STRUCTURAL BASIS OF INDUCTION OF IMMUNE REACTIONS IN EMOTIONAL STRESS

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Previous investigations [2, 13] showed that emotional stress leads to an increase in permeability of the blood-brain barrier (BBB). Ruptures of the wall of the intracerebral vessels have been found in the mesencephalic reticular formation of rats exposed to prolonged emotional stress. It can be tentatively suggested that structural lesions of the brain vessels caused by emotional stress permit penetration of specific brain proteins into the general circulation, and of blood plasma proteins and cells into the parenchyma of the brain, so that the conditions are created for induction of immune reactions.

Previous investigations showed [5, 9] that sensitization is formed after trauma to BBB and synthesis of antibodies to the tissue antigens of the brain and to neurotransmitters is induced. To test this hypothesis, we studied the morphology of the mesencephalic reticular formation of rats exposed to emotional stress and also induction of synthesis of autoantibodies to neuroantigens and neurotransmitters and sensitization to brain tissue antigens.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-300 g. The rats were immobilized on a board [1, 14] for 2 days with interruptions. The total duration of three immobilizations was 38 h. The rats were divided into three groups. Animals of group 1 (10 experimental and 10 control) were used for morphological investigation immediately after the end of immobilization. The rats were decapitated and the brain quickly removed and fixed for light-optical investigation in 10% neutral formalin. Serial frontal sections through the mesencephalon were stained with hematoxylin, Luxol fast blue, and cresyl violet (the method of Klüver and Barrera).

Material for electron-microscopic investigation was fixed by immersion of a fragment of the mesencephalic reticular formation, isolated under a magnifying glass, in a 2.5% solution of glutaraldehyde in phosphate buffer, pH 7.2, and subsequently treated by the standard method.

In the animals of group 2 (22 experimental, 18 control) an immunological investigation was carried out 2 weeks (eight rats) and 1 month (32 rats) after exposure to emotional stress. Antibodies were determined to tissue antigens (TA) of homologous brain in the complement fixation test in the cold, with a control for TA of the liver, the tissue of which has common determinants with brain antigens. Antibodies to noradrenalin and serotonin were determined in the passive hemagglutination test (PHT) [10]. Sheep's red blood cells sensitized with noradrenalin or serotonin, covalently bound with bovine serum albumin (BSA) by a modified method in [15] were used as the diagnostic preparation. Sera from rabbits hyperimmunized with the corresponding conjugates of the monoamines with BSA were used as standard control sera. The titer of antibodies to the conjugates in the corresponding sera was 1:1024 and 1:8192. The PHT was carried out in a microtitrator with the necessary control tests for nonspecific agglutination of the diagnostic preparation with normal serum, for stability of the diagnostic preparation in medium for the PHT, and for the carrier protein of the antigen.

Antibody titers were expressed in $\log_2$ of twofold dilutions of the sera: $\log_2$-1 for 1:2, $\log_2$-2 for 1:4, and so on. To to estimate increased immediate-type sensitivity of specific

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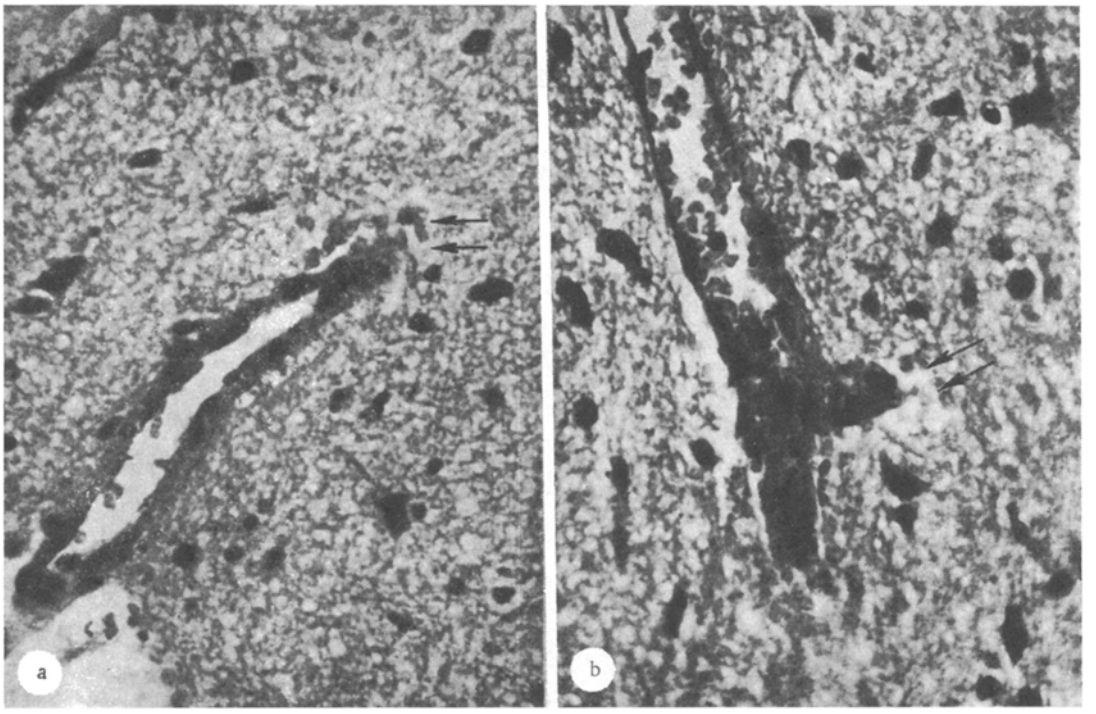

Fig. 1. Rupture of wall of arteriolovenular anastomosis and diapedesis of erythrocytes: a) hematoxylin-eosin, 200 ×; b) Klüver-Barrera method with counterstaining, 400 ×.

reaction of the basophils (SRB) to brain TA was investigated by Shelley's indirect and direct methods in the modification in [3].

EXPERIMENTAL RESULTS

Ruptures of arterioles and of arteriolovenular anastomoses with escape of blood cells into the paremchyma of the brain were found in the mesencephalic reticular formation of three of the five rats chosen for light microscopy (Fig. 1a, b). Lesions of the vessel walls in the brain parenchyma, ruptures of the walls, and desquamation of endothelial ultrastructures into the lumen of the vessels were found electron-microscopically in all five experimental animals. Elements of the neuropil were in direct contact with plasma (Fig. 2) or blood cells lying in the brain parenchyma (Fig. 3).

In the control animals, no damaged intracerebral vessels could be found either light- or electron-microscopically.

SRB and antibodies to brain TA were found 2 weeks after exposure to stress in 20% of cases. After 1 month these reactions were found in the majority of experimental animals (Table 1). Complement-fixing antibodies to neuroantigens were found in a titer of 1:10-1:40 in 53% of the experimental animals but were not found in the control in a single case. In 71% of rats exposed to stress increased sensitivity to TA was found in the SRB test. Antibodies to noradrenalin and serotonin were found in 28% of experimental animals in a titer of

TABLE 1. Immune Reactions and Neurosensitization 1 Month after Exposure to Stress (M ± m)

Animals	SRB	Antibody titers ($\log_2$) to		
		brain TA	noradrenalin	serotonin
Experimental:				
whole group	21±6,0*	1,9±0,47*	1,9±0,6*	1,75±0,73*
subgroup with immune reactions	33,4±5,8*	3,6±0,21*	6,0±0,08*	5,6±0,98*
Control	3,0±2,6	0	0,27±0,27	0,22±0,22

Legend. *p < 0.05 compared with control.

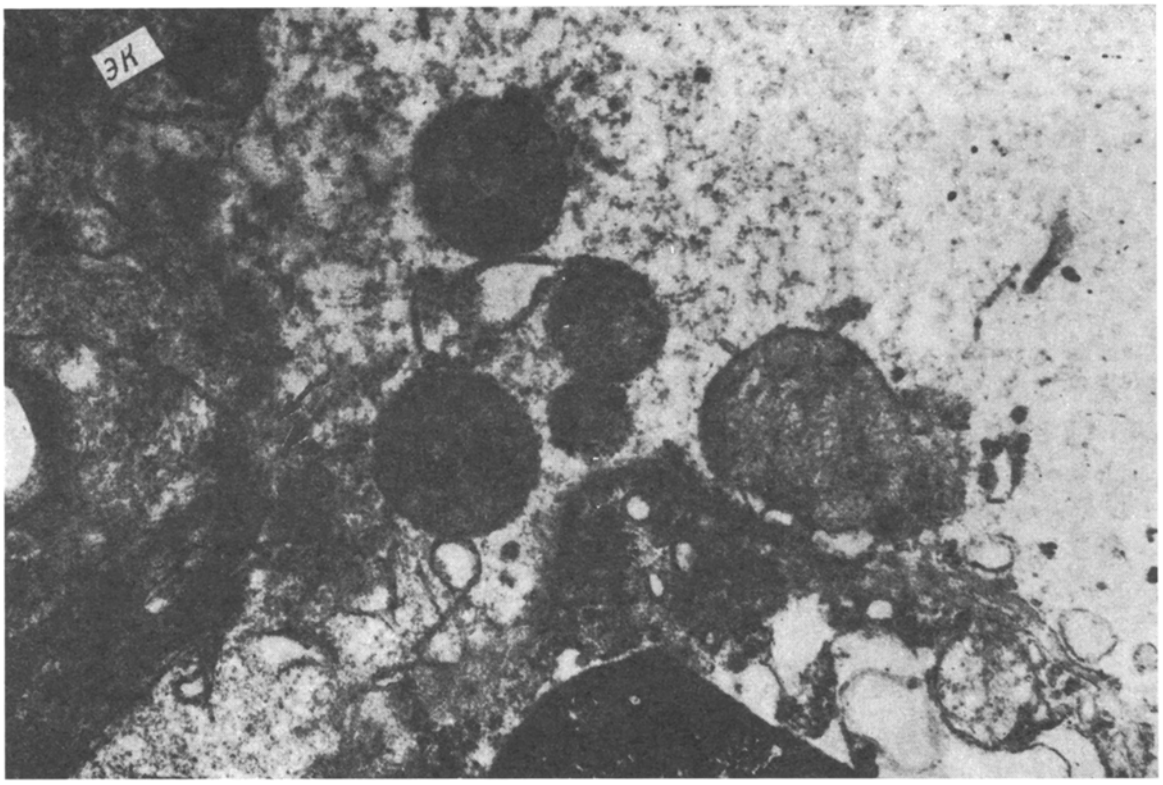

Fig. 2. Destruction of part of a capillary endothelial cell (EC; indicated by arrows). Fragments of cell membranes and mitochondria visible in capillary lumen. 35,000 ×.

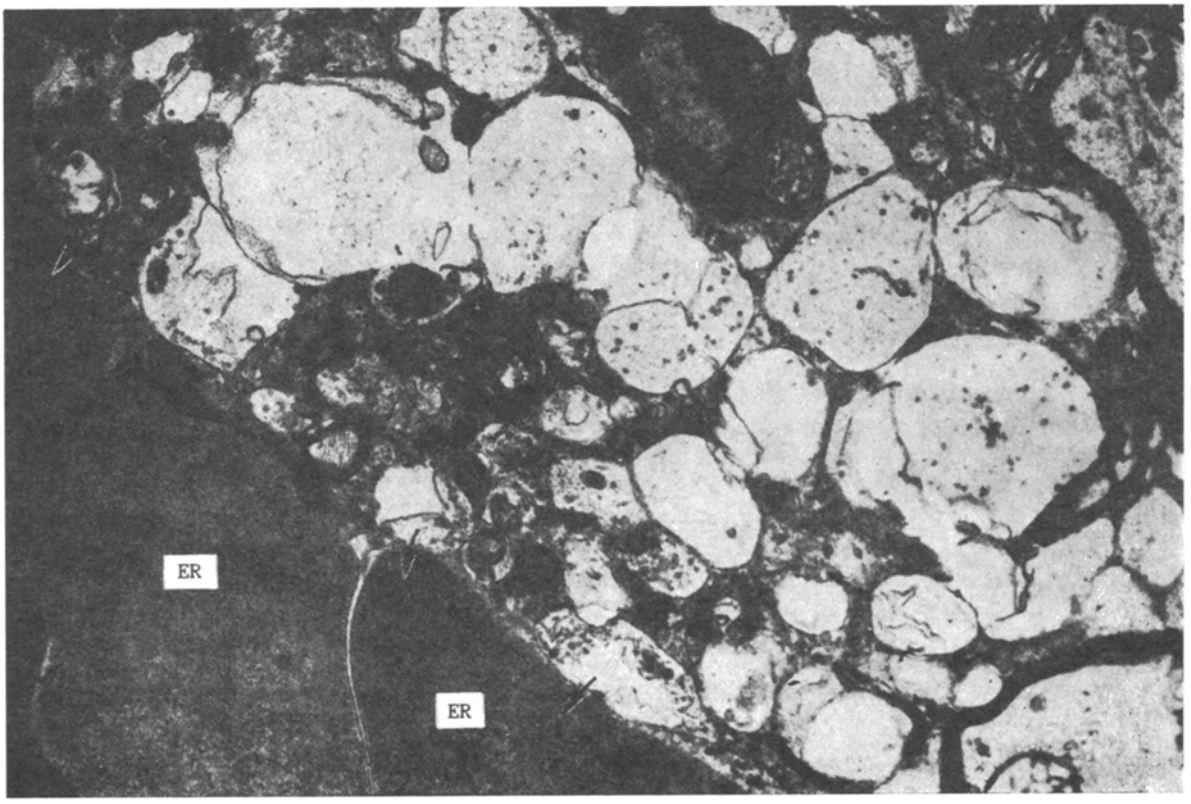

Fig. 3. Accumulation of erythrocytes (ER) in brain parenchyma, 35,000 ×.

1:16-1:256. In the control these antibodies were found in only one rat. Characteristically, antibodies to neurotransmitters and brain TA were found in the same experimental animals.

The results of the morphological investigations thus confirmed previous data according to which long-term emotional stress can induce lesions of the intracerebral vessels of the mesencephalic reticular formation. In some animals exposed to emotional stress, sensitization and antibodies to brain TA were found starting from the 2nd week after stress.

There is evidence in the literature of the presence of antibodies to brain tissue antigen after psychoemotional tension in clinically healthy individuals [8, 11] and in animals exposed to emotional-painful stress [6, 7]. However, the concrete mechanisms of this phenomenon have not yet been explained.

It can be concluded from the data given above that one of the real mechanisms of induction of immune responses to neuroantibodies arising as a result of emotional stress is an increase in permeability of BBB together with growth injuries to the wall of the intracerebral vessels, enabling contact between specific brain proteins and cells of the immune system.

Sensitization caused by emotional stress and the presence of antibodies to neuroantigens in the blood stream can be regarded as a risk factor for the development of immunopathology, if data on the enhancing pathogenic effect of preliminary neurosensitization on the time course of pathological processes forms of activity in brain structures in response to intracerebral injection of antisera to neuroantigens [12, 16] are taken into consideration. After stress, repeated disturbances of the integrity of BBB of varied origin, destructive changes in brain tissue, and activation of autoimmune reactions in the presence of secondary immunodeficiencies — all these factors may create the conditions for development of ummunopathological processes.

LITERATURE CITED

1. A. D. Ado and A. Kh. Kangurin, Problems in General Virology [in Russian], Moscow (1959), pp. 16-19.
2. T. I. Belova and G. Jonsson, Usp. Fiziol. Nauk, 16, No. 2, 61 (1985).
3. E. A. Venglinskaya and S. M. Shchegol', Byull. Eksp. Biol. Med., No. 11, 599 (1979).
4. I. V. Gannishkina, Immunologic Aspects of Trauma and Vascular Lesions of the Brain [in Russian], Moscow (1974).
5. V. A. Evseev and S. V. Magaeva, Vestn. Akad. Med. Nauk SSSR, No. 8, 18 (1985).
6. A. Z. Ivanshina, L. Z. Gaskin, and D. D. Orlovskaya, Zh. Nevropatol. Psikhiat., No. 7, 983 (1973).
7. G. I. Kolyaskina, S. G. Kushnir, L. Z. Gaskin, et al., Problems in the Immunopathology of Nervous and Mental Diseases [in Russian], Moscow (1968), pp. 230-231.
8. Yu. B. Maksimal'do, Stress and Mental Pathology [in Russian], Moscow (1983), pp. 28-32.
9. A. V. Martynenko, S. V. Magaeva, L. A. Basharova, and M. V. Martynenko, Byull. Eksp. Biol. Med., No. 7, 10 (1986).
10. O. Yu. Polevaya, L. A. Basharova, and I. E. Kovalev, Khim.-farm. Zh., No. 7, 32 (1981).
11. S. F. Semenov, R. D. Kogan, L. I. Brusina, et al., Stress and Mental Pathology [in Russian], Moscow (1983), pp. 23-28.
12. J. A. Aarli and A. Fontana, Epilepsia (Amsterdam), 21, 451 (1980).
13. T. I. Belova (T. J. Belova) and G. Jonsson, Acta Physiol. Scand., 116, 21 (1982).
14. R. Kvetnansky and L. Mikulaj, Endocrinology, 87, 738 (1980).
15. K. L. Melmon, G. Weinstein, H. R. Bourne, et al., Pharmacology, 12, 701 (1976).
16. M. M. Rapport, S. E. Karpiak, and S. P. Mahadik, Fed. Proc., 38, 2391 (1979).