ISSN 1819-7124, Neurochemical Journal, 2007, Vol. 1, No. 3, pp. 260–263. © Pleiades Publishing, Ltd., 2007.
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EXPERIMENTAL ARTICLES

Estimation of the Antioxidant Effect of the Nootropic Dipeptide Noopept on the Model of Fe^{2+}-induced Chemiluminescence of Lipoproteins of Human Serum in vitro

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Received September 15, 2006

Abstract—The antioxidative activity of the nootropic dipeptide Noopept (the ethyl ether of *N*-phenylacetyl-*L*-prolylglycine, GVS-111) was studied on a model of Fe^{2+}-induced chemiluminescence (CL) of serum lipoproteins from healthy human donors in vitro. Efficiency of the following antioxidant agents has been compared: Noopept, pyracetam (the non-peptide prototype of Noopept), the PBN antioxidant (*N*-tert-butyl-α-phenylnitron that acts as a free radical trap), and mannitol, which is used as a neuroprotective drug in the treatment of brain edema. Noopept was shown to modulate the duration of the CL latent period (τ) that reflected an endogenous antioxidant potential. Noopept and PBN caused a maximum increase in this parameter of 3.4 and 3 times relative to the control, respectively. At the same time, Noopept appeared to be more effective, because it acted at lower concentrations compared to PBN. Our results demonstrated that Noopept protected human serum lipoproteins from Fe^{2+}-induced lipid peroxidation. This effect, in combination with its previously found nootropic and neuroprotective properties, made this systemically active dipeptide a promising clinical drug for the treatment of stroke and other disorders associated with deficiency of the endogenous antioxidant system.

Key words: Noopept, natural and synthetic antioxidants, chemiluminescence, human serum lipoproteins

DOI: 10.1134/S1819712407030154

INTRODUCTION

Activation of lipid peroxidation is an important component of the metabolic cascade that develops during various brain disorders such as stroke, brain trauma, neurodegenerative diseases, and neural infections. Therefore antioxidative drugs are indispensable in clinical practice. It is well established that many nootropic agents, such as pyracetam [1], combine the ability to restore damaged cognitive functions with the neuroprotective effect. Normalization of interrelations between the prooxidant and antioxidant systems may play a significant role in realization of this effect. The antioxidant properties of pyracetam have also been described in the literature [2, 3]. However, an insufficient efficiency of pyracetam and the lack of convincing data about the mechanism of its action have provoked a search for other nootropics with higher biological stability [4]. Studies on carnosine and related compounds confirmed the high biologic activity of dipeptides [5]. An original approach to searching for highly effective nootropics has been developed at the Institute of Pharmacology of the Russian Academy of Medical Sciences. It is based on the synthesis of dipeptides containing one of the endogenous pirrolidine carbonic amino acids, pyroglutamate, or proline [6, 7]. This approach, based on the hypothesis of a peptidergic mechanism of the pyracetam effect, was used for preparation of a series of *N*-acyl derivatives of proline. Noopept (ethyl ester of *N*-phenylacetyl-*L*-prolylglycine) was selected for further studies owing to its pronounced antiamnesic and neuroprotective effects [8, 9].

Noopept was experimentally shown to surpass pyracetam both in its effective dose (Noopept acts at a dose 1000-fold less than that of pyracetam) and in the spectrum of its mnemonic activity. Pyracetam facilitates only the early phases of information processing, whereas Noopept also affects the process of consolidation, storage and extraction of information in the brain [10]. Previously, experimental evidence was obtained for its capacity for preventing LPO activation and the deficiency of antioxidant system induced by animal immobilization [11, 12]. The pronounced neuroprotective effect of Noopept was demonstrated during experimental brain ischemia [13] and on various models of neuronal cultures. Noopept prevents accelerated neu-

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Abbreviations: CL, chemiluminescence; LPO, lipid peroxidation; PBN, *N*-tert-butyl-α-phenylnitron.

ronal death and increases neuronal survival [14]. Its antioxidant activity, its capacity to block the neurotoxic effects of calcium ions [15], and glutamate [16], inhibition of glutamate release [17], its anti-inflammatory effect [18] and its ability to improve the rheological properties of blood [19] were shown to be the basis of this effect.

Application of new neuroprotectors to clinical practice is limited by the lack of methods of determination of their anti-oxidant potential. This study was aimed at quantitative determination of a Noopept anti-oxidant effect on the model of Fe^{2+}-induced CL of serum lipoproteins from healthy donors in vitro.

MATERIALS AND METHODS

We used a chemiluminescent analysis of the Fe^{2+}-induced oxidation of blood lipoproteins of healthy donors to estimate the effects of Noopept on LPO parameters. We also used the following comparison agents: pyracetam standard nootropic agent [1]; PBN, which serves as a free radical trap [20], and mannitol, which is used in clinical practice as a neuroprotector mainly during brain edema [21].

Chemiluminescence analysis was conducted as described previously [22–24]. Serum was obtained from the blood of volunteers on an empty stomach. Following this, 200 μl of the serum were supplemented with 2000 μl of 0.28% $CaCl_2$ and 40 μl of 1% heparin. After centrifugation at 3000 rpm for 15 min, the suspension of lipoproteins of low and very low density was obtained and used as the oxidation substrate. Phosphate buffer (pH 7.45, 900 μl) containing 60 mM KH_2PO_4 and 105 mM KCl was added to the lipoprotein suspension. A cuvette with the lipoprotein suspension was placed into a Luminometer-1251 chemiluminometer (LKB, Sweden) and the background chemiluminescence was recorded in duplicates or triplicates. Chemiluminescence was initiated by the addition of $FeSO_4$ at a final concentration of 2.5 mM. The following parameters were measured: amplitude of the fast CL flash (h, mB), which characterizes the level of the pre-formed LPO products (mainly lipid hydroperoxides), the duration of the latent CL period (τ, c) indicative of the antioxidant potential, and the maximal CL value (H, mB) characteristic of the lipoprotein capacity for peroxidation. The rate of lipoprotein oxidation was calculated from the slope of the CL curve (in relative units per second). Solutions of the examined compounds (pH 7.45) in combination with compensating volumes of phosphate buffer were added to the examined samples 30 sec before $FeSO_4$ addition. Effects of the examined compounds were compared in the concentration range from 10^{-6} to 2×10^{-3} M. Changes in the CL parameters were expressed as a percentage related to the control values for the samples diluted with the corresponding volumes of the standard buffer.

The Biostat computer program was applied for statistical data processing using the parametric Student *t*-test. The results were presented as $M \pm S.E.M.$

RESULTS AND DISCUSSION

The examined compounds affected the chemiluminescence in a different degree. As one can see from Fig. 1a, PBN significantly inhibited the value of CL initial flash (h). It was augmented proportionally to the concentration of this drug (by 92% with respect to the control at the maximum concentration). These results witness to the ability of this drug to interact with preformed peroxides. Pyracetam and Noopept did not significantly change this index in the tested concentration range.

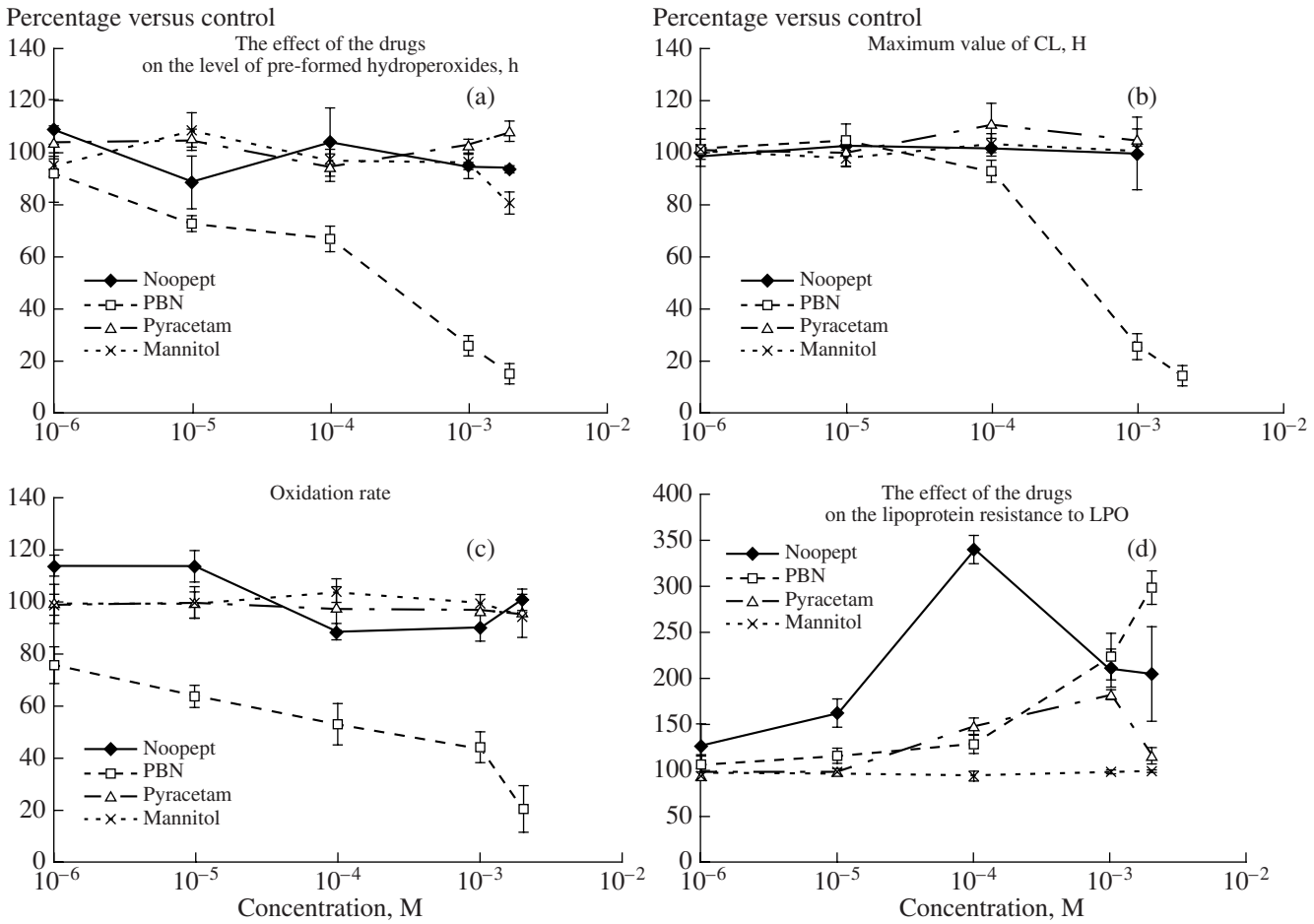
The maximum LPO value (H) (Fig. 1b) was exclusively inhibited only by PBN, and this effect began to be detectable at its higher concentrations in the sample ($10^{-4} - 2 \times 10^{-3}$ M). The ability of PBN to inhibit the rapid CL flash (h) and to decrease the maximal LPO value (H) confirms its characteristics as a free radical trap and an LPO inhibitor. We have previously described a similar effect for trolox, which is a hydrophilic synthetic analog of vitamin E [25] and is well-known as another antioxidant of direct action.

The lipoprotein oxidation rate (Fig. 1c) was also significantly decreased in a dose-dependent manner after PBN addition to the incubation medium. Pyracetam and Noopept did not affect this CL parameter.

The ability of Noopept and the other compounds (except mannitol, which was inactive according to all measured parameters) to increase duration of the latent CL phase (τ) appeared to be the most important. This effect reflects an endogenous antioxidant potential in the studied structural elements of human blood (Fig. 1d). The maximum increase in duration of the latent period was observed for Noopept (3.4 fold versus the control) and PBN (3 fold versus the control), which indicates the ability of these compounds to protect human plasma lipoproteins from the Fe^{2+}-induced lipid peroxidation. Noopept proved to be the most effective, because it acted at lower concentrations in the sample than PBN.

All the effective drugs operated in a dose-dependent manner (see Fig. 1d). At the same time, this dependence was linear for PBN, whereas it showed a bell shape for Noopept, which has been observed for many other biologically active peptides [26]. Pyracetam was shown to have a similar pattern, which also suggests the involvement of a peptidergic component in the mechanism of its action [6].

The effect of Noopept revealed in this study is in good agreement with the properties of this dipeptide as described previously. Thus, incubation of neurons isolated from the cortical tissue of 17-21-week-old healthy human embryos previously affected by hydrogen peroxide or $FeSO_4$, as well as the fetus of a Down syn-



The effect of Noopept, pyracetam, PBN, and mannitol on parameters of the Fe^{2+}-induced chemiluminescence of lipoproteins from blood serum of healthy donors. The results are presented as a percentage versus the control (the control is 100%). a, the level of preformed lipid hydroperoxides (h); b, maximum value of LPO (H); c, the rate of lipoprotein oxidation; d, lipoprotein resistance to LPO (τ). The concentration (M) is plotted on the X axis.

drome carrier prevented the development of oxidative stress and significantly increased neuron survival in the presence of Noopept (10^{-6} M) [14]. It can be supposed that the antioxidant effect of Noopept plays an important role in its antiapoptotic action. Preventively injected Noopept was also shown to increase the activity of SOD and other components of the antioxidant system (catalase and ceruloplasmin) in the brain tissue and in the blood plasma of experimental animals in a model of immobilization stress [12]. It is believed that this drug facilitates the initiation of additional protective mechanisms, both in vivo and in a continuous neuron culture, which provides stability to the damaging effect of free radical forms of oxygen.

These data on Noopept's ability to maintain endogenous antioxidant potential in human blood lipoproteins are presented for the first time. They not only suggest the expediency of the use of this drug for the treatment of a wide spectrum of types of brain damage, which are characterized by the deficiency of the endogenous antioxidant defense, but attract attention to other possibilities of its application as well. Atherosclerosis is known to be one of the health hazards of the development of vascular pathology of the brain and heart [27], and the oxidative modification of atherogenic class low-density lipoproteins is an essential element of its pathogenesis. Noopept may decrease the damaging effect of oxidized lipoproteins on the vascular wall.

This study confirmed that the method of Fe^{2+}-induced chemiluminescence of blood serum lipoproteins is an adequate model for the quantitative determination of lipid peroxidation and the characteristics of the antioxidant effect of biologically active compounds. This model allows the in vitro identification of the differences in the mechanisms of action of these compounds. This method can allow the prediction of antioxidant efficiency under clinical conditions.

Thus, Noopept surpasses other drugs in its ability to increase the activity of endogenous antioxidant protective mechanisms. These results, in combination with the previously observed nootrophic and neuroprotector

properties of Noopept, indicate that it is a promising drug for clinical practice.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project no. 03-04-49049). The authors are grateful to Prof. A. A. Boldyrev for his help in the performance of the experiments and his support in the preparation of the manuscript.

REFERENCES

1. Giurgea, C., *Actualites pharmacologiques*, 1972, vol. 25, pp. 115–156.
2. Promyslov, M.S. and Demchuk, M.L., *Mol. Chem. Neuropathol.*, 1995, vol. 25, pp. 69–80.
3. Kolpikova, O.C., Farkhutdinov, R.R., Magzhanov, R.V., *Korsakov Zh. Nevrologii i Psikhiatrii*, 2002, vol. 102, no. 8, pp. 22–25.
4. Gualtieri F., Manetti, D., Romanelli, M.N., et al., *Curr. Pharmaceutical Design*, 2002, vol. 8, pp. 125–138.
5. Boldyrev, A.A., *Karnozin i zashchita tkanei ot okislitel'nogo stressa* (Carnosine and Tissue Defefence from the Oxidative Stress), Moscow: Dialog-Moscow State University, 1999, pp. 37.
6. Gudasheva, T.A., Trofimov, S.S., Ienkina, F.B., et al., *Khim.-Farm. Zh.*, 1985, no. 11, pp. 1319–1322.
7. Gudasheva, T.A., Voronina, T.A., Ostrovskaya, R.U., et al., *Eur. J. Med. Chem.*, 1996, vol. 31, pp. 151–157.
8. Seredenin, S.B., Voronina, T.A., Ostrovskaya, R.U., et al., US Patent K2 5,439, 930, 1995.
9. Ostrovskaya, R.U., Gudasheva, T.A., Voronina, T.A., et al., *Exp. Klin. Farmacol.*, 2002, vol. 65, no. 5, pp. 66–72.
10. Ostrovskaya, R.U., Gudacheva, T.A., Kravchenko, E.V., et al., Biological Basis of Individual Sensitivity to Psychotropic Drugs, Abstract of Papers, *Golden Ring Conference*, Seredenin, S.B., Longo, V., Gaviragi, G., Eds., 1994, vol. 1, pp. 79–91.
11. Lysenko, A.V., Uskova, N.I., Ostrovskaya, R.U., et al., *Exp. Klin. Farmacol.*, 1997, vol. 60, no. 3, pp 15–18.
12. Mendzheritskii, A.M., Lysenko, A.V., Demyanenko, S.V., et al., *Neurochimiya*, 2003, vol. 20, no. 4, pp. 281–286.
13. Ostrovskaya, R., Romanova, G., Barskov, I., et al., *Behavioural Pharmacol.*, 1999, vol. 10, pp. 549–553.
14. Pelsman, A., Hoyo-Vadillo, Gudasheva, T., et al., *Int. J. Devel. Neurosci.*, 2003, vol. 803, pp. 1–8.
15. Bukanova, Ju., Solnzeva, E., Skrebitsky, V.G., *Int. J. Neuropsychopharm.*, 2002, vol. 5, pp. 229–237.
16. Andreeva, N.A., Stelmashuk, E.V., Isaev, N.K., et al., *Byull. Eksp. Biol.*, 2000, vol. 130, no. 10, pp. 418–421.
17. Us, K.S., Klodt, P.M., Kudrin, V.S., et al., *Int. Symp."Hyppocampus and memory"*, Pushchino, 2006, p. 111.
18. Kovalenko, L.P., Miramedova, M.G., Ostrovskaya, R.U., et al., *Eksp. Klin. Farmacol.*, 2002, vol. 65, no. 2, pp. 53–55.
19. Ostrovskaya, R.U., Lyapina, L.A., Gudasheva, T.A., et al., *Eksp. Klin. Farmacol.*, 2002, vol. 65, no. 2, pp. 34–37.
20. Schulz, J., Henshaw, R., Siwek, D., *J. Neurochem.*, 1995, vol. 64, pp. 2239–2247.
21. Zausinger, S., Westermaier, T., Plesnila, N., *Stroke*, 2003, vol. 34, no. 6, pp. 1526–1532.
22. Vladimirov, Yu.A., Archakov, A.I., *Perekisnoe okislenie lipidov v biologicheskikh membranakh* (Lipid Peroxidation in Biological Membranes), Moscow: Nauka, 1972, pp. 57–58.
23. Vladimirov, Y.A. *Proc. Int. Symp. on Natural Antioxidants. Molecular Mechanisms and Health Effects*, Parcker, L., Traber, M.G., and Xin, W., Eds., Champaign, Illinois: AOCS Press, 1995, pp. 125–144.
24. Fedorova, T.N., Rebrova, O.Yu., Larskii, E.G., *Lab. Delo*, 1991, no. 3, pp. 37–39.
25. Fedorova, T.N., *Eksp. Klin. Farmacol.*, 2003, no. 5, pp. 56–58.
26. Ostrovskaya, R.U., Mirzoev, T.Kh., Firova, F.A., et al., *Eksp. Klin. Farmacol.*, 2001, vol. 64, no. 2, pp. 11–14.
27. Tikhadze, A.K., Clinical-Experimental Study of Free Radical Oxidation of Lipids at Atherosclerosis and Antioxidant Correction of Metabolic Disorders of Lipoperoxides, *Extended Abstract of Doctor Sci. (Med.) Dissertation*, Moscow: Research Inst., 1999.