Cerebrovascular Injuries Induced by Activation of Platelets and Leukocytes In Vivo and Their Correction by Neurotropin

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ABSTRACT—Ischemia-like brain damage was induced in cats by selective injection of 4β-phorbol-12β-myristate-13α-acetate (PMA) into the left carotid artery. PMA-injection provoked significant decreases in platelet and neutrophil counts due to their intravascular aggregation. Platelet and neutrophil aggregates caused brain edema with accumulation of sodium fluorescein in the cerebrospinal fluid and ipsilateral derangement of the cerebral energy state in the parietal cortex. Neurotropin administration decreased the changes in platelet and neutrophil counts and prevented the developments of both brain edema and cerebral energy failure.

Keywords: Neurotropin, Blood cell, Cerebrovascular injury

Neurotropin is a non-protein extract obtained from the rabbit skin tissue treated with vaccinia virus (1). Its many pharmacological actions include analgesic, anti-allergic, and immunomodulating effects and effects on autonomic nervous function. Recently a beneficial effect of neurotropin on ischemic cerebrovascular disorders was found (2). It is not clear how neurotropin influences the circulatory homeostasis of the brain in pathological states. Therefore the present study evaluates the effects of neurotropin on brain tissue damage in experimental circulatory disease induced by intravascular blood cell aggregation, which is the way that cerebrovascular disorders are provoked in many cases (3).

Blood cell embolism was induced with 4β-phorbol-12β-myristate-13α-acetate (PMA, Sigma). This agent provokes aggregation and secretion of both platelets and neutrophils, and its administration causes blockage of the microvessels by cell aggregates, resulting in the development of ischemia and edema (4). PMA was dissolved in dimethyl sulfoxide (0.5 mg/ml) and was injected into the left carotid artery of cats anesthetized with ketamine (10 mg/kg) at the dose 40 μg/kg (2 ml) in 30 sec, as was recommended by O'Flaherty et al. (4). The injection of saline instead of PMA had no effect on the parameters studied. Immediately before and at 3, 10, 20, 30 and 60 min after injection of PMA, 0.2-ml arterial blood samples were withdrawn, and the numbers of platelets and leukocytes were counted according to O'Flaherty et al. (4). Neurotropin (Nippon Zoki Pharmaceutical Co., Osaka) was given intravenously (3 mg/kg) at 5 min before PMA injection. This concentration is optimal for exerting the effects of neurotropin (2, 5). The injectable solution of neurotropin (5 mg/ml in physiological saline) was used. In the control, an analogous volume of saline was infused.

Analysis of energy metabolism of the parietal cortex was performed according to Folbergova et al. (6). The contents of ATP, ADP, AMP, and lactate were analyzed by enzymatic fluorometric techniques (6, 7). The energy charge of the adenine nucleotide pool (EC) was calculated according to Atkinsson (8). A degree of the blood brain barrier damage was estimated by an increase in sodium fluorescein concentration in cerebrospinal fluid at 60 min after injection (9). Sodium fluorescein solution (3 ml, 15 mg/kg) was injected intravenously.

As shown in Fig. 1, PMA injection significantly decreased both platelet and leukocyte counts. These decreases were already evident at 3 min after administration of PMA; and even at 60 min, the number of platelets and especially that of leukocytes did not completely return to the baseline levels. In the presence of neurotropin, the fall in the number of circulating platelets and leukocytes decreased, although not completely.

The values of the energy charge and lactate level in the parietal cortex of both hemispheres are shown in...
Fig. 1. Time courses of PMA-induced (40 μg/ml injected into the left carotid artery) changes in platelet and leukocyte counts in non-pretreated cats and in cats pretreated with neurotropin (3 mg/kg at 5 min before PMA injection). *P < 0.05 versus the changes in counts in non-pretreated animals.

Fig. 2. In the PMA-injected hemisphere, a decrease in the energy charge and an increase in the lactate level in comparison with the control (P < 0.01) were observed. In the contralateral noninjected hemisphere, these changes were significantly less marked and did not statistically differ from the control. Pretreatment of the animals with neurotropin resulted in the disappearance of the changes in energy metabolites. No interhemispherical differences in both energy charge and lactate level were observed, and their values were similar to those of the control (Fig. 2). Simultaneously, neurotropin prevented blood brain barrier damage. PMA-injection increased sodium fluorescein concentration in the cerebrospinal fluid from 7.9 ± 1.9 to 54.7 ± 4.8 ng/ml (P < 0.001) in the nonpretreated animals and only to 13.2 ± 3.1 ng/ml (P > 0.05) in the neurotropin-pretreated animals.

The data obtained in this study confirms the PMA capability to induce significant and prolonged reduction in the number of circulating platelets and leukocytes with deterioration of the brain tissue functional state due to the embolism by the cell aggregates (4). The degradation of labile phosphates and the increase in lactate level that we observed after PMA injection demonstrated the presence of ischemia-like brain damage, especially in the injected hemispheres. In addition, the PMA-induced cell embolism also provoked brain edema, and this was confirmed by the significant increase of sodium fluorescein concentration in the cerebral fluid.

Neurotropin administration diminishes PMA-induced reduction in the number of circulating platelets and
leukocytes. Probably neurotropin can depress both platelet and neutrophil aggregabilities. Some authors have also found the inhibition of platelet aggregability and neutrophil activation by neurotropin in vitro (10, 11). However, under our conditions, neurotropin did not reduce cell aggregation completely, whereas it completely prevented the degradation of labile phosphates, the increase of the lactate level and the intensity of the entry of sodium fluorescein into the brain. Hence, neurotropin did not reduce PMA-induced cell aggregation to an extent sufficient enough to totally explain its effect on brain damage. It is likely that in addition to the decrease in the cell aggregation, neurotropin prevents brain failure by directly acting on the capillary bed and brain tissue. Further studies will be necessary to elucidate these mechanisms.

The overall the finding that neurotropin may correct cerebrovascular failure induced by cell embolism explains its beneficial effect in patients with ischemic stroke (12). It may be suggested that the application of neurotropin in patients with cerebrovascular disorders appears to have potential.

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