PROTECTION OF RAT BRAIN β-ADRENORECEPTORS DURING EMOTIONAL-PAINFUL STRESS BY AN ANTIOXIDANT OF THE STERICALLY HINDERED PHENOL CLASS

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The damaging action of stress factors on the brain is manifested, in particular, by disturbances of function of its membrane structures, One manifestation of this type of damage is a change in the state of the receptors, including β-adrenoreceptors [6, 8]. Damage to membranous structures in stress accounts for the urgency of the search for ways and means of protection against the action of stress factors. Establishment of the key role of lipid peroxidation (LPO) in the realization of stress-induced membrane damage has made possible the investigation of the possibility that antioxidants may have a protective action in stress. It has been demonstrated, for instance, that ionol (2,6-di-*tert*-butyl-4-methylphenol) prevents disturbance of the state of β-adrenoreceptors in emotional-painful stress (EPS) [9]. Nevertheless, the realization of this approach has been limited by the fact that existing phenolic antioxidants (and ionol, in particular) are not sufficiently effective, i.e., they act in quite high concentrations which disturb the barrier properties of the lipid bilayer in vitro [3, 7].

In the search for more effective agents, we undertook a comprehensive study of the protective properties of a new antioxidant of the sterically hindered phenol class, the isoquinoline derivative U18.

EXPERIMENTAL METHOD

Experiments were carried out on 40 noninbred male albino rats. The animals were divided into four equal groups: control, EPS, control + U18, and EPS + U18, The antioxidant was given 40 min before the beginning of EPS in isotonic NaCl solution in a dose of 1 mg/kg body weight intraperitoneally. Instead of the antioxidant, animals of the first two groups received an equal volume of isotonic solution. EPS was created by means of unavoidable electric shocks applied to the animals' limbs for 2 h, as described in [2]. The animals were then immobilized in tubes, their blood pressure was measured at the base of the tail, and their heart rate and respiration rate were recorded by photoelectric electroplethysmography [5]. After 1 h the animals were decapitated. The cerebral cortex was used to isolate the coarse microsomal fraction by a method based on that in [12, 14]. The state of the membrane β-adrenoreceptors of this fraction was assessed relative to binding of ^{3}H-dehydroalprenolol, values of K_D and B_{max} being calculated by the method in [15]. Concentrations of products reacting with 2-thiobarbituric acid (TBA) [13], fluorescent Schiff's bases [11], and superoxide-scavenging activity (SSA) [10] were determined in the coarse microsomal fraction of the brain. Concentrations of TBA active products and SSA also were determined in serum from blood obtained at decapitation.

The reagents used were as follows: ^{3}H-dehydroalprenolol from "Amersham," sucrose, HEPES, Tris, and TBA from "Serva," and NaCl and KCl from "Merck." The remaining preparations were of Soviet manufacture and not below the chemically pure grade.

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TABLE 1. Effect of Stress and U18 on State of β-Adrenoreceptors of Rat Brain Synaptic Membranes

Experimental conditions	Parameters of binding of ^{3}H-dehydroal	
	K_D, nM	B_{max}, fmoles/mg protein
Control	7,3±0,2	10,5±0,5
	7,8±0,2	4,5±0,4**
Stress	10,0±0,1**	4,5±0,2**
U18 + stress	8,3±0,1*	3,8±0,4**

Legend. Here and in Table 3, significance of differences from control: *p < 0.05, **p < 0.01.

TABLE 2. Effect of Stress and U18 on Parameters of LPO in Rat Brain and Blood

Parameter	Control	Experimental conditions		
		U18	stress	U18 + control
TBA-active products in brain homogenate	100	53,2±10,6**	223,4±24,3**	95,9±9
Schiff's bases in brain homogenate	100	92,1±3,1*	159,7±5,6**	103,5±3
SSA in brain homogenate	100	216,4±19,0**	69,9±8,5**	155,8±14,
TBA active products in blood serum	100	64,3±8,9**	244,9±17,5**	125,6±10,5
SSA in blood serum	100	182,3±15,6**	65,6±10,3**	119,8±9,8

Legend. All values given as percentages of corresponding values in control group. Significance of differences from control values: *p < 0.01, **p < 0.001

TABLE 3. Effect of Stress and U18 on Autonomic Parameters in Rats

Experimental conditions	control	Experimental conditions		
		U18	stress	U18+ stress
Blood pressure, mm Hg	120,0±7,6	108,0±6,4*	100,0±6,3**	127,3±7,9
Hildebrandt's index, rel. units	6,8±0,3	6,5±0,2	8,2±0,5**	7,0±0,4

EXPERIMENTAL RESULTS

It will be clear from Table 1 that EPS caused changes in the properties of the brain β-adrenoreceptors, expressed as a significant increase of K_D and decrease of B_{max}. Injection of U18 into the control animals led to a comparable decrease in B_{max} which, however, was not accompanied by any significant change in K_D. Injection of U18 before stress prevented the increase in K_D but not the decrease in B_{max}. It must be noted that U18 in the control had an effect on the state of the β-adrenoreceptors similar to the action of EPS. This fact is in agreement with known ideas relating to the similar effect of adaptogens and stress in certain of its phases on membrane structures [1, 4].

As was pointed out above, injury to β-adrenoreceptors during EPS was due to activation of LPO. In fact, as can be seen in Table 2, EPS led to a significant accumulation of LPO products in the brain and blood, accompanied by a decrease in SSA — the most important component of the antioxidative system of the body. U18 increased SSA, and this evidently was responsible for preventing activation of LPO as a result of EPS. The antioxidative effect of U18 was much stronger in the brain (virtually complete normalization of LPO) than in the blood.

The protective effects of U18 at the brain membrane level corresponded to its general adaptogenic action (Table 3). U18 prevented the fall of blood pressure and sharp increase in Hildebrandt's index (ratio of heart rate to respiration rate) induced by EPS.

Thus the antioxidant U18 in a dose of 1 mg/kg has a marked protective action during EPS, manifested as normalization of autonomic parameters, parameters of LPO in the blood and brain, and the state of the brain β-adrenoreceptors. It must be emphasized that protection of β-adrenoreceptors by U18 under conditions of EPS is not complete, for when the value of K_D returns to normal the number of binding sites still remains low. This is evidently due to solubilization of receptors from the membranes as a result of accumulation of products of hydrolysis of phospholipids by phospholipase A2 in them, which is characteristic of EPS [8].

Allowing for the fact that the protective effect of U18 is achieved in doses 1 or 2 orders of magnitude below doses of ionol, when used in EPS [6], this new compound can be regarded as promising for use in the protection of membranes against free-radical injury.

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