in the erythrocytes of animals receiving picamilon was more physiological, a fact which distinguishes this compound favorably from the other nootropic drugs. Piracetam did not lower the LPC content when almost doubled during stress. This was evidently due to its ability to activate phospholipase A_2 [13]. However, it virtually completely preserved the concentrations of the most labile fractions during stress, namely PEA and PC. The GABA analog B-44 also prevented a decrease in the content of total PL and a change in the relative content of their fractions, but this effect was weaker than that of piracetam and picamilon. This suggests that besides the other mechanisms of stabilization of functional activity of membrane-bound enzymes which we have demonstrated, B-44 may also have other mechanisms for this purpose.

The results indicate a marked membrane-protective action of the nootropic drugs studied in chronic stress, realized both in the membranous structures of the brain and in the erythrocytes. This confirms the opinion of a number of workers to the effect that nootropic drugs possess extracerebral properties, and that they have a general metabolic action at the whole body level [2, 13]. These data may provide an experimental basis for their widespread application as drugs for the prevention of extremal states accompanied by structural changes in membranes.

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