have not yet been clarified. Nevertheless, activation of one of the main enzymes of the AOES is undoubtedly important for compensating for the disturbances in LPO regulation, which occur in epileptic patients [9]. This effect of TP is most likely largely responsible for its anticonvulsant properties.

Thus, the treatment of epileptic patients with α-tocopherol in addition to traditional anticonvulsants leads to increased blood activity of superoxide dismutase, which explains to a certain extent its anticonvulsant properties.

REFERENCES

1. A. N. Erin, M. M. Spirin, L. B. Tabidze, *et al.*, *Biokhimiya*, **48**, № 11, 1855-1861 (1983).
2. I. I. Ivanov, in: *Biomembranes: Structure, Function, and Methods of Investigation* [in Russian], Riga (1977), pp. 248-260.
3. V. M. Kovalenko, G. N. Kryzhanovskii, V. S. Kovalenko, E. V. Nikushkin, *et al.*, *Zh. Nevropatol. Psikhiatr.*, **84**, № 6, 892-897 (1984).
4. G. N. Kryzhanovskii, E. V. Nikushkin, V. A. Voronko, *et al.*, *Ibid.*, pp. 806-809.
5. G. N. Kryzhanovskii, I. R. Tupeev, *et al.*, *Byull. Eksp. Biol. Med*,. **104**, № 4, 396-398 (1987).
6. V. Z. Lankin, A. K. Tikhadze, D. R. Rakita, *et al.*, *Biokhimiya*, **48**, № 9, 1555-1559 (1983).
7. A. L. Megrabyan, V. G. Mkhitaryan, M. G. Amadyan, *et al.*, *Zh. Nevropatol. Psikhiatr.*, **86**, № 9, 1407-1410 (1986).
8. E. V. Nikushkin and G. N. Kryzhanovskii, *Pat. Fiziol.*, № 6, 19-24 (1987).
9. E. V. Nikushkin, G. N. Kryzhanovskii, I. R. Tupeev, *et al.*, *Byull. Eksp. Biol. Med.*, **103**, № 3, 297-299 (1987).
10. I. R. Tupeev, M. M. Bordyukov, G. N. Kryzhanovskii, and E. V. Nikushkin, *Ibid.*, **100**, № 11, 538-541 (1985).
11. R. R. Shakarishvili, *Zh. Nevropatol. Psikhiatr.*, **87**, № 6, 825-828 (1987).

Correction of Neuroimmune Reactions by Regulation of Lipid Peroxidation

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The activation of free-radical oxidation (FRO) is of great pathogenic importance [8] in the development of stress-induced alterations and immune disorders in which stress plays a significant role. Experimental allergic encephalomyelitis (EAE) is a model of neuroimmune damage to the brain and is accompanied by an enhancement of FRO [2]. It is known, that guinea pigs possess a high sensitivity to EAE (which may be overcome by a vitamin E-deficient diet), while albino rats show tolerance to the reproduction of this process.

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A comparative study of the stress sensitivity of these species of animals to the model of emotional-pain stress is of interest. The dynamics of autoneurosensitization was used as the criteria of resistance, and the state of FRO processes was examined in parallel.

MATERIALS AND METHODS

Experiments were carried out on male albino rats weighing 150-200g and on guinea pigs of both sexes weighing 250-300 g kept under vivarium conditions in the fall and winter period. Emotional-pain stress (EPS) was reproduced according to a

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TABLE 1. Changes of Content of LPO and AO Products in Rats and Guinea Pigs in Stress ($M \pm m$, $n = 6-12$)

Index	Dienic conjugates, mmol/mg lipids	Schiff bases,% of standard/mg lipids	TBA-active products per mol/mg protein	SOD, EA/ml erythrocytic mass or mg brain protein	GP, mmol/min×mg protein	Vitamin E, mmol/liter serum	Antibody titer
Rats							
Control							
Blood	17.05±1.71	15.46±0.9	4.09±0.17	208.51±8.64	8.98±0.55	10.09±0.38	neg.
Brain	27.37±2.46	13.17±0.93	1.75±0.12	22.96±0.87	2.73±0.40		100%
EPS							
Blood	35.20±2.35*	34.99±2.65*	6.40±0.31*	306.92±9.84*	14.48±0.91*	12.19±0.51*	1:20
							20%
Brain	44.39±1.96*	27.54±2.10*	2.34±0.40*	35.94±1.75*	7.51±0.59*–	–	
EPS + SOD							
Blood	24.49±1.34**	16.52±0.77**	4.32±0.29**	223.29±17.46**	8.79±0.69**	9.55±0.38*	neg.
Brain	35.63±1.88**	14.98±0.76**	1.45±0.07*	25.99±1.46*	4.63±0.46*	–	–
EPS+DSIP							
Blood	–	–	–	308.71±6.82**	5.85±0.85**	8.96±0.13*	neg
Brain	–	–	–	26.47±1.12**	4.63±1.22**	–	–
Lipid diet							
Blood	220.80±27.78*	19.68±1.59*	–	264.60±7.08*	15.14±1.3*	7.76±0.62*	1:20
Brain	42.12±4.94*	27.68±2.09*	–	27.02±1.52*	4.01±0.40*	–	20%
							–
Lipid diet + EPS							
Blood	180.6±18.99	22.88±1.90	–	222.84±7.33	16.3±1.10	7.50±0.90	1:40
							1:80
Brain	72.82±4.84	47.49±3.26	–	21.26±1.43	11.92±1.10	–	100%
							–
Guinea Pigs							
Control							
Blood	27.95±2.48	12.99±0.73	4.57±0.26	198.43±5.15	11.87±1.17	9.58±0.30	neg.
Brain	32.99±2.76	10.72±1.0	1.87±0.08	26.35±0.89	6.89±0.48	–	–
EPS							
Blood	63.20±4.01*	37.32±2.85*	8.99±0.22	138.65±11.32*	6.95±0.28*	7.65±0.23*	1:40
							1:80
							80%
Brain	89.50±11.52*	28.39±2.88*	2.90±0.1*	31.08±1.01*	16.14±0.99	–	–
EPS + SOD							
Blood	34.07±1.75**	10.30±1.85**	6.27±0.29**	220.0±13.2**	8.24±0.25**	9.49±0.33**	1:20
							1:40
							40%
Brain	39.52±2.74**	17.44±1.46**	2.19±0.11**	25.43±0.77**	9.71±0.45**	–	–
EPS + DSIP							
Blood	–	–	–	282.38±9.37**	13.19±1.47**	9.73±0.84*	1:20
							30%
Brain	–	–	–	41.09±3.65**	4.12±0.42**	–	–

Note. neg. means negative titer, * means $p<0.05$ in comparison with control; ** means $p<0.05$ vis–a–vis stress

method described elsewhere [13] with some modifications of the experimental chamber. Stress action lasted 6h and one day later the animals were decapitated. Chemiluminescence (ChL) parameters measured in the blood serum and in homogenate of the cerebral cortex were as follows [4]: spontaneous luminescence (S) reflecting the intensity of endogenous lipid peroxydation (LPO) following a spontaneous course; fast burst (h), which was noted for administration of Fe^{2+} ions to the system with a value depending on the level of hydroperoxides; duration of the latent period (L), depending both on the agents accelerating the oxidation of iron and on the level of antioxidants; the index of the slow burst (H), reflecting the level of LPO, activated by Fe^{2+} ions; the tangent of the slope of the initial phase of the slow burst, depending inversely on the level of antioxidants (AO) in the system (tg α). The level of LPO was determined from the accumulation of thiobarbituric acid (TBA). The content of TBA-active products was detected in the serum [14], and the rate of spontaneous LPO was measured in the brain tissue [3]. The products of dienic conjugation [11] and fluorescent LPO products [12] were determined in an extract of the total lipids of serum and brain tissue. The activity of superoxide dismutase (SOD) [6] and glutathione peroxidase (GP) [5], the content of vitamin E in

the serum [10], and the number of antibodies in the complement-fixation reaction (CFR) [7] were determinted.

The data were statistically processed using the Student t test for small samples. The rank correlation coefficient was calculated in order to determine whether there was a correlation between the indexes of LPO and AO.

RESULTS

The level of determined indexes of primary and secondary LPO products in the serum after stress was significantly higher compared to the control in guinea pigs than in rats, while the activity of enzymes of the AO system and the level of vitamin E decreased in guinea pigs but increased in rats (Table 1). The differences between all measured indexes in percent are significant in rats and guinea pigs . The elevation of the content of LPO products in the brain tissue was more pronounced in guinea pigs than in rats, but the tendency of activation of the AO system enzymes was similar. A comparative analysis of the ChL indexes reflecting the level of LPO and AO activation offered further proof of the different degree of activation of LPO and the AO system in the animals under study (Fig. 1). The determination of the correlation coefficients between S and tg α values; H and tg α; h and tg α; and L and tg α pointed to the existence of a connection (from weak to significant) between the parameters of LPO and the AO system. The more pronounced activation of the free-radical processes in guinea pigs subjected to EPS may be related to the drop of the AO level.

One of the aims of the present investigation was to study the association of LPO processes with the induction of neuroimmune processes. Antibodies to brain antigens in guinea pigs were determined 7, 14, and 21 days after exposure to stress in 80% of animals. Antibrain antibodies in 1:20 titers were found just in 20% of the stressed animals only on the 14th day.

Our data are in agreement with those reported previously [1], where LPO activation induced by EPS was also accompanied by the production of antibodies to brain antigens, as well as with the findings of another workers who discovered damage to the intracerebral vessels upon morphological examination along with the activation of LPO and production of antibodies. This enables us to assume that one of the actual mechanisms of the induction of immune reactions to neuroantigens in stress is the activation of free-radical processes, resulting in the destruction of cell membranes and

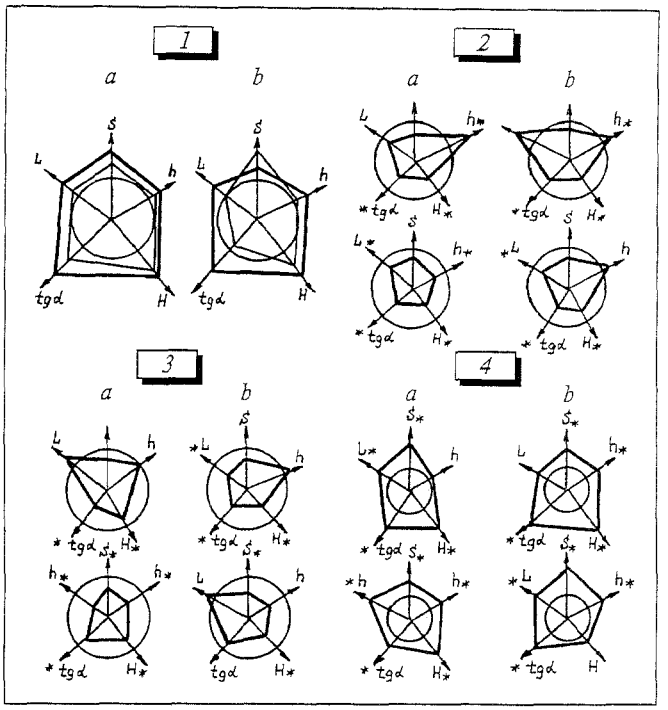

Fig. 1. Changes of chemiluminescence indexes in rats (*a*) and guinea pigs (*b*). *1*) EPS; *2*) EPS + SOD; *3*) EPS + DSIP; *4*) EPS + lipid diet. * means $p<0.05$ in comparison with control.

impairment of the blood-brain barrier (BBB) permeability, creating the possibility of contact between specific brain proteins and cells of the immune system. EPS-induced neurosensitization may be considered a risk factor for the development of immunopathology because repeated damage to BBB integrity of various genesis may create conditions for the development of a neuroimmune process.

Activation of FRO in damage to the nervous system and under stress theoretically justifies the use of drugs which eliminate or prevent alterative effects of already formed radicals. A promising preparation is the enzyme SOD, which was used by us as a modifier of the nonspecific adaptive reactions developing in response to stress. SOD is considered to be a most effective AO due to its capacity to utilize superoxide radicals as a substrate.

We found that a one-trial i.p. administration of SOD in a dose of 1 mg/kg immediately before exposure to stress resulted in a stabilization of the AO system and a normalization of the free-radical processes (Table 1, Fig. 1).

Together with the regulatory effect of SOD enzyme on the LPO system and stabilization of AO in guinea pigs, the degree of neurosensitization decreased, and antibrain antibodies were noted in only 40% of the animals, while they were absent in rats (Table 1).

Thus, the assumption that the production of antibrain antibodies is related to the stress-induced

damage of neuronal membranes, blood vessels, and other structures of the nervous tissue due to FRO enhancement is corróborated. The methods used in the study will evidently help seek out agents raising resistance to the development of negative consequences of EPS. At present, neuropeptides are considered to be such agents.

In our studies we demonstrated the possibility of boosting resistance to EPS under the influence of one neuropeptide, namely, delta-sleep-inducing (DSIP). It has been established that a one-trial i.p. administration of DSIP in a dose of 12 mg/100 g animal weight 1 h before stress prevented the activation of LPO in the blood and brain of guinea pigs and rats, stabilized the AO system, and had a prophylactic effect on the development of neurosensitization (Table 1, Fig. 1). There was a decrease of AO activity of the SOD and GP enzymes, as well as of the level of α-tocopherol in rats both in the blood (by 48, 59, and 27%, respectively) and in the brain tissue (SOD by 26, GP by 38% in comparison with the stressed group). The data obtained on the change of SOD activity in guinea pigs treated with DSIP are of interest. SOD activity in erythrocytes and in the brain tissue was not only not decreased, as was the case in stress, but was even significantly higher than in the control group. The activity of GP and the level of vitamin E remained on the control level.

The next experimental series was performed to study the changes in FRO processes in the blood and brain of rats kept on lipid diet, impairing the resistance of animals to stress and reproduction of EAE.

After 4 weeks of the diet, rats exhibited the signs of E-avitaminosis, namely loss of body weight, sluggishness, and yellowing and shedding of hair. The level of vitamin E in the serum of such rats proved to be decreased by 23.1%. A marked increase of LPO products was determined both in the blood and in the brain tissue. The activity of the AO system enzymes increased (Table 1, Fig. 1). Stress introduced in rats kept on the lipid diet resulted in a more pronounced activation of free-radical processes and in a drop of the AO level. The principal parameters of free-radical processes after stress in hypovitaminized rats were similar to those in guinea pigs. All stressed rats kept on the lipid diet had the same titer of antibrain antibodies on the 14th day as the stressed guinea pigs. These data confirmed the important role of LPO in destroying neuromembrane integrity.

The data obtained in the experiments on the development of EAE in 70% of albino rats kept on a lipid diet against the background of pronounced activation of FRO, after immunization with encephalitogenic emulsion, prove once again that changes of the capacity of AO system, and, the resulting activation of LPO processes, may be a factor necessary for the realization of the neuroimmune process.

Thus, the overcoming of the natural resistance of albino rats to EAE is related to the drop of the AO level. The correctness of this thesis is confirmed by the possibility of preventing EAE in guinea pigs by administering vitamin E, SOD, and unitiol [15].

Thus, the basis of stress resistance and tolerance of EAE is the strength of the AO systems, capable of inhibiting the excessive intensification of FRO processes, which are to be considered a trigger mechanism of a neuroimmune process. An informative test for assessment of the antistressor effects of various agents is the determination of the degree of neurosensitization.

REFERENCES

1. E. D. Bogdanova, V. E. Kagan, I. Ya. Kuliev, *et al.*, *Immunologiya*, № 2, 65-66 (1981).
2. G. A. Vilkov, E. M. Stepanenko, and G. N. Kryzhanovskii, *Byull. Eksp. Biol. Med.*, № 9, 288-290 (1987).
3. Yu. A. Vladimirov, V. I. Olenev, and T. B. Suslova, in: *The Superweak Luminescence of Blood Serum in Clinical Diagnostics* [in Russian], Moscow (1974), pp. 6-33.
4. Yu. A. Vladimirov and A. I. Archakov, *Lipid Peroxidation in Biological Membranes* [in Russian], Moscow (1972).
5. A. R. Gavrilova and N. Kh. Khmara, *Lab. Delo*, 721-723 (1986).
6. E. G. Dubinina, *Ukr. Biokhim. Zh.*, **60**, № 3, 20-25 (1988).
7. N. I. Kuznetsova and S. F. Semenov, *Zh. Nevropatol. Psikhiatr.*, № 6, 869-874 (1961).
8. E. V. Nikushkin, G. N. Kryzhanovskii, N. R. Tupeev, *et al.*, *Byull. Eksp. Biol. Med.*, № 3, 297-299 (1987).
9. L. P. Prilipko, A. N. Erin, B. S. Belyaev, *et al.*, *Zh. Nevropatol. Psikhiatr.*, № 1, 100-104 (1987).
10. R. I. Chernyauskine, Z. Z. Vartkyavichene, and P. S. Gribauskas, *Lab. Delo*, № 6, 362-364 (1984).
11. J. L. Boland and H. P. Koch, *J. Chem. Soc.*, № 7, 445-449 (1945).
12. A. S. Csallany and K. L. Agaz, *Lipids*, № 11, 412 (1976).
13. O. Desiderato, J. R. McKinnon, and H. J. Nisson, *J. Comp. Physiol. Psychol.*, **87**, 208-214 (1974).
14. K. Saton, *Clin. Chim. Acta*, № 1, 37-43 (1978).
15. G. A. Vilcov, *Constituent Congress International Society for Pathophysiology*, Moscow (1991), pp. 192-193.