In Vivo Efficiency of Semax in Global Cerebral Ischemia

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The efficiency of neuropeptide preparation Semax was studied on an animal model of global cerebral ischemia caused by irreversible bilateral occlusion of the common carotid arteries. Semax significantly alleviated neurological deficit and slightly improved postischemic survival. These effects can be attributed to the antihypoxic and neurotrophic effects of the preparation.

Key Words: *bilateral occlusion of the common carotid arteries; neurological deficit; neuroprotection; Semax*

Recent studies have opened up a new understanding of the mechanisms of damage to the nervous tissue under conditions of acute cerebral ischemia. Two basic mechanisms of neuronal death are now intensely investigated: necrosis and apoptosis [3,5]. A promising approach to the neuroprotective therapy in ischemia is stimulation of neurotrophic processes, which prevents the development of both apoptotic and necrotic changes in neurons. An important role in this approach is assigned to neuropeptide preparations [4,9].

The aim of this study was to assess the efficiency of neuropeptide preparation Semax ($ACTH_{4-10}$ analog without hormonal activity [1]), in an animal model of global ischemia induced by irreversible bilateral occlusion of the common carotid arteries. Semax as a medicinal preparation was developed and synthesized at the Institute of Molecular Genetics (Russian Academy of Sciences) in 1981.

MATERIALS AND METHODS

Randomized blind study was carried out on 40 male Wistar rats weighing 80-100 g. Irreversible bilateral occlusion of the common carotid arteries was performed under ether narcosis. After surgical approach the arteries were simultaneously ligated with a silk ligature. Standard surgery took no more than 7-10 min and the rats rapidly recovered from narcosis.

The test drugs were injected intraperitoneally (daily dose in 1 ml volume). The animals were divided into 4 groups (10 rats per each): group 1 received Semax, group 2 — saline (control); group 3 —cerebrolysin (reference group); group 4 comprised sham-operated animals. Group 1 rats received 300 μg/kg Semax divided into 4 fractions: 15, 60, 120 min and 5 h after occlusion. The dosage and fractional administration were chosen on the basis of clinical and experimental data showing that Semax exerts a nootropic effect in small doses (3-30 μg/kg) and antihypoxic and neurotrophic effects in high doses (150-300 μg/kg) [1,2,7]. Group 3 rats received cerebrolysin 15 and 60 min after occlusion (total dose 2.5 ml/kg). This preparation contains low-molecular-weight peptides with a pronounced neurotrophic effect [9]. Group 2 rats (controls) received 0.5 ml saline 15 and 60 min postocclusion. The sham-operated rats (the same anesthesia and surgery without ligation) received intraperitoneal saline. Blind assessment of neurological deficit was performed every 30 min for 24 h using C. P. McGrow [8] and Rudolphi *et al.* [6] scales. According to C. P. McGrow's scale [8], the stroke index increased with the appearance of signs of neurological deficit. According to Rudolphi's *et al.* scale [6], the neurological

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scores ranged from 9 (norm) to 0 (death). Limited mobility, ptosis (bi- or unilateral), circling, hyperactivity, rotatory, tonic and clonic seizures, and coma with weak or absent nociceptive responses were considered as principal symptoms of neurological deficit.

The dynamics of neurological deficit was analyzed using ANOVA (analysis of variance) and factor analysis. Mortality and neurological symptoms were assessed using Fisher's test.

RESULTS

During the first 3 h after bilateral occlusion of the carotid arteries the rotatory and clonic seizures were observed in 80% control animals (group 2), 40% Semax-treated rats (p=0.08), and in 50% cerebrolysin-treated rats (p=0.17).

The rats of groups 3 and 2 did not differ by neurological scores measured over 24 hours [6,8] (Fig. 1). In group 1, the mean stroke index remained below and Rudolphi score remained above the corresponding indices in groups 2 and 3 throughout the 24-h observation period. The difference between groups 1 and 2 was significant from 1.5 to 6.5 h postocclusion.

In groups 2 and 3, 50% lethal outcomes were observed within the first 7 h after occlusion, while in group 1 all animals survived this period, but 50% of them died from 8 to 10 h postocclusion. Twenty four hours postocclusion, the mortality in the control group was 100%, in group 3 — 90% , and in group 1 — 70%. The mean survival time in the control group was 9.1±1.7 h, in group 3 — 11.5±2.3 h, and in group 1 — 14.2±2.5 h. The mean McGrow's scores at the moment of death were comparable in all groups: 19.4± 0.9 (group 2, control), 19.9±0.5 (group 3, cerebrolysin), and 20±1.1 (group 1, Semax). In should be noted that 24 h after occlusion group 1 rats showed higher McGrow's scores (by 10 points) than group 3 animals, which suggest that Semax prolonged survival of animals with a pronounced neurological deficit.

Thus, the neuropeptide preparation Semax had a positive effect on animals with global cerebral ischemia and alleviated symptoms of neurological deficit during the first 6.5 h. The tendency toward the increase in survival time and the period until the first

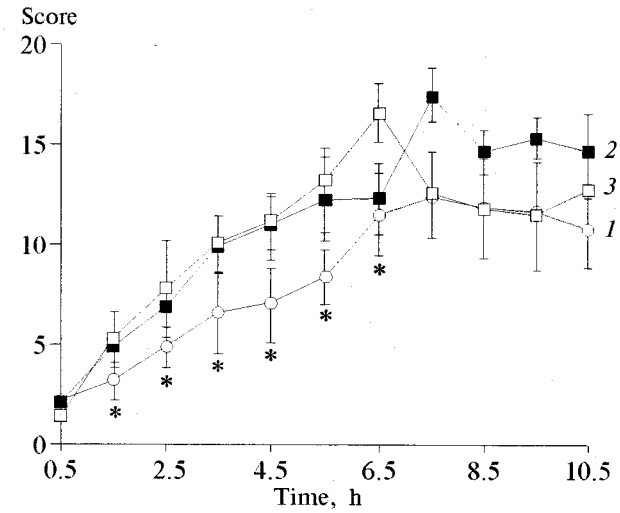

Fig. 1. Dynamics of neurological deficit (McGrow's score [8]) in rats after simultaneous bilateral occlusion of the common carotid arteries. Groups 1, 2, and 3 are represented by the corresponding curves. p<0.03 in comparison with the control (ANOVA).

lethal outcome can also be attributed to the neuroprotective properties of Semax. These data need further confirmation with a greater number of observations. However, they provide the basis for experimental studies of Semax efficiency in focal *in vivo* ischemia and experimental ischemic stroke.

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