Relationship between Changes in Rat Behavior and Integral Biochemical Indexes Determined by Laser Correlation Spectroscopy after Photothrombosis of the Prefrontal Cortex

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Experiments on rats showed that Noopept improved retention and retrieval of conditioned passive avoidance response after phototrombosis of the prefrontal cortex (a procedure impairing retention of memory traces). The impairment of mnesic functions was accompanied by changes in integral biochemical indexes of the plasma determined by laser correlation spectroscopy. Treatment of behavioral disorders with Noopepet normalized biochemical indexes.

Key Words: *photothrombosis; prefrontal cortex; Noopepet; conditioned passive avoidance response; laser correlation spectroscopy*

The prefrontal cortex is responsible for spatial orientation and plays a key role in the mechanisms of learning and memory [3,12]. Previous studies showed that local ischemic damage to the prefrontal cortex (photothrombosis) produced local morphological defect and impaired cognitive function in the central nervous system (CNS). These changes were not accompanied by locomotor disorders or suppression of unconditioned responses.

Apart from direct damage to the nervous tissue photothrombosis causes a variety of secondary disorders related to delayed metabolic and neurochemical changes. The method of laser correlation spectroscopy (LCS) is used to evaluate the directionality and severity of metabolic changes during vascular disorders in the brain. LCS is based on studying of the contribution of particles with different size into the integral light-scattering spectrum and allows evaluation of the subcellular composition of biological fluids. The ratio between various components of biological fluids can serve as a diagnostic and prognostic criterion for different pathologies.

Behavioral disorders were treated with Noopept. This original preparation synthesized at the Institute of Pharmacology possesses nootropic and neuroprotective activities [13].

Here we studied the relationship between disturbances in conditioned behavior of rats after bilateral photothrombosis of the prefrontal cortex, evaluated the efficiency of correction with Noopept, and measured integral biochemical indexes of the plasma.

MATERIALS AND METHODS

Experiments were performed on 40 male outbred rats weighing 220-250 g. The animals were divided into 3 groups: sham operation (n=11, group 1); photothrombosis of the prefrontal cortex and administration of physiological saline (n=18, group 2); and photothrombosis and Noopept treatment (n=11, group 3).

The method of photothrombosis is based on photostimulation of thrombus formation in vessels of rat cerebral cortex after the interaction between the pho-

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tosensitive dye rose Bengal administered into the vascular bed (3%, 40 mg/kg intravenously) and focused beam from a halogen lamp (560 nm) directed to the intact cranial surface. This reaction leads to the release of free oxygen, damage to the vascular endothelium, platelet aggregation, thrombus formation, and occlusion of vessels (experimental ischemic injury of the cerebral cortex). Photochemical damage to the prefrontal cortex was produced in animals intraperitoneally narcotized with 300 mg/kg chloral hydrate. During surgery, body temperature was maintained at 37°C.

Physiological saline and Noopept (0.5 mg/kg) were injected intraperitoneally 1 h after bilateral focal ischemic damage to the prefrontal cortex. In the follow-up period the test preparations were administered daily for 9 days.

Horizontal locomotor activity of rats in the open field was recorded on an automatic RODEO-1 device for 5 min. Functional state of CNS was determined by conditioned passive avoidance response (CPAR), i.e. by the latency of transition from light compartment to dark compartment (in sec). The rats was considered as trained if it stayed in the light compartment over 300 sec. CPAR was trained before photothrombosis of the prefrontal cortex. The rats were tested before and 9 days after damage.

The animals were decapitated on day 9 after surgery. Whole blood (100 μl) was taken and mixed with physiological saline (400 μl). Samples were incubated at room temperature for 30 min and centrifuged at 5000 rpm for 15 min. The supernatant was stored at -20°C. Before measurements the samples were defrosted in a thermostat at 37°C and repeatedly centrifuged. The supernatant (200 μl) was placed in a cuvette of a LKS-03-INTOKS spectrometer. The measurements were performed in a frequency range of 16,538 Hz (2000 integrations). The spectrum was regularized using Spectrometer and Blood softwares. This study allowed us to construct a histogram (ordinate: percent of particles in the spectrum of light scattering; abscissa: size of particles, nanometers). According to the classification of LCS for blood plasma, particles were divided by size into 5 informative zones: zone I, <10 nm; zone II, 11-30 nm; zone III, 31-70 nm; zone IV, 70-150 nm; and zone V, >150 nm. The measurements [2] showed that zone I mainly includes low-molecular-weight monomeric albumins and free glycolipid complexes. Zone II contained globular proteins and low-molecular-weight lipoprotein complexes. High-molecular-weight lipoprotein complexes, nucleoproteins, and very-low-molecular-weight immune complexes were localized in zone III. Zone IV includes medium-sized constitutive immune complexes. Zone V appears when the process of immunopoiesis resulted in the formation of high-molecular-weight complexes.

The results were analyzed by nonparametric Student's test for paired and independent variables and Mann—Whitney test (Statistica 5.0 software).

RESULTS

The initial value of horizontal activity did not differ in rats of groups 1, 2, and 3 (203.09±13.74, 184.33±11.53, and 175.81±13.04, respectively). Horizontal activity of group 1 and 3 animals decreased to 102.00±20.71 and 89.45±11.97, respectively, 9 days after surgery ($p<0.01$, Student's t test for paired variables). In group 2 rats horizontal activity remained practically

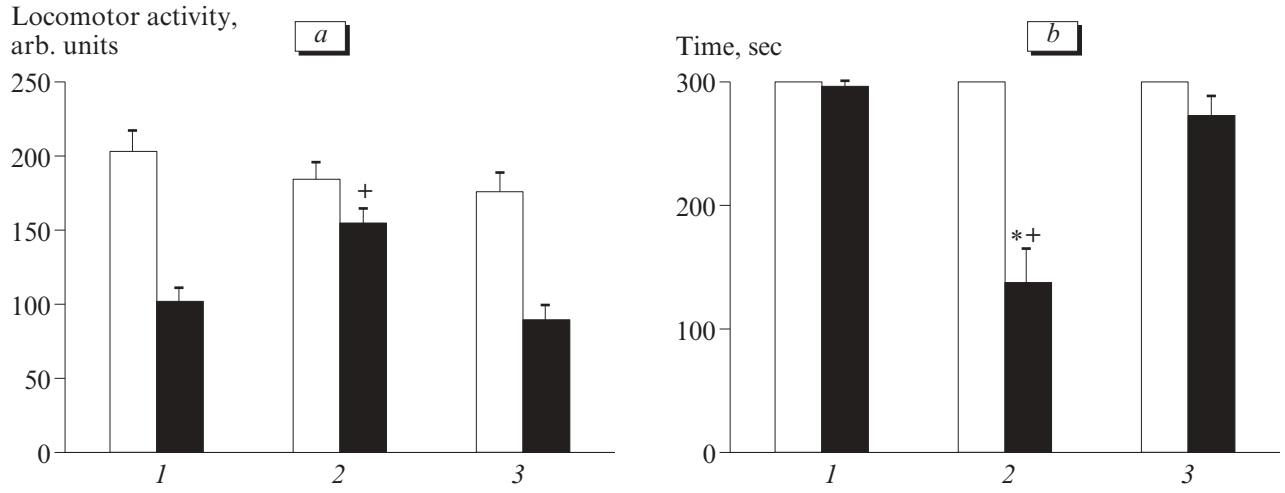

Fig. 1. Changes in locomotor activity (*a*) and latency of conditioned passive avoidance response (CPAR, *b*) during photothrombosis of the prefrontal cortex: sham operation (*1*), ischemia (*2*), and ischemia+Noopept (*3*). (*a*) Light bars: baseline level of locomotor activity; dark bars: 9 days after surgery. (*b*) Light bars: CPAR latency before surgery; dark bars: 9 days after surgery. *$p<0.01$ compared to the baseline value (t test for paired variables). +$p<0.05$ (*a*) and +$p<0.001$ (*b*) compared to sham-operated animals and ischemia+Noopept group (t test for independent variables).

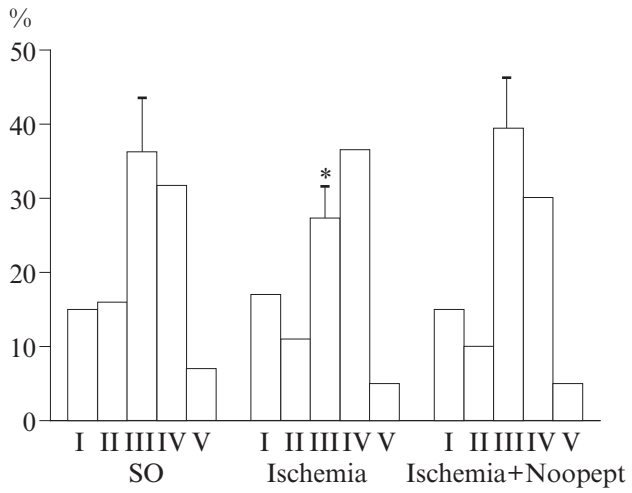

Fig. 2. Distribution of plasma particles in the light scattering spectrum. Abscissa: particle size. Ordinate: contribution to light scattering spectrum, %. *p<0.05 compared to the ischemia+Noopept group (Mann—Whiteny test). SO, sham operation.

unchanged (154.77±20.17). Therefore, group 2 rats displayed higher locomotor activity compared to group 1 and 3 animals (p<0.05, Student's t test for independent variables, Fig. 1, *a*). The latency of CPAR before surgery was 300 sec. In group 1 rats this index remained unchanged 9 days after sham operation (296.36±3.38 sec). However, the latency of CPAR in group 2 animals markedly decreased 9 days after photothrombosis of vessels in the prefrontal cortex and differed from the initial value (137.5±27.4 sec, p<0.0001, Student's t test for pairwise variables). Daily treatment with Noopept prevented the decrease in CPAR latency in group 3 rats. The latency of CPAR in rats with ischemia receiving Noopept for 9 days did not differ from the initial value (272.72±15.79 sec, Fig. 1, *b*). These results illustrate that on day 9 after surgery, the latency of CPAR in group 2 rats was much lower than in group 1 and 3 animals (p<0.0001 and p<0.001, respectively, Student's t test for independent variables). Ischemia of the prefrontal cortex caused by photothrombosis was accompanied by impairment of mnesic functions. Similar behavioral changes were revealed in animals of various species with ischemic brain injury [11]. In our experiments locomotor activity of rats in the open field remained practically unchanged after photothrombosis of the prefrontal cortex. Locomotor activity of sham-operated animals decreased during repeated testing. These results are consistent with changes in locomotor activity of rats during repeated testing in the open field [6]. Learning capacity and memory were impaired in rats with ischemia of the prefrontal cortex. Noopept prevented the development of mnesic dysfunction, which indicates that this preparation possesses antiamnesic activity. Noopept is superior to the classic nootropic drug piracetam in effective doses and modulation of cognitive function in the brain [13].

Photothrombosis of the prefrontal cortex not only impaired conditioned activity of rats, but also decreased the number of light-scattering particles in zone III (high-molecular-weight lipoprotein complexes, nucleoproteins, and very-low-molecular-weight immune complexes) and increased the count of particles in zone IV (autoantibodies, Fig. 2). Noopept treatment after ischemic damage to the prefrontal cortex produced an antiamnesic effect and increased the number of zone III particles in the plasma from 27.3±3.8 to 39.4±5.4 (p<0.05, Mann—Whitney test).

The contribution of zone IV particles in the light scattering spectrum tended to decrease to normal. Our findings suggest that the ratio between the total content of autoantibodies and low-molecular-weight lipoprotein complexes estimated by LCS can serve as a biochemical marker for ischemic brain injury.

Previous studies revealed a correlation between autoimmunization and ischemic damage to the brain tissue. For example, the amount of antibodies against heat shock proteins markedly increases in the blood of patients over the first 48 h after ischemic stroke [7,10]. The titer of antibodies to β_2-glycoprotein increases during ischemic stroke [9]. The increased titer of antibodies against phosphatidylserine [14] and cardiolipin [8] reflects high risk of ischemic stroke. A relationship was found between changes in the content of autoimmune and lipoprotein components in the blood and recovery of conditioned activity after photothrombosis of the prefrontal cortex. These findings suggest that the present approach to the study of experimental and clinical samples holds much promise for early diagnostics of high-risk individuals.

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