ISSN 0096-3925, Moscow University Biological Sciences Bulletin, 2009, Vol. 64, No. 4, pp. 137–140. © Allerton Press, Inc., 2009.
Original Russian Text © Z.V. Bakaeva, K.E. Baglikova, P.A. Klimova, L.A. Andreeva, G.E. Samonina, 2009, published in Vestnik Moskovskogo Universiteta. Biologiya, 2009, No. 4, pp. 3–7.

The Comparison of Gastroprotective Properties of Semax and Its Metabolites

Z. V. Bakaeva[a], K. E. Baglikova[b], P. A. Klimova[c], L. A. Andreeva[c], and G. E. Samonina[a]

[a] *Department of Human and Animal Physiology, Biological Faculty, Moscow State University, Moscow, 119992 Russia*
e-mail: g_samonina@gmail.com
[b] *Kalmyk State University, Elista*
e-mail: kermenev@mail.ru
[c] *Institute of Molecular Genetics, Russian Academy of Sciences, Moscow*
e-mail: landr@img.ras.ru

Received December 25, 2006

Abstract—Semax (MEHFPGP) was shown to increase gastric mucosal homeostasis to the action of such ulcerogenic factors as ethanol and stress. In the case of the stress model of ulcer formation, Semax and its two metabolites—HFPGP and FPGP—at the wide range of doses (0.06–3.7 μmol/kg) have demonstrated protective antiulcerogenic properties. In the case of ethanol model of ulcer formation, only Semax in two used doses (0.06 and 0.37 μmol/kg) reported reliable protective antiulcerogenic property. It was supposed that Semax's gastroprotective activity directed to peripheral mechanisms of ulcerogenesis did not depend on its metabolites' activities. On the contrary, Semax's gastroprotective activity directed to the central mechanisms of ulcerogenesis might be also caused by gastroprotective activities of HFPGP and FPGP metabolites.

Key words: semax, metabolites, gastroprotective activity

DOI: 10.3103/S0096392509040014

INTRODUCTION

Semax has been synthesized via addition of Pro-Gly-Pro sequence to the C-end of unstable Met-Glu-His-Phe peptide—fragment of adrenocorticotropic hormone ACTH (4–7). Peptide MEHFPGP synthesized as reported above demonstrated high resistance to proteases action that allowed to significantly increase the duration of its action (Ashmarin et al., 1997).

Semax characterized by the absence of hormonal activity was shown to stimulate animals' training in different tests—in normal animals and in those with pathology (Yasnetsov et al., 1995; Ashmarin et al., 1997)—to demonstrate antihypoxic and antiamnestic properties (Kaplan et al., 1992; Yasnetsov et al., 1998) and to improve blood circulation in human and animals (Ashmarin et al., 1997; Khugaeva and Aleksandrin, 1997). In clinical practices Semax was used for patients' treatment with ischemic apoplexy (Myasoedov et al., 1999).

According to the study of Semax, influence on processes of blood coagulation and thrombosis and conjunction of PGP sequence to $AKTG_{4-7}$ resulted in both increase in its preliminary biological activity and in its addition with PGP effects. Thus, Semax was shown to possess somewhat lower (compared to glyprolines) but significantly marked anticoagulant, fibrinolytic, and antithrombotic activities in vivo. Since Semax is neutral with respect to homeostasis parameters in vitro, it could be supposed that Semax hydrolysis to PGP is essential for its influence on fibrinolysis and thrombosis processes (Ashmarin et al., 1996). PGP itself was reported to be classic glyproline with markedly expressed antithrombotic, fibrinolytic, and anticoagulant properties (Pastorova et al., 1998).

Semax was shown to enhance gastric mucosal (GM) resistance to action of several ulcerogenic factors such as ethanol and stress. Inhibition of acetic ulcers formation, as well as increase in its healing, under the action of Semax was also reported (Zhuikova et al., 2000).

It has been observed that Semax' half-life is less than 1 h in the presence of plasmatic membranes from rat brain. Peptide biodegradation mainly occurs by deletion of the N-end aminoacids from the primary nucleotide sequence. The main products of heptapeptide biodegradation are HFPGP, FPGP, and PGP with the prevalence of pentapeptide and tripeptide forms (Dolotov et al., 2004).

PGP protective properties with respect to gastric mucosal (GM) were demonstrated on various classical experimental models of ulcerogenesis (Abramova et al., 1996; Samonina et al., 2000; Bakaeva, Samonina, 2005). PGP metabolites—PG and GP dipeptides—might be detected in rats' blood after 30 min (Zolotarev et al., 2003) that possess their own differentiated gastroprotective activity. PG was assumed to

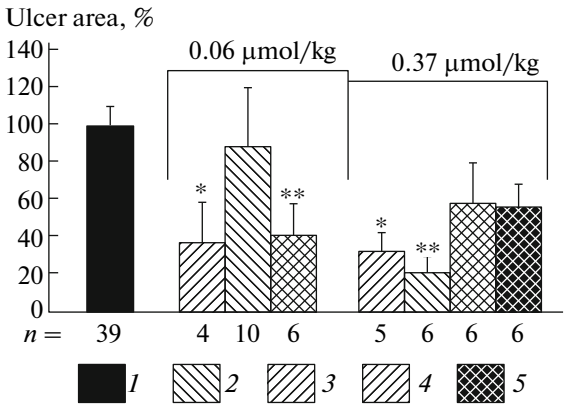

Fig. 1. Influence of Semax and its metabolites on ethanol-induced ulcerogenesis in rats. $*P < 0.05$; $**P < 0.01$ compared to control, n is the number of animals in the group. *1* is control, *2* is MEHFPGP, *3* is HFPGP, *4* is FPGP, and *5* is PGP.

be more effective on the models of gastric mucosal injury mainly caused by the disturbance in peripheral mechanisms of gastric homeostasis maintenance (Abramova et al., 1996).

Since antiulcerogenic activity of other Semax metabolites has not been studied previously, the present study aimed to investigate the possible influence of these peptides on GM resistance to such injuring factors as ethanol and stress, and to analyze the role of Semax derivatives with respect to formation of gastroprotective properties.

MATERIAL AND METHODS

Activity of Semax (MEHFPGP) and its metabolites—HFPGP, FPGP, and PGP—synthesized at the Institute of Molecular Genetics of the Russian Academy of Sciences has been investigated via two experimental models of ulcerogenesis: ethanol model in 0.06 and 0.37 μmol/kg doses and stress model in 0.06, 0.37 and 3.7 μmol/kg doses. Increase of Semax and its metabolites classic clinical dose (0.06 μmol/kg) to 0.37 and 3.7 μmol/kg allowed to compare antiulcerogenic effects of investigated peptides with glyproline effects (PGP, PG, and GP)—agents known for their antiulcerogenic properties.

In the first case, males from outbred white rates were used as model animals, while, in the second case, males from Wistar line with weight 200–300 g were investigated. Animals were deprived of food one day prior to the experiment, while those subjected to participation in the ethanol model were also deprived of water, and placed into the chamber with lattice bottom for coprophagy prevention. Case and control animals were peritoneally injected either with the studied preparation or physiological solution in the quantity of 0.5 ml/200 g of weight one hour prior ulcerogenesis.

One hour after preparation injection, rats were either intragastrically injected with 1ml/200g of weight 96% ethanol or placed into water-containing pool (water temperature was 21°C) for 30 min. Subsequently animals were wiped and placed into warm heated chambers. Animals were killed by ether after one hour.

Every ulcer area in mm^2 has been assessed via binocular with ocular micrometer. Summarized ulcer area was counted in every stomach. Since mean ulcer areas in control animals varied significantly in different experiments, ulcer areas were normalized, i.e., were presented in percent to mean control meaning in every distinct experiment. Mean ulcer area was estimated for every group, i.e., ulcer injury in percent to control one. Antiulcerogenic preparation action was presented as reverse number, i.e., percent explaining decrease in mean area of experimentally induced ulcers compared to control due to peptides action. Test of statistically significant differences between groups was carried out under Statistica 5.0 LSD-test software.

Animal experiments were performed correspondingly to bioethical principals and normative documents recommended by the European Science Foundation and the Declaration of Helsinki of Humanic Relation to Animals.

RESULTS AND DISCUSSION

One hour after ethanol injection, large injuries on GM were detected involving significant widely stretching hemorrhages and erosions. In the case of stress model of ulcer induction, spot erosions on mucous membrane sized from 0.1×0.1 mm to 1×1 mm named stressor ulcers in the published data were generated.

Induction of ulcer injuries is caused by involvement of both peripheral and central mechanisms possessing different impact in stomach ulcerogenesis. Occurrence of ethanol-induced ulcers in GM was shown to be mainly due to peripheral mechanisms of ulcerogenesis (Sibilia et al., 2003), while stress influence of swimming in cold water was shown to be mainly due to the central mechanisms (Mayer, 2000).

Ethanol model of ulcerogenesis. The group predominantly subjected to Semax injection in classic clinical doses of 0.06 μmol/kg was characterized by the occurrence of ethanol-induced injury equal to 36% compared to the ulcer area equal to 100% in control animals (Fig. 1). Antiulcerogenic effect comprised 64%. Slightly lower antiulcerogenic effect (60%) was observed in the case of FPGP tetrapeptide injection. HFPGP pentapeptide has not demonstrated any influence on ulcerogenesis.

Increase in Semax dose by six times resulted in no change in its gastroprotective action (Fig. 1). Interestingly, with an injection of penta- and tetrapeptides in a 0.37 μmol/kg dose, the inversion of their action was

detected. In the case of HFPGP, significant gastroprotective action (80%) was observed, while FPGP injection demonstrated only the tendency of this action.

Gastroprotective effect of PGP tripeptide is known to be stable at the wide dose range from 0.5 to 53 μmol/kg. Analogous properties were shown for PG dipeptide, while GP dipeptide decreased ethanol-induced injuries only in the case of dose increase to 53 μmol/kg (Zhuikova et al., 2003).

Probably, subsequent FPGP hydrolysis does not result in PGP metabolite synthesis since no action of this tetrapeptide used in classic dose 3.7 μmol/kg was observed (not shown in Fig. 1), while markedly expressed antiulcerogenic activity was detected for tripeptide (Zhuikova et al., 2003). FPGP effect, or its absence to be more precise, reminds of indifferent properties of GP with respect to injuries induced by peripheral mechanisms of ulcerogenesis. Hence, it might be supposed that the product of FPGP hydrolysis is GP characterized by the absence of ulceroprotective properties in the case of ethanol model of ulcerogenesis. It could not be excluded that the methods used by authors (Dolotov et al., 2004) for investigation of Semax biodegradation in the presence of plasmatic membranes from rat's brain have not allowed to detect short dipeptides in the incubation mixture.

Accordingly, Semax protection of GM from ethanol-induced injuries is probably defined by heptapeptide's action in total, while its metabolites were shown to possess their own dose-dependent gastroprotective effects. Considering our previous findings, antiulcer activity was reported for such tetrapeptides as GPGG (Abramova et al., 1996) and GPGP (Baglikova et al., 2009); however, FPGP could not be referred to glyprolines due to the presence of phenylalanine in its molecule.

Stress water immersion model of ulcerogenesis. In the case of stress model, Semax was demonstrated to possess approximately equal antiulcerogenic activity at 0.06 and 0.37 μmol/kg doses—60%—while at 3.7 μmol/kg dose activity was 89% (Fig. 2). This effect is 18% higher than the effect reported from the action of the mostly active to stress-induced ulcers glyproline—GP dipeptide used in classic dose 3.7 μmol/kg (Zhuikova et al., 2003a). Semax derivatives HFPGP and FPGP demonstrated significant gastroprotective effects at dose 0.06 μmol/kg—92 and 90%, respectively. Dose increase by either 6 or 60 times resulted in almost unchanged properties. Antiulcerogenic effects comparison between MEHFPGP and its metabolites—HFPGP, FPGP, and PGP—injected at 3.7 μmol/kg dose revealed reliably enhancement in GM resistance to stress action with the maximal tendency in effectiveness for MEHFPGP.

Accordingly, Semax metabolites—HFPGP and FPGP—were demonstrated to possess their own gastroprotective potential at the large range of doses—from 0.06 to 3.7 μmol/kg—in the case of peritoneal injection and might be involved into MEHFPGP protective properties directed to the stomach mucous membrane against stress action. Significantly large protective percent of heptapeptide injection was revealed only using the largest acceptable dose.

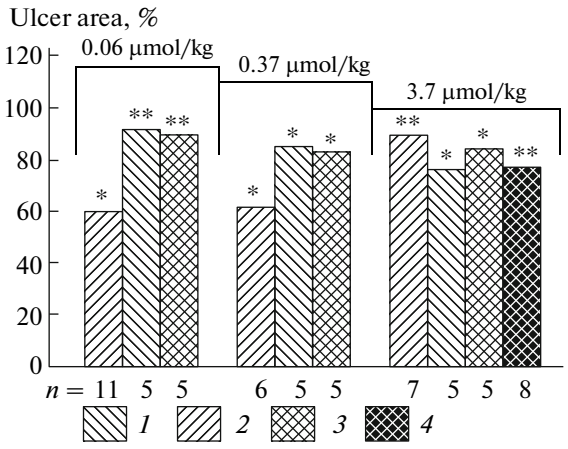

Fig. 2. Influence of Semax and its metabolites on stress-induced ulcerogenesis in rats. $*P < 0.005$; $**P < 0.001$ compared to control, n is the number of animals in the group. *1* is MEHFPGP, *2* is HFPGP, *3* is FPGP, and *4* is PGP. Other designations the same as in Fig. 1.

Thus, revealed data confirms the presence of Semax's gastroprotective properties under both peripheral and central level. The main mechanisms of antiulcerogenic Semax action might include its influence on blood flow (Zhuikova et al., 2002), on stomach secretion (Zhuikova et al., 2003a, b), and on CNS (Yasnetsov et al., 1995; Ashmarin et al., 1997).

It should be supposed that antiulcerogenic Semax activity directed to injuries induced by peripheral mechanisms of ulcerogenesis is mainly determined by the effects of heptapeptide itself and does not depend on hydrolysis products. Gastroprtotective action of MEHFPGP directed to the ulcer induced by central disturbances in gastric mucosal homeostasis might be related to HFPGP and PGP metabolites release.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 06-04-48833.

REFERENCES

Abramova, M.A., Samonina, G.E., and Ashmarin, I.P., Proline and Simple Proline-Containing Neuropeptide Fragments Modulate the State of Gastric Mucosa via Central and Peripheral Mechanisms, *Neirokhimiya,* 1996, vol. 13, no. 3, pp. 209–214.

Ashmarin, I.P., Lyapina, L.A., and Pastorova, V.E., Modulation of Hemostatic Reactions in Vitro and in Vivo by Members of Regulatory Peptide Families, *Vestn. Ross. Akad. Med. Nauk*, 1996, issue 6, pp. 50–57.

Ashmarin, I.P., Nezavibat'ko, V.N., Myasoedov, N.F., Kamenskii, A.A., Grivennikov, I.A., Ponomareva-Stepnaya, M.A., Andreeva, L.A., Kaplan, A.Ya., Koshelev, V.B., and Ryasina, T.V., Semax, a Nootropic Analogue of Adrenocorticotropin 4–10 (15-Year Experience of Development and Study), *Vyssh. Nervn. Deyat. im. I. P. Pavlova*, 1997, vol. 47, no. 2, pp. 420–430.

Baglikova, K.E., Trufanova, A.V., Bakaeva, Z.V., Samonina, G.E., Andreeva, L.A., Klimova, P.A., and Vas'kovskii, B.V., The Influence of Hydroxyproline- and Proline-Containing Glyprolines on Ethanol-Induced Gastric Lesions in Rats, *Vestn. Mosk. Univ., Ser.: Biol.*, 2009, vol. 64, no. 1, pp. 3–8.

Bakaeva, Z.V. and Samonina, G.E., Effect of Glyprolins on Development and Healing of Acetate Ulcers in Rats, *Patofiziol. i Eksperiment. Terapiya,* 2005, no. 2, pp. 25–27.

Dolotov, O.V., Zolotarev, Yu.A., Dorokhova, E.M., Andreeva, L.A., Alfeeva, L.Yu., Grivennikov, I.A., and Myasoedov, N.F., The Binding of Semax, ACTH 4–10 Heptapeptide, to Plasma Membranes of the Rat Forebrain Basal Nuclei and Its Biodegradation, *Bioorg. Khim.*, 2004, vol. 30, no. 3, pp. 241–246.

Kaplan, A.Ya., Koshelev, V.B., Nezavibat'ko, V.N., and Ashmarin, I.P., Increased Resistance to Hypoxia Effected by the Neuropeptide Preparation SEMAX, *Fiziol. Chel.*, 1992, vol. 18, no. 5, pp. 104–107.

Khugaeva, V.K. and Aleksandrin, V.V., Relationship between the Therapeutic Effect of the Peptide Preparation Semax and the Severity of Brain Ischemia, *Byul. Eksp. Biol. Med.*, 1997, vol. 124, no. 7, pp. 39–42.

Mayer, E.A., The Neurobiology of Stress and Gastrointestinal Disease (Review), *Gut*, 2000, vol. 47, pp. 861–869.

Myasoedov, N.F., Skvortsova, V.I., Nasonov, E.L., Zhuravleva, E.Yu., Grivennikov, I.A., Arsen'eva, E.L., and Sukhanov, I.I., Study of Mechanisms of Neuroprotective Effect of Semax in the Acute Period of Ischemic Stroke, *Zhurn. Nevrol. Psikhiat.,* 1999, vol. 99, no. 5, pp. 15–19.

Pastorova, V.E., Lyapina, L.A., Smolina, T.Yu., and Ashmarin, I.P., Anticoagulant and Fibrinolytic Effects of Short Proline-Containing Peptides, *Izv. Akad. Nauk, Ser. Biol.,* 1998, vol. 25, no. 3, pp. 390–394.

Samonina, G., Lyapina, L., Kopylova, G., Pastorova, V., Bakaeva, Z., Jeliaznik, N., Zuykova, S., and Ashmarin, I., Protection of Gastric Mucosal Integrity by Gelatin and Simple Proline-Containing Peptides, *Pathophysiology*, 2000, issue 7, pp. 69–73.

Sibilia, V., Rindi, G., Pagani, F., Rapetti, D., Locatelli, V., Torsello, A., Campanini, N., Deghenghi, R., and Netti, C., Ghrelin Protects against Ethanol-Induced Gastric Ulcers in Rats: Studies on the Mechanisms of Action, *Endocrinology,* 2003, vol. 144, no. 1, pp. 353–359.

Yasnetsov, V.V., Krylova, I.N., and Provornova, N.A., Pharmacological Treatment of Memory Disorders Caused by Hypoxia and Cerebral Ischemia in Rats, *Kosm. Biol. Aviakosm. Med.,* 1998, no. 1, pp. 55–60.

Yasnetsov, V.V., Popov, V.M., Kisileva, N.M., Kamenskii, A.A., and Nezavibat'ko, V.N., Vestibular-Protective and Anti-amnesia Properties of Adrenocorticotropic Hormone Fragments and Their Analogs, *Byul. Eksp. Biol. Med.*, 1995, vol. 119, no. 6, pp. 634–636.

Zhuikova, S.E., Bakaeva, Z.V., and Samonina, G.E., Differential Antiulcer Effects of Possible Metabolites of Peptide PGP–GP and GP in Ethanol- and Stress-Induced Ulceration Models in Rats, *Vestn. Mosk. Univ., Ser. Biol.*, 2003a, issue 2, pp. 20–22.

Zhuikova, S.E., Khropycheva, R.P., Zolotarev, V.A., Polenov, S.A., and Samonina, G.E., New Peptide Regulatory of Gastric Secretion in Rats (Amylin, PGP, and Semax), *Eksp. Klinich. Gastroenterol.*, 2003b, issue 2, pp. 86–91.

Zhuikova, S.E., Sergeev, V.I., Samonina, G.E., and Myasoedov, N.F., Effect of Semax on Indomethacin-Induced Ulceration in Rats and a Possible Mechanism of Its Action, *Byul. Eksperiment. Biol. Med.*, 2002, vol. 133, no. 6, pp. 665–667.

Zhuikova, S.E., Smirnova, E.A., Bakaeva, Z.V., and Samonina, G.E., Effect of Semax on Homeostasis of Albino Rat Gastric Mucosa, *Byul. Eksper. Biol. Med.*, 2000, vol. 129, no. 10, pp. 300–302.

Zolotarev, Yu.A., Zhuikova, S.E., Ashmarin, I.P., Myasoedov, N.F., Vas'kovskii, B.V., and Samonina, G.E., Metabolism of PGP Peptide after Administration via Different Routes, *Byul. Eksp. Biol. Med.*, 2003, vol. 135, no. 4, pp. 423–426.