CEREBROVASCULAR LESION CAUSED BY ACTIVATION OF PLATELETS AND LEUKOCYTES AND THEIR CORRECTION BY NEUROTROPIN

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Neurotropin is a new antistressor consisting of an extract of rabbit skin, artificially infected by vaccinia virus [8]. Its pharmacodynamic profile includes widely different effects, including antiallergic, immunomodulating, etc. Neurotropin has been used with good results in patients with cerebrovascular lesions, including patients suffering from stroke, but the mechanisms of its therapeutic action are not clear [8].

The aim of the present investigation was to study the effect of neurotropin on circulatory homeostasis of the brain in a model of its disturbance by induction of intravascular activation and aggregation of platelets and neutrophils. This phenomenon is one of the leading mechanisms of cerebrovascular catastrophes [1].

EXPERIMENTAL METHOD

Experiments were carried out on cats anesthetized with ketamine (10 mg/kg). A model of cerebral embolism by aggregates of blood cells was created by intracarotid injection of 4β-phorbol-12β-myristate-13α-acetate (PMA). This agent provokes aggregation and secretion of both platelets and neutrophils, with the development of massive occlusion of the microcirculatory bed and ischemic tissue damage [10]. PMA (40 μg/kg) was injected into the left carotid artery after ligation of its branches. Before injection and 3, 10, 20, 30, and 60 min thereafter, 0.2 ml of arterial blood was withdrawn, and the number of single platelets and leukocytes in it was counted with the aid of a "Laborscel" particle counter (Hungary). Neurotropin (Nippon Zoki Pharmaceutical Co., Japan) was injected intravenously (3 mg/kg) 5 min before injection of PMA. The energy metabolism of the brain was analyzed separately for the hemisphere on the side of injection of PMA and the contralateral hemisphere, in accordance with recommendations in [7]. In parts of the parietal cortex, after extraction with HCl-methanol, and then with perchloric acid [6, 7], ATP, ADP, AMP, and lactate were determined. Concentrations of these compounds were determined by an enzymic fluorescence method [6], using an RF-540 spectrofluorometer ("Shimadzu," Japan). The energy potential of the tissue was calculated as in [2]. In tests carried out in vitro, the effect of neurotropin on platelet and neutrophil aggregation was studied as in [3] on a "Payton" two-channel aggregometer. Free radical generation in the neutrophils was studied spectrophotometrically, using cytochrome c [4]. Platelets were activated by ADP (10^{-6} mole/liter), neutrophils by hexachlorocyclohexane ($2 \cdot 10^{-5}$ mole/liter), which activates neutrophils and stimulates synthesis of leukotriene B_4 in them [1].

EXPERIMENTAL RESULTS

The study of the effect of neurotropin on platelet and neutrophil aggregation in vitro showed that in a final concentration of 100 μg/ml neurotropin inhibited platelet aggregation by 36.4 $\pm$ 4.2% ($p < 0.05$). In lower concentrations neurotropin had no effect. No significant action of neurotropin likewise was discovered on free radical generation in neutrophils, and their aggregation was inhibited by neurotropin (100 μg/ml) only by 15.0 $\pm$ 7.9%. In vivo, however, the

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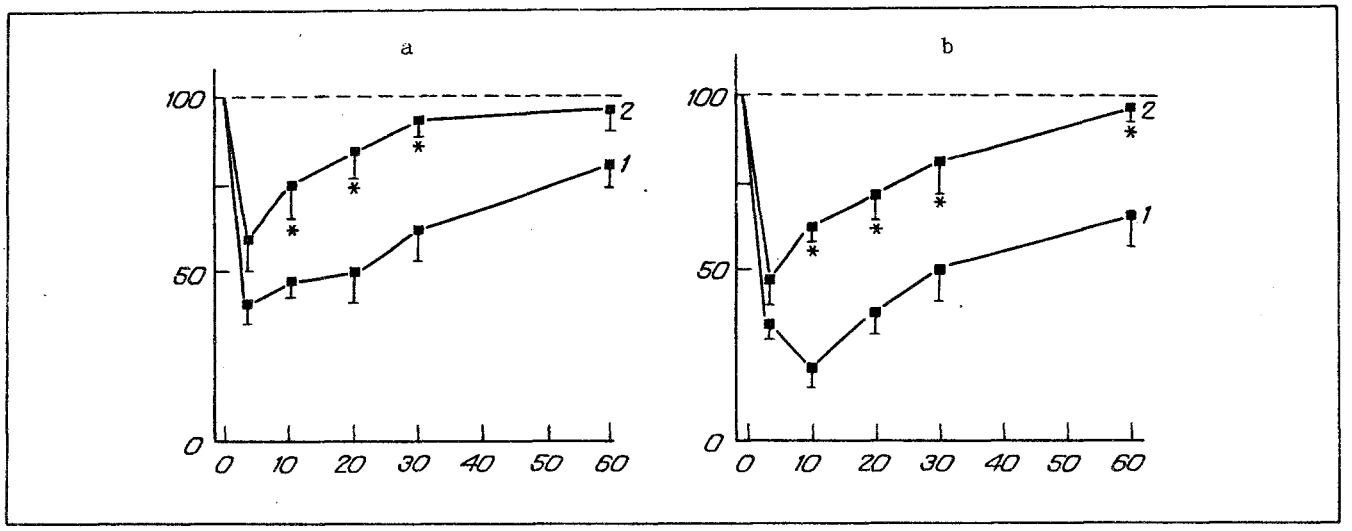

Fig. 1. Changes in number of single platelets (a) and leukocytes (b) after injection of PMA into cat carotid artery. 1) Changes in number of cells under the influence of PMA; 2) changes in number of cells under the influence of PMA after preliminary injection of neurotropin. Abscissa, time after injection of PMA (in min); ordinate, changes in number of cells (in % of initial number).

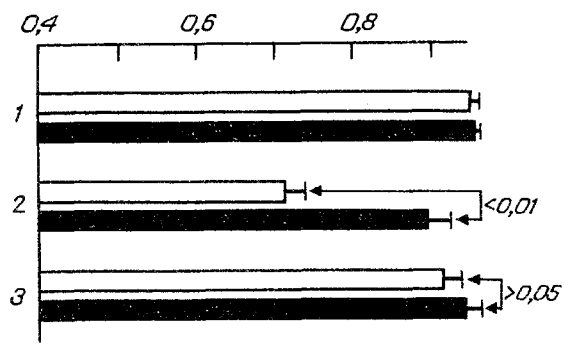

Fig. 2

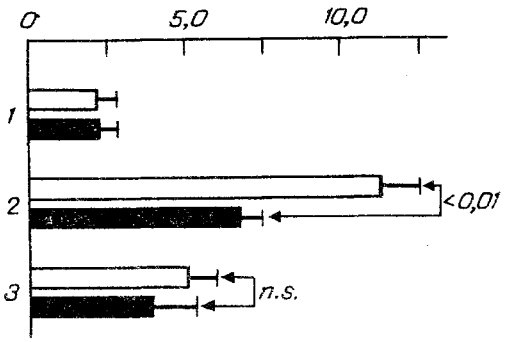

Fig. 3

Fig. 2. Changes in energy potential of cerebral cortex 60 min after injection of PMA on side of injection (unshaded columns) and in contralateral hemisphere (black columns). 1) Control level of energy potential; 2) under influence of PMA; 3) under influence of PMA preceded by injection of neurotropin. Abscissa, level of energy potential (relative units).

Fig. 3. Changes in lactate level in cerebral cortex 60 min after injection of PMA, on side of injection (unshaded columns) and in contralateral hemisphere (black columns). 1) Control level of energy potential; 2) under influence of PMA; 3) under influence of PMA preceded by injection of neurotropin. Abscissa, lactate level (in μmoles/g tissue).

antiaggregative effect of neurotropin was stronger. Injection of PMA led to a sharp decrease in the number of single platelets and leukocytes in the blood stream, which began as early as the 3rd minute after injection of the preparation and persisted until 60 min (Fig. 1). This phenomenon reflects the formation of aggregates of blood cells and their sequestration in the microcirculatory bed [10]. In the presence of neurotropin a significant decrease was observed in the degree of reduction of the number of platelets and leukocytes at the 3rd minute after injection of PMA, and normalization of the number of single cells toward the 60th minute (Fig. 1).

Analysis of the state of energy metabolism of the cerebral cortex showed reduction of the energy potential after injection of PMA in both cerebral hemispheres, which was significantly greater in the hemisphere on the side of injection of PMA (Fig. 2). There was a parallel rise of the lactate level, which also was greater on the side of injection of PMA (Fig. 3). Preliminary injection of neurotropin abolished virtually completely the changes induced by PMA in both energy potential and lactate level in both cerebral hemispheres (Figs. 2 and 3).

The results are evidence that injection of PMA leads to the development of ischemic damage of brain tissue, and that this model can be used to study the effect of drugs on the processes of formation of an ischemic focus, linked with changes in the aggregate state of the blood. It must be pointed out that the use of PMA is preferable to injection of ADP, as has been practiced by other workers [4], since PMA, unlike ADP, induces aggregation not only of platelets, but also of leukocytes, and the ischemia induced by it corresponds more closely to real conditions. This analysis of the effect of neurotropin on this model shows that it can prevent the development of cerebral ischemia induced by aggregates of blood cells. This may be due to reduction of the degree of intravascular aggregation of platelets and leukocytes; this effect of neurotropin, moreover, is unlikely to be associated with its direct action on the cells. The fact that neurotropin in vitro has no marked effect on them, but in vivo it prevents the development of aggregation suggests that its effect is based on regulation of cell functions through certain intermediate factors. These may perhaps include the system of eicosanoids, kinins, etc., which are effectively controlled by neurotropin [8].

It must also be pointed out that prevention of disturbances of brain metabolism by neurotropin may be associated not only with reduction of aggregation of the blood cells. Even in the presence of neurotropin, although aggregation is reduced, it nevertheless develops to a certain degree, whereas unfavorable changes of brain energy metabolism are almost completely abolished. The beneficial effect of neurotropin may perhaps be connected not only with its action on intravascular activation of the blood cells, but also with a direct effect on brain tissue. Whatever the case, the investigation has shown that neurotropin may be a promising preparation for the prevention of cerebrovascular disorders, and its antistressor activity makes it particularly suitable for clinical use [8].

LITERATURE CITED

1. É. S. Gabrielyan and S. É. Akopov, Blood Cells and the Circulation [in Russian], Erevan (1985).
2. D. Atkinson, Biochemistry, **7**, 4030 (1968).
3. G. Born, Nature, **194**, 927 (1962).
4. D. English, J. Roloff, and J. Lukens, Blood, **58**, 129 (1981).
5. C. Fieschi, N. Battistini, F. Volante, et al., Stroke, **6**, 617 (1975).
6. J. Folbergova, V. MacMillan, and B. Shiesö, J. Neurochem., **19**, 2497 (1972).
7. K. Fredriksson, I. Rosen, B. Johansson, and T. Wieloch, Stroke, **16**.
8. Introduction to Neurotropin, Osaka (1989), p. 23.
9. J. Meade, J. Harvey, and J. Boot, Biochem. Pharmacol., **33**, 289 (1984).
10. J. O'Flaherty, S. Cousart, A. Lineberger, et al., Am. J. Path., **101**, 79 (1980).