ROLE OF LIPIDS IN CHANGES IN PROPERTIES OF BRAIN β-ADRENORECEPTORS UNDER THE INFLUENCE OF EMOTIONAL-PAINFUL STRESS

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One of the consequences of stress is a marked decrease in adrenoreceptor activity [2]. This effect may be based on a disturbance of mechanisms that participate in the transmembrane transmission of signals from the adrenoreceptors to the effector enzyme systems (adenylate cyclase, proteinkinases, etc.), in the realization of which an important role is ascribed to lipids of the plasma membranes of nerve cells [6]. At the same time, in stress substantial changes are known to take place in lipid metabolism, and among the more important of these changes are activation of lipases (and phosphorylases) and activation of lipid peroxidation (LPO) [1, 3].

For the reasons given above, and considering the evidence of sharp changes in the properties of neuronal receptors during LPO and the action of phospholipase A_2 [8], it was decided to estimate the contribution of each of these factors in changes in the properties of brain β-adrenoreceptors during severe emotional–painful stress (EPS).

EXPERIMENTAL METHOD

Male Wistar rats weighing 160-180 g were exposed for 6 h to EPS [5], and decapitated 2 h after the end of exposure. The gray matter of the cerebral cortex was homogenized in a Potter–Elvehjem homogenizer in 10 volumes of medium containing 0.32 M sucrose, 1 mM EDTA, and 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged for 10 min at 1000g. The supernatant was collected and the residue homogenized under the same conditions and centrifuged. The operation was repeated once. The three supernatants thus obtained were pooled and centrifuged for 20 min at 10,000g. The residue was reprecipitated in 50 mM Tris-HCl and subjected for 2 h to hypo-osmotic shock in 6 mM Tris-HCl, pH 7.4. After sedimentation for 20 min at 20,000g, a coarse fraction of synaptosomal membranes was obtained. The protein concentration was determined by the biuret method. Ligand–receptor interaction was analyzed by the use of [^{3}H]dihydroalprenolol. After incubation for 20 min at 37°C the samples were filtered through glass gF/B filters. Radioactivity of the filters was measured by the scintillation method. Specific binding of the ligand was determined as the difference between its binding in the absence and in the presence of an excess of the nonradioactive antagonist D,L-propranolol. Lipids were extracted [4], then subjected to thin-layer chromatography on plates with kieselguhr (No. 5721, from Merck, West Germany). Phospholipids were fractionated in a chloroform–methanol–H_2O system in the ratio 65:25:4, and neutral lipids in a hexane–ether–acetic acid system in the ratio 70:30:1. The spots were developed by sprinkling the plates with 10% H_2SO_4 in methanol and then heating them to 150°C. Scanning was carried out on an "Opton" chromatograph equipped with "W + W" 3218 integrating automatic writer. The fatty acid composition of the lipids was determined after methylation on a Perkin-Elmer F22 gas–liquid chromatograph.

EXPERIMENTAL RESULTS

The study of binding of [^{3}H]dihydroalprenolol by membranes of the coarse synaptosomal fraction of rat brain showed that EPS causes a decrease in the level of specific binding of

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TABLE 1. Changes in Binding of [^{3}H]dihydroalprenolol by β-Adrenoreceptors of Rat Brain Synaptosomal Fraction after EPS (M ± m)

Experimental conditions	Dissociation constant nM	Specific binding, fmoles/mg protein
Control	0,90±0,12	1000±140
EPS	1,40±0,12	580±83
	0,10±0,01	120±21
4-methyl-2,6-di-tert-butylphenol + EPS	0,90±0,10	780±91

TABLE 2. Lipid Composition (in percent) of Rat Cerebral Cortex under Normal Conditions and after EPS (M ± m)

Experimental conditions	Control	EPS
Free fatty acids	2,2±0,6	2,4±0,3
Cholesterol	37,7±6,1	35,4±4,4
Other neutral lipids	7,1±1,0	7,7±0,8
Gangliosides	14,5±0,9	12,6±0,8
Phosphatidylethanolamine	18,7±3,0	19,2±3,2
Phosphatidylcholine	10,4±1,7	11,2±2,1
Phosphatidylinositol	1,4±0,2	1,2±0,3
Phosphatidylserine	6,9±1,8	8,5±2,3
Sphingomyelin	0,8±0,3	1,4±0,3
Lysophosphatidylcholine	0,3±0,1	0,4±0,1

TABLE 3. Concentration of Primary and End Products of LPO in Rat Cerebral Cortex before and after EPS (M ± m)

Experimental conditions	Diene conjugates, optical density units at 232 nm	Hydroperoxides of phospholipids, nmoles/mg lipids	Fluorescence of Schiff bases, relative units
Control	0,15±0,02	0,52±0,09	1,00±0,12
EPS	0,45±0,05	1,93±0,33	2,65±0,41
4-methyl-2,6-di-tert-butylphenol	0,15±0,02	0,44±0,10	1,31±0 09
4-methyl-2,6-di-tert-butylphenol + EPS	0,25±0,04	0,50±0,10	1,82±0 29

the ligand. The number of specific binding sites with dissociation constant of 0.9-1.4 nM 2 h after the end of EPS did not exceed 60% of the initial value; the decrease in binding of the ligand, moreover, was based both on a change in the number of specific binding sites and in their affinity for the ligand (Table 1).

Changes arising in the brain lipids during EPS were analyzed in a series of experiments. It follows from Table 2 that as a result of EPS no changes took place in the composition of the brain membrane lipids: the concentrations of the main classes of phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine), of gangliosides, and of neutral lipids was the same in the control and after EPS. The absence of changes in the concentration of free fatty acids and of monoacyl glycerophosphatides — hydrolysis products of phospholipids by phospholipases A_2 — must be particularly emphasized. Analysis of the fatty-acid composition of the lipids showed that EPS does not change the fatty-acid spectrum of the lipids. Meanwhile determination of products of free-radical oxidation of lipids indicated substantial activation of LPO by EPS (Table 3). The question arises: why does the accumulation of LPO products observed during EPS not come to light on analysis of the lipid and fatty-acid composition? These facts can evidently be explained on the grounds that during accumulation of 1.93 ± 0.33 nmoles of hydroperoxides per milligram lipids (i.e., during maximal accumulation of LPO products in the brain, recorded during EPS), not more than 0.2% of the lipids undergoes oxidative modification, and this amount lies outside the limits of sensitivity of chromatographic methods of lipid analysis and within the limits of individual scatter of the data.

Of the two mechanisms of modification of the lipid bilayer of the membranes, capable of causing a fall in the level of specific binding of [^{3}H]dihydroalprenolol, which were examined it is evident that only one, namely activation of LPO, is a realistic cause of this effect during EPS. This conclusion was confirmed experimentally by the results in Tables 1 and 3, showing the ability of the antioxidant 4-methyl-2,6-di-tert-butylphenol, administered to the animals beforehand, to prevent both the accumulation of LPO products caused by EPS and the lowering of the level of specific binding of [^{3}H]dihydroalprenolol at the same time.

According to recent data [6] hydrolysis of phosphatidylcholine by phospholipase A_2 with the formation of free fatty acids and monoacyl glycerophosphatides always takes place during transmission of signals from β-adrenoreceptors to adenylate cyclase and it is an essential condition of this process, and one which takes place intensively in nerve tissues during activation of the CNS under stress conditions. The absence of any signs of accumulation of enzymic hydrolysis products in brain cell membranes during EPS may indicate the existence of

highly active phospholipid repair systems (acyltransferase, cyclo-oxygenase), enabling the system to return to its initial state and thus preventing the accumulation of hydrolyzed residues of phospholipids and free fatty acids in the membranes, where they could change the properties of the receptors.

LITERATURE CITED

1. M. L. Libe, E. D. Bogdanova, A. E. Rozenberg, et al., Byull. Éksp. Biol. Med., No. 11, 552 (1981).
2. F. Z .Meerson, M. S. Gorina, and A. M. Zeland, Byull. Éksp.,Biol. Med., No. 11, 528 (1979).
3. F. Z. Meerson, V. E. Kagan, L. L. Prilipko, et al., Byull. Éksp. Biol. Med., No. 10, 404 (1979).
4. E. G. Bligh and W. S. T. Dyer, Can. J. Biochem., 37, 911 (1959).
5. O. Desiderato, J. R. MacKinnon, and H. Hisson, J. Comp. Physiol. Psychol., 87, 208 (1974).
6. F. Hirata and J. Axelrod, Science, 209, 1082 (1980).
7. E. Ueno and K. Kurijama, Neuropharmacology, 20, No. 12A, 1169 (1981).

EFFECT OF IMMOBILIZATION STRESS ON THE ADRENERGIC INNERVATION OF THE RAT MESENTERY AND DURA MATER

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Adrenergic (sympathetic) innervation structures are inseparable components of peripheral catecholaminergic systems which, together with central systems, play an important role in the mechanisms of onset and development of the response to stressors. Processes of tissue metabolism and the adaptive activity of the organism at different levels of its integration are directly linked with the trophic and trigger (effector) function of peripheral adrenergic nerves [1-3, 9]. Meanwhile the problem of the morphological and functional state of the peripheral adrenergic innervation under extremal conditions has not attracted the attention it deserves from the research worker. There have been only single investigations which have shown directly or indirectly that it participates in the general reaction of the body to stress [4, 5]. Essentially, however, the dynamics of mediator activity of adrenergic nerves at the various stages of stress has not been seriously studied, nor have any reliable criteria been developed for the objective assessment of the corresponding processes.

Accordingly, in the investigation described below a model of immobilization stress was used to study the adrenergic innervation of the rat mesentery and dura mater.

EXPERIMENTAL METHOD

Experiments were conducted on male rats weighing 180 ± 30 g. The animals were immobilized for 1, 4, 6, 16, and 24 h. The duration of the experiments was determined by analysis of the dynamics of the somatic manifestations of the animal's stressor reaction in this particular model of immobilization stress established previously by the writers [6]. Total preparations of mesentery of the small intestine and dura mater were studied. Adrenergic structures were detected histochemically by the fluorescence-microscopic method of Falk and Hillarp, in the writers' modification [10]. The morphological and functional state of the adrenergic innervation of the mesentery and dura mater was estimated by qualitative (visual) and quantitative methods. The latter consisted of determining the intensity of luminescence of the adrenergic nerves by means of an FÉU-19 photosensitive attachment to the ML-2 lumines-

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