C-Terminal Pro-Gly-Pro Tripeptide in Contrast to Full-Length Neuropeptide Semax Exhibits No Neuroprotective Effect in Experimental Cerebral Ischemia

O. E. Fadyukova, A. Kadi, Ou Bai, G. M. Andzhusheva*, and V. B. Koshelev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 4, pp. 413-415, April, 2005
Original article submitted April 23, 2004

The C-terminal fragment Pro-Gly-Pro of semax does not modulate the development of symptoms of neurological deficiency and mortality in rats with incomplete global cerebral ischemia. Hence, previously revealed neuroprotective effects of semax are mainly determined by corticotropin $ACTH_{4-7}$ fragment.

Key Words: *Pro-Gly-Pro; semax; cerebral ischemia*

Neuropeptide drug semax (synthetic analog of $ACTH_{4-10}$) exhibiting no hormonal activity contains a C-terminal Pro-Gly-Pro (PGP) tripeptide determining molecular stability of the preparation [2,15]. Semax possesses nootropic and neuroprotective activities [4]. Experiments showed that semax improved animal resistance to acute hypoxia [7] and decreased the severity of neurological deficit in experimental cerebral ischemia [12]. Clinical use of semax reduced mortality, improves outcomes in brain stroke, and promotes recovery of impaired neurological functions in patients with ischemic stroke [4]. Semax produces an antiulcer effect by promoting healing gastric ulcers in humans [6] and experimental ulcers in animals [5]. Similarly to semax, its C-terminal PGP fragment is also characterized by protective effects in ulcers caused by central (stress) and peripheral mechanisms (induced by ethanol and indomethacin) [1]. A possible mechanisms of antiulcerogenic effect of PGP is improvement of the bloodflow in the gastric mucosa [9]. It remains unclear whether the stabilizing C-terminal PGP fragment of semax possesses an independent neuroprotective activity and whether the protective effects of semax in cerebral ischemia are due to these characteristics of the fragment.

We studied the effect of PGP tripeptide on the development of neurological deficit (ND) symptoms in rats with incomplete global ischemia of the brain.

MATERIALS AND METHODS

The study was carried out on 19 random-bred rats (161±2 g). Incomplete global ischemia of the brain was induced by irreversible bilateral occlusion of the common carotid arteries [14]. The right and left common carotid arteries were separated through an incision on the neck in all animals under ether narcosis; two ligatures were made on both arteries, the vessels were ligated simultaneously and crossed between the ligatures. The duration of operation was 7-10 min, and animals rapidly (within 15-20 min) awakened after the end of operation and narcosis. The animals of the experimental group (n=10) were injected with PGP (synthesized at Laboratory of Regulatory Peptides, Institute of Molecular Genetics, Russian Academy of Sciences) in a total dose of 2 mg/kg (2 intraperitoneal injections in a volume of 0.3 ml/150 g 15 min and 1 h after ligation of the common carotid arteries). Controls (n=10) were injected with saline in the same volume according to the same protocol.

Faculty of Fundamental Medicine; *Biological Faculty, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** olevfa@fbm.msu.ru. O. E. Fadyukova

0007-4888/05/1394-0421

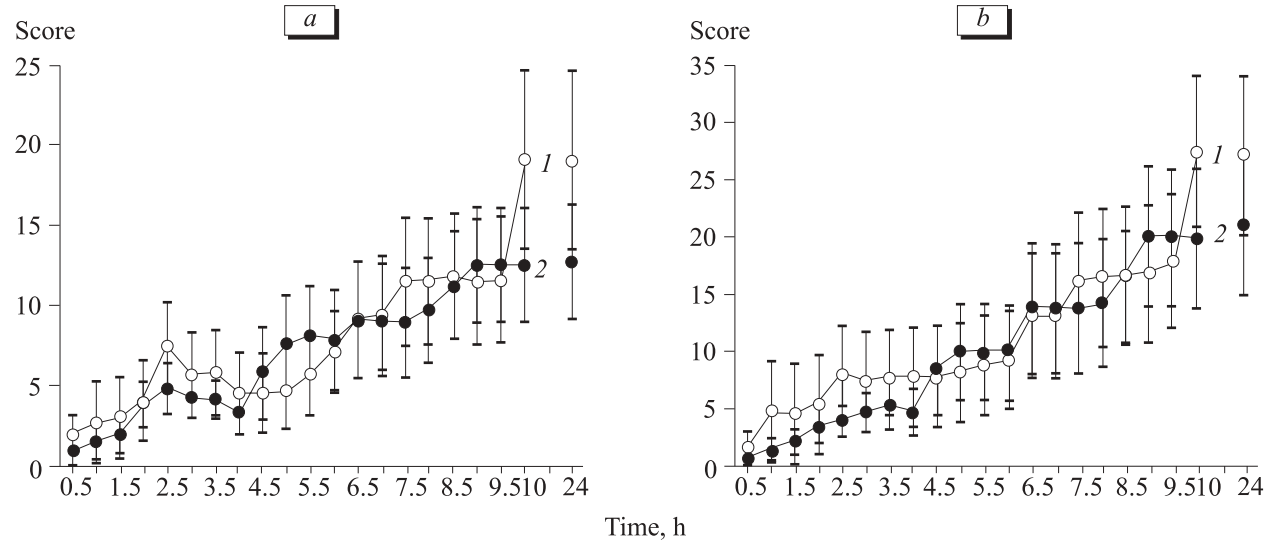

Fig. 1. McGraw's (*a*) and K. Yu. Sarkisova scores (*b*) of neurological deficiency in rats with cerebral ischemia injected with saline (*1*; *n*=9) or PGP (*2*; *n*=10).

After surgery the animals were placed into cages and the development of ND symptoms was scored visually every 30 min for 10 h [10,13]. The score increased with the appearance of ND signs and their progress. ND symptoms were evaluated by the blind method (without information about rat division into groups). The main signs of ND included low motor activity, ptosis, forced movements (whirling, jumps, convulsive and rotating seizures), pareses of the limbs, and coma.

The data were statistically processed using Mann—Whitney nonparametric test.

RESULTS

The symptoms of ND evaluated by McGraw score (Fig. 1, *a*) and by Sarkisova score (Fig. 1, *b*) in rats with cerebral ischemia treated with PGP did not differ from those in rats injected with saline during the entire period of observation.

The time of appearance of convulsive attacks in animals with ND progressing during 10 h of observation was 5.4±1.2 (*n*=5) and 6.3±1.5 h (*n*=6) in experimental and control groups, respectively (the differences are insignificant).

Half of lethal outcomes were recorded by the 9th h of observation in the experimental group and by the 10th hour in control rats; 60% animals with cerebral ischemia treated with PGP and 67% controls died 24 h after carotid occlusion. The mean life span of experimental animals by this term was 15±2.9 h and that of controls 13±2.9 h.

Apart from nootropic effect, semax therapy of rats with experimental cerebral ischemia was associated with protective effects: the severity of ND was lower during the first 6.5 h [12], which correlated with the decrease in NO hyperproduction observed in ischemia [11]. Clinical studies showed high efficiency of semax during the acute period of ischemic stroke [4]. It activated the antiinflammatory component of postischemic reactions in the brain [8] and its antihypoxic effect consisted in improvement of brain resistance to hypoxia [7]. It cannot be excluded that the neuroprotective effects of semax are due to the presence of the PGP fragment in the molecule. Semax and PGP possess antiulcerogenic effect preventing the development of experimental gastric ulcers and promoting ulcer healing in experimental animals and patients [1,5,6,9]. The protective effect of PGP in gastric ulcer is attributed to its capacity to correct the bloodflow in the gastric mucosa and to relax blood vessels [3,9]. We detected no neuroprotective effect of PGP in rats with cerebral ischemia: symptoms of ND progressed similarly in experimental rats injected with PGP and in controls injected with saline. We observed no effect of the tripeptide on the incidence and time of lethal outcomes. Hence, therapy with PGP (the C-terminal fragment of semax) produces no neuroprotective effect in cerebral ischemia, in contrast to whole semax. It can be concluded that previously described neuroprotective effects of semax are mainly due to the presence of the $ACTH_{4-7}$ fragment in its molecule.

REFERENCES

1. M. A. Abramova, G. E. Samonina, and I. P. Ashmarin, *Neirokhimiya,* **13**, No. 3, 209-214 (1996).
2. I. P. Ashmarin, V. N. Nezavibat'ko, N. F. Myasoedov, *et al.*, *Zh. Vyssh. Nervn. Deyat.*, **47**, No. 2, 420-430 (1997).
3. Z. V. Bakaeva, K. E. Badmaeva, I. Yu. Sergeev, and G. E. Samonina, *Byull. Eksp. Biol. Med.*, **135**, No. 4, 390-393 (2003).

4. E. I. Gusev and V. I. Skvortsova, *Brain Ischemia* [in Russian], Moscow (2001).
5. S. E. Zhuikova, E. A. Smirnova, Z. V. Bakaeva, *et al.*, *Byull. Eksp. Biol. Med.*, **130**, No. 9, 300-302 (2000).
6. I. O. Ivanikov, M. E. Brekhova, G. E. Samonina, *et al.*, *Ibid.*, **134**, No. 7, 83-84 (2002).
7. A. Ya. Kaplan, V. B. Koshelev, V. N. Nezavibat'ko, and I. P. Ashmarin, *Fiziol. Chel.*, **18**, No. 5, 104-107 (1992).
8. N. F. Myasoedov, V. I. Skvortsova, E. L. Nasonov, *et al.*, *Zh. Nevrol. Psikhiatr.*, No. 5, 15-19 (1999).
9. G. E. Samonina, G. N. Kopylova, V. I. Sergeev, *et al.*, *Ros. Fiziol. Zh.*, **87**, No. 11, 1488-1492 (2001).
10. K. Yu. Sarkisova, B. Opitz, and P. Oeme, *Byull. Eksp. Biol. Med.*, **121**, No. 4, 399-403 (1996).
11. O. E. Fadyukova, A. A. Alekseev, V. G. Bashkatova, *et al.*, *Eksp. Klin. Farmakol.*, **64**, No. 2, 31-34 (2001).
12. E. V. Yakovleva, V. S. Kuzenkov, V. N. Fedorov, *et al.*, *Byull. Eksp. Biol. Med.*, **128**, No. 8, 172-174 (1999).
13. C. P. McGraw, *Arch. Neurol.*, **34**, 334-336 (1977).
14. K.-A. Hossman, *Cardiovasc. Res.*, **39**, 106-120 (1998).
15. V. N. Potaman, L. Y. Alfeeva, A. A. Kamensky, *et al.*, *Biochem. Biophys. Res. Commun.*, **176**, No. 2, 741-746 (1991).