Neuroprotective and Antiamnesic Effects of Semax during Experimental Ischemic Infarction of the Cerebral Cortex

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Semax had a pronounced neuroprotective and antiamnesic effect during focal photoinduced ischemia of the prefrontal cortex. Intranasal administration of Semax for 6 days decreased the volume of cortical infarction and improved retention and performance of conditioned passive avoidance response.

Key Words: *experimental ischemic infarction of the cerebral cortex; photoinduced thrombosis; amnesia; neuroprotection; Semax*

Nootropic drug Semax synthesized on the basis of synthetic peptide Met-Glu-His-Phe-Pro-Gly-Pro ($ACTH_{4-10}$ analogue) [1] is now extensively used in medical practice in Russia. Semax improves learning and memory and surpasses the prototype by several pharmacological properties [10]. Semax not only modulates cognitive function, but also exhibits neuroprotective activity [4,9,12]. Published data show that the use of Semax in combination therapy for carotid ischemic strokes decreases mortality rate, improves clinical outcome of stroke, and increases the degree of functional recovery [4]. Previous studies showed that intraperitoneal injection of Semax decreases the severity of neurological disorders and improves survival of animals with global cerebral ischemia induced by occlusion of both common carotid arteries [9]. This peptide improved recovery of behavioral reaction in rats receiving the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine selectively damaging dopaminergic neurons of the substantia nigra [12]. However, there is direct evidence of the neuroprotective effect of Semax during focal cerebral ischemia.

Photoinduced thrombosis of blood vessels in the cerebral cortex (method of photothrombosis) is an experimental model most closely reproducing clinical signs of ischemic cerebral infarction [14]. This noninvasive procedure allows selecting the required area of ischemic cortical infarction, which gives reliable quantitative data on the severity of brain injury and dynamics of pathological and reparative processes in the ischemic focus. This model allows evaluation of the neuroprotective and antiamnesic effects of drugs for pharmacological correction of ischemic injury to the brain [13].

Here we studied the neuroprotective and antiamnesic effects of Semax on rats with bilateral focal ischemic infarction of the prefrontal cortex leading to pronounced cognitive deficit [7].

MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 200-250 g. The animals were maintained

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in a vivarium under natural light/dark conditions and had free access to food and water.

Bilateral focal ischemic infarction of the prefrontal cortex (areas Fr1 and Fr2) was induced by the method of photothrombosis [14]. The animals were intraperitoneally narcotized with 300 mg/kg chloral hydrate. Photosensitizing dye Bengal rose (3% solution, 40 mg/kg; Sigma) was injected into the jugular vein. Animal's head was fixed in a stereotaxis, the skin was cut longitudinally and the periosteum was separated. A fiber-optic light guide (light beam diameter 3 mm) was placed at a distance of 1 mm from the cranial surface (2 mm rostral to bregma, 2 mm lateral to the sagittal suture). The cranial surface was bilaterally irradiated with cold light for 15 min (250-W lamp). Sham-operated rats were subjected to the same manipulations except Bengal rose injection.

Experimental animals were divided into 3 groups. Group 1 consisted of sham-operated animals (n=11). Group 2 animals (n=12) intranasally received distilled water (7 μl) 15 min and 1 h after photothrombosis. Distilled water was administered daily for 6 days. Group 3 animals (n=10) intranasally received Semax in a dose of 250 μg/kg (7 μl) after photothrombosis. This scheme of Semax administration provided for a possibility of modulating the processes occurring in the early and delayed stages after ischemia modeling. Semax was withdrawn 48 h before testing to exclude short-term effects of this drug on memory and behavior.

Horizontal and vertical locomotor activity (LA) of rats was studied in an open field using an automatic RODEO-1 setup. The procedure was performed before passive avoidance (PA) conditioning. LA was repeatedly studied 8 days after photothrombosis (before the test for PA retention).

Passive avoidance was conditioned as described elsewhere [2]. The response latency was estimated as the time from the start of the test to the moment when the rat passed the hole between the light and dark compartments of the chamber. On day 1 of training the rat was placed in the illuminated compartment (100-W lamp). The rat examined this area and moved to the dark compartment (latency before training). The door was closed, and the rat remained in this compartment for 5 min. The procedure was repeated after 1 h, but during this session the rat was immediately removed from the dark compartment. On day 2, the procedure was performed 2 times at a 1-h interval. When the rat repeatedly entered the dark compartment, the door was closed. Electric current (1.3 mA, 50 Hz) was delivered via a metal-grid floor for 5 sec. PA was considered to be elicited when the latency of transition was ≥300 sec. The animals with lower latency were excluded from the study. The neuroprotective effect of Semax was studied 8 days after infarction modeling.

For visual evaluation of the size of ischemic infarction, nonfixed brain was stained with 1% triphenyltetrazolium chloride (Sigma) at 37°C for 15 min. The volume (V) of infarction was calculated from the areas of ischemic injury on serial cross-sections of the brain (100 μ) stained with 1% cresyl violet:

$$V=d\times\Sigma A_i,$$

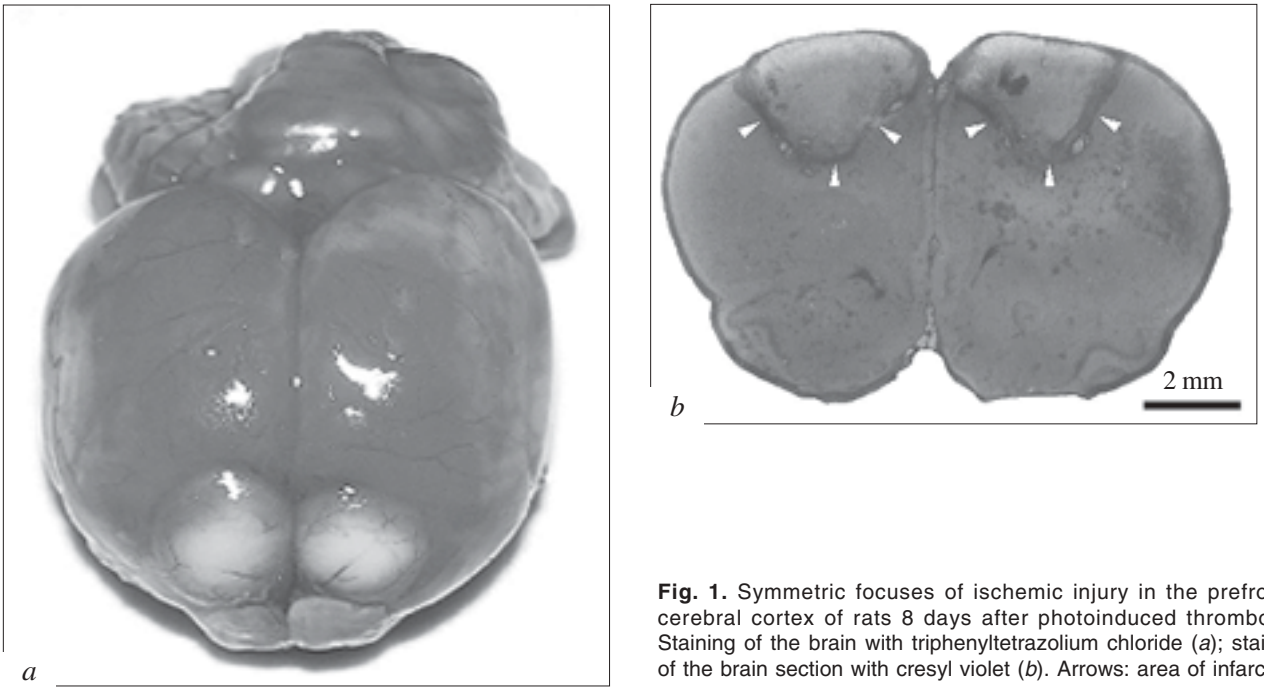

Fig. 1. Symmetric focuses of ischemic injury in the prefrontal cerebral cortex of rats 8 days after photoinduced thrombosis. Staining of the brain with triphenyltetrazolium chloride (*a*); staining of the brain section with cresyl violet (*b*). Arrows: area of infarction.

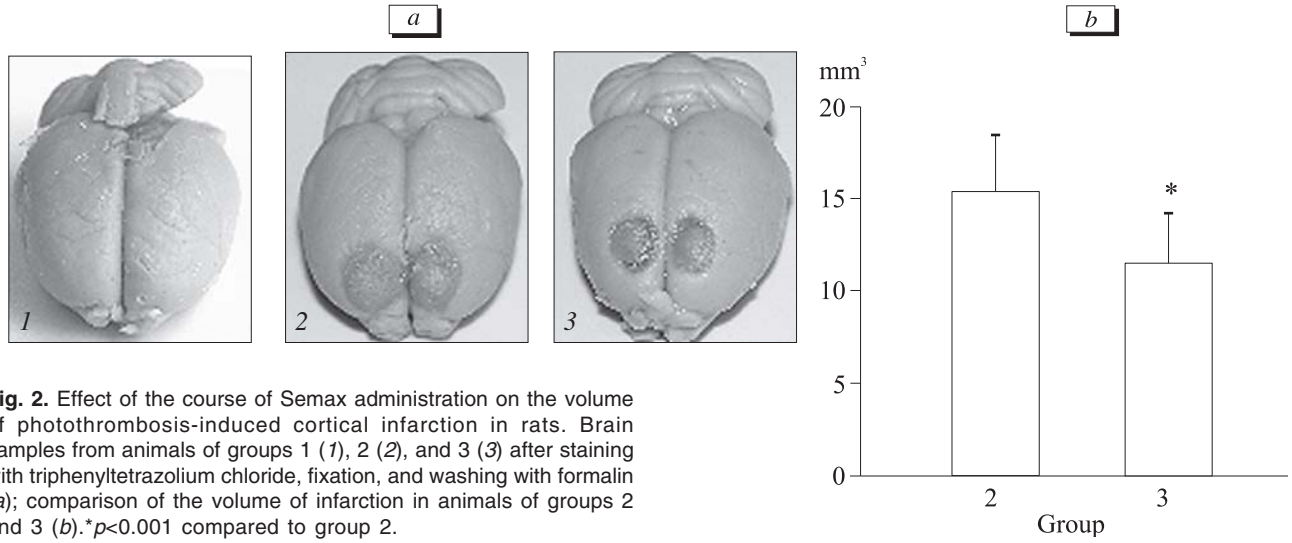

Fig. 2. Effect of the course of Semax administration on the volume of photothrombosis-induced cortical infarction in rats. Brain samples from animals of groups 1 (*1*), 2 (*2*), and 3 (*3*) after staining with triphenyltetrazolium chloride, fixation, and washing with formalin (*a*); comparison of the volume of infarction in animals of groups 2 and 3 (*b*).*$p<0.001$ compared to group 2.

where ΣA_i is the total area of damage in all sections; and d is the width of sections.

The data were processed using Statistica 6.0 software. Normality of distribution was tested using Shapiro—Wilk W test. LA and PA latency were analyzed using Mann-Whitney U test (independent variables) and Wilcoxon test (paired comparison of linked samples). The difference between the volumes of infarction was analyzed using Student's t test.

RESULTS

Bilateral injury of the prefrontal cortex after photothrombosis led to the formation of local ischemic infarction involving the whole width of the cortex and was separated from the surrounding intact tissue by a well-defined boundary (Fig. 1).

The volumes of injury in rats of groups 2 and 3 were compared 8 days after induction of cortical infarction. Semax decreased the volume of infarction by 25% (Fig. 2).

Significant behavioral changes were revealed 8 days after photothrombosis, whereas before PA conditioning the animals of experimental groups demonstrated similar LA (Table 1). The latency and LA decreased compared to the basal level (Fig. 3, Table 1). LA decreased in animals of all groups. No intergroup differences were found in LA before and after focal cortical infarction. This probably suggests that the decrease in LA is associated with the reaction of animals to anesthesia and surgical manipulations, rather than to damage to the prefrontal cortex.

Systemic administration of Semax after bilateral local ischemic infarction significantly improved PA retention and performance (Fig. 3). This effect was also observed 48 h after withdrawal of the drug, which attests to the formation of stable changes probably related to a decrease in the severity of

TABLE 1. LA of Rats before and after Bilateral Photoinduced Thrombosis of the Prefrontal Cortex

Group	LA, arb. units			
	horizontal		vertical	
	before photothrombosis	day 9 after photothrombosis	before photothrombosis	day 9 after photothrombosis
1	**202** (146-209-257)	**151** (121-154-214)	**26.5** (10-26-38)	**15.5** (7-16-23)
2	**231** (211-225-267)	**151**** (87-149-202)	**22.7** (12-21-22)	**15.6** (2-8-11)
3	**239** (201-226-273)	**156*** (65-172-233)	**25.4** (14-*22*-30)	**14.1** (8-12-15)

Note. The data are presented as means (lower quartile-median-upper quartile). *$p<0.05$ and **$p<0.01$ compared to LA before photothrombosis.

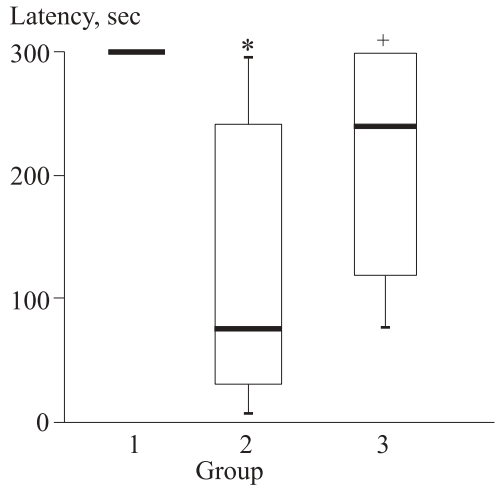

Fig. 3. Effect of the course of Semax treatment on PA retention and performance in rats with photothrombosis. Horizontal line, median; bars, interquartile interval; lower vertical line, 10%; upper vertical line, 90%. *$p<0.001$ compared to group 1; $^{+}p<0.05$ compared to group 2 (Mann—Whitney *U* test).

damage to cortical structures and changes at the level of the transmitter, metabolic, or trophic system.

Our results indicate that Semax produces a pronounced neuroprotective and antiamnesic effect during focal ischemia of the cerebral cortex, which is probably determined by its capacity to decrease the degree of ischemic inflammatory reactions [6, 11], stimulate synthesis of neutrophins [5], and prevent neuronal death [3,8]. These data provide experimental evidence that Semax can be used in the acute period of cerebral ischemic stroke.

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