ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2007, Vol. 33, No. 6, pp. 537–543. © Pleiades Publishing, Inc., 2007.
Original Russian Text © P.S. Gromovykh, L.S. Guzevatykh, K.V. Shevchenko, L.A. Andreeva, L.Yu. Alfeeva, V.P. Shevchenko, I.Yu. Nagaev, T.A. Voronina, N.F. Myasoedov, 2007, published in Bioorganicheskaya Khimiya, 2007, Vol. 33, No. 6, pp. 581–587.

Synthesis, Pharmacokinetics, and Metabolism of the *C*-Terminal Tripeptide of Dermorphin and Its Diastereomer

P. S. Gromovykh[a,1], L. S. Guzevatykh[b], K. V. Shevchenko[a], L. A. Andreeva[a], L. Yu. Alfeeva[a], V. P. Shevchenko[a], I. Yu. Nagaev[a], T. A. Voronina[b], and N. F. Myasoedov[a]

[a] *Institute of Molecular Genetics, Russian Academy of Sciences, pl. Akademika Kurchatova 2, Moscow, 123182 Russia*
[b] *Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, ul. Baltiiskaya 8, Moscow, 125315 Russia*

Received September 21, 2006; in final form, April 25, 2007

Abstract—A tritium-labeled *C*-terminal fragment of dermorphin (H-Tyr-[3,4-^{3}H]Pro-Ser-NH_2) and its isomer (H-Tyr-*D*-[3,4-^{3}H]Pro-Ser-NH_2) with molar radioactivity of 35 Ci/mmol were synthesized, and their pharmacokinetics and metabolism in rat organs were studied after their intramuscular injections. The tripeptides were detected in the blood only for 5 min after the injection, and maximum contents of both compounds (approximately 5% of the total amount of the injected label) were registered in the kidneys after 20 min. Both stereomers were shown to penetrate into the brain. We failed to detect any radioactive metabolite, except proline, due to rapid proteolytic degradation of these peptides.

Key words: dermorphin, C-terminal fragment, dehydroproline analogue, synthesis, labeling with tritium, metabolism, pharmacokinetics

DOI: 10.1134/S1068162007060015

INTRODUCTION

Dermorphin (H-Tyr-*D*-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) is an endogenous opioid peptide with a high biological activity. Dermorphin and its analogue, [Hyp^6]dermorphin, were first isolated from skin of South American frogs [1].[2] Dermorphins were also found in the central nervous system and peripheral organs of warm-blooded animals [2]. Dermorphin affects the thermoregulation system of warm-blooded animals upon its peripheral administration and exhibit strong analgesic effect, which is 290 and 10–25 times higher than the effect of morphine after the central and peripheral administration, respectively [3]. Dermorphin also exhibits a wide spectrum of physiological activities: it affects functions of cardiovascular, respiratory, digestive, and secretory systems and various forms of animal behavior [4].

Studies of dermorphin pharmacokinetics upon its intravenous administration in rats demonstrated that it has a short lifespan in blood ($t_{1/2}$ = 1.3 min) and is accumulated in liver and kidneys. It was shown that only a third of dermorphin remains intact, two thirds are its degradation products. The Gly–Tyr bond of the peptide is mainly cleaved by enzymes of brain homogenate [5], which leads to the formation of *N*-terminal tetrapeptide and *C*-terminal tripeptide.

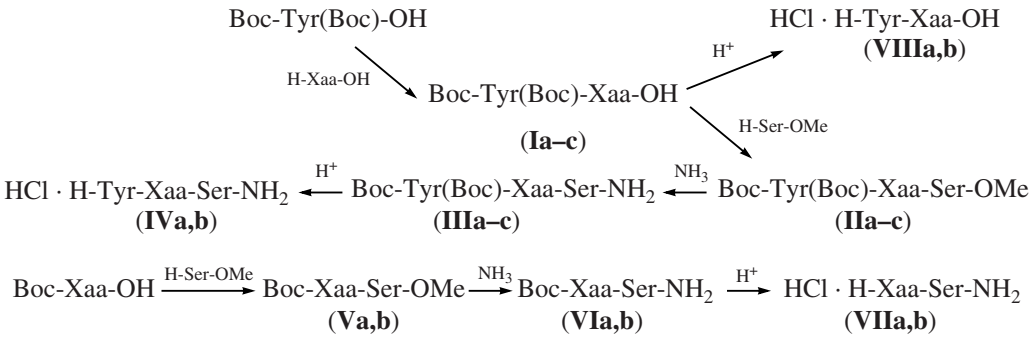
The *N*-terminal tetrapeptide is known to be a minimal fragment with the analgesic activity [2]. There is no information on biological properties of the *C*-terminal tripeptide and its stereomer (H-Tyr-*D*-Pro-Ser-NH_2) in literature. We have here studied the pharmacokinetics and metabolism of H-Tyr-Pro-Ser-NH_2 and H-Tyr-*D*-Pro-Ser-NH_2 in rat organs upon their intramuscular injections and their ability to penetrate into the rat brain.

RESULTS AND DISCUSSION

It was necessary to synthesize the Boc-Tyr(Boc)-*DL*-3,4-ΔPro-Ser-NH_2 precursor (**IIIc**) of the isomeric tripeptides with the labeled Pro residue for the studies of metabolism of the *C*-terminal fragment of dermorphin in different organs of rats. We also synthesized all the corresponding nonradioactive tri- and dipeptides: H-Tyr-Pro-Ser-NH_2 (**IVa**), H-Tyr-*D*-Pro-Ser-NH_2 (**IVb**), H-Pro-Ser-NH_2 (**VIIa**), H-Pro-*D*-Ser-NH_2 (**VIIb**), H-Tyr-Pro-OH (**VIIIa**), and H-Tyr-*D*-Pro-OH (**VIIIb**) as chromatographic standards for the metabolic studies (scheme).

[1] Corresponding author; fax: +7 (499) 196-0221; e-mail: nagaev@img.ras.ru.

[2] Abbreviations: 3,4-ΔPro, 3,4-dehydroproline; [3,4-^{3}H]Pro, 3,4-bistritioproline.



Scheme. Synthesis of the H-Tyr-Pro-Ser-NH_2 *C*-terminal tripeptide of dermorphin (**IVa**), its analogues, and relative dipeptides.

Dipeptides (**Ia**)–(**Ic**), (**VIa**), and (**VIb**) were synthesized using the methods of mixed anhydrides and active esters [6–8]. Tripeptides (**IIIa**)–(**IIIc**) were prepared by the coupling of dipeptides (**Ia**)–(**Ic**) with the serine methyl ester and subsequent ammonolysis. The necessary standards of free peptides (**VIa**), (**VIb**), (**VIIa**), (**VIIb**), (**VIIIa**), and (**VIIIb**) hydrochlorides were obtained as a result of deprotection of the corresponding Boc-derivatives. They were characterized by mass spectrometry and HPLC (the retention times are given in Table 1).

One can see from Table 1 that the isomeric [3,4-^{3}H](**IVa**) and [3,4-^{3}H](**IVb**) labeled with tritium can be obtained by the HPLC fractionation of the diastereomeric mixture formed after the tritium hydrogenation and subsequent deprotection of the derivative of racemic dehydroproline (**IIIc**). In addition, a clear fractionation of the studied tripeptide and most of the potential metabolites proceeds under the specially chosen HPLC conditions. As expected, chromatographic mobilities of diastereomers (**VIIIa**) and (**VIIIb**) significantly differ. However, hydrophilic dipeptides (**VIIa**) and (**VIIb**) are weakly retained on the reversed phase and cannot be resolved by HPLC.

We introduced the tritium label into the dehydroproline precursor (**IIIc**) by the solid phase and liquid phase hydrogenation [9] with tritium gas in the presence of a catalyst. The liquid phase hydrogenation in the atmosphere of tritium gas in dioxane for 3 h at room temperature in the presence of 5% $PdO/BaSO_4$ proved to be the best synthetic method with optimum balance between the yield and molar radioactivity of the target peptide. The resulting mixture of the labeled diastereomers was deprotected and fractionated by HPLC, and labeled peptides [3,4-^{3}H](**IVa**) and [3,4-^{3}H](**IVb**) were obtained. Their molar radioactivities (35 Ci/mmol) was sufficient for the studies of pharmacokinetics and metabolism.

Table 1. Retention times of the tripeptides and their fragments at HPLC (for conditions, see the Experimental section)

Substance	Retention times, min	
	series **a** with *L*-Pro	series **b** with *D*-Pro
H-Tyr-**Pro**-Ser-NH_2 (**IV**)	13.56	14.21
H-Tyr-**Pro**-OH (**VIII**)	22.28	26.43
H-**Pro**-Ser-NH_2 (**VII**)	1.49	1.49
H-Pro-OH	1.76	1.76

The labeled peptides [3,4-^{3}H] (**IVa**) and [3,4-^{3}H](**IVb**) were intramuscularly injected in rats for studies of penetration and cleavage rate of these peptides in the rat organs and blood. The rats were decapitated after the definite intervals after the administration of labeled peptides, and the organs and blood samples were taken.

The samples of organs and blood were homogenized at cooling in liquid nitrogen. The solution containing the known quantities of the corresponding unlabeled peptides was added to the samples to monitor the completeness of extraction of the peptide material. The contents of the peptides were determined after the extraction, and the corresponding corrections were entered. The peptides were extracted from the samples with a 4 : 1 acetonitrile–water mixture and centrifuged. The supernatant was evaporated. The residue was dissolved in a 1 : 1 methanol–water mixture and analyzed by HPLC. An aliquot (0.1 volume) was preliminarily taken for the determination of radioactivity of the biological sample. The results revealed peculiarities of pharmacokinetics and metabolism of the tripeptides (Table 2, Figs. 1, 2). The contents of tripeptides in the brain were calculated by the technique given in [10, 11], according to which the blood volume of the brain is 2.6% of the blood volume in the whole organism.

Peptides [3,4-^{3}H](**IVa**) and [3,4-^{3}H](**IVb**) were found to be detected in blood only for the first five min, whereas the maximum contents of the tripeptides in brain, lungs, kidneys, and liver of rats were observed

Table 2. Change in the percent content of isomers (**IVa**) and (**IVb**) with time in the rat organs (radioactivity of the tripeptide administered in rats is taken to be 100%)

Substance	Organ	Time, min			
		5	20	40	80
(**IVa**)	Blood	0.31 ± 0.05	0	0	–
	Kidneys	0.8 ± 0.1	3.6 ± 0.5	2.8 ± 0.5	0.23 ± 0.03
	Liver	0.35 ± 0.08	0.53 ± 0.07	0.5 ± 0.2	0.04 ± 0.02
	Brain	0.047 ± 0.007	0.059 ± 0.004	0.032 ± 0.003	–
	Heart	0.28 ± 0.05	0.07 ± 0.02	0.03 ± 0.02	–
	Lungs	0.09 ± 0.01	0.22 ± 0.05	0.07 ± 0.02	–
(**IVb**)	Blood	0.42 ± 0.09	0	0	–
	Kidneys	2.4 ± 0.4	5 ± 2	4.904 ± 0.004	0.8 ± 0.2
	Liver	0.18 ± 0.05	0.6 ± 0.3	0.26 ± 0.09	1.50 ± 0.04
	Brain	0.08 ± 0.06	0.13 ± 0.04	0.062 ± 0.007	–
	Heart	0.30 ± 0.02	0.31 ± 0.08	0.13 ± 0.01	–
	Lungs	0.41 ± 0.07	0.5 ± 0.1	0.218 ± 0.008	–

after 20 min. Content of the *D*-Pro-containing peptide [3,4-^{3}H](**IVb**) in the brain and lungs is about two times higher than that of [3,4-^{3}H](**IVa**) (0.13 and 0.06% in the brain and 0.49 and 0.22% in lungs, respectively). Similar amounts of both isomers are accumulated in heart within the first five min, but the content of the *L*-isomer quickly decreases later, whereas the content of the *D*-isomer remains practically constant. This indicates a higher proteolytic stability of the latter. Similarly, the labeled *L*-proline is formed from [3,4-^{3}H](**IVa**) faster than *D*-proline from [3,4-^{3}H](**IVb**).

We cannot find any radioactive metabolites of the tripeptides in the organs and blood of rats, except for the proline isomers (Figs. 3, 4). This fact unexpectedly indicates a high rate of the proteolytic cleavage of both peptide bonds of such short peptides independently of the proline configuration.

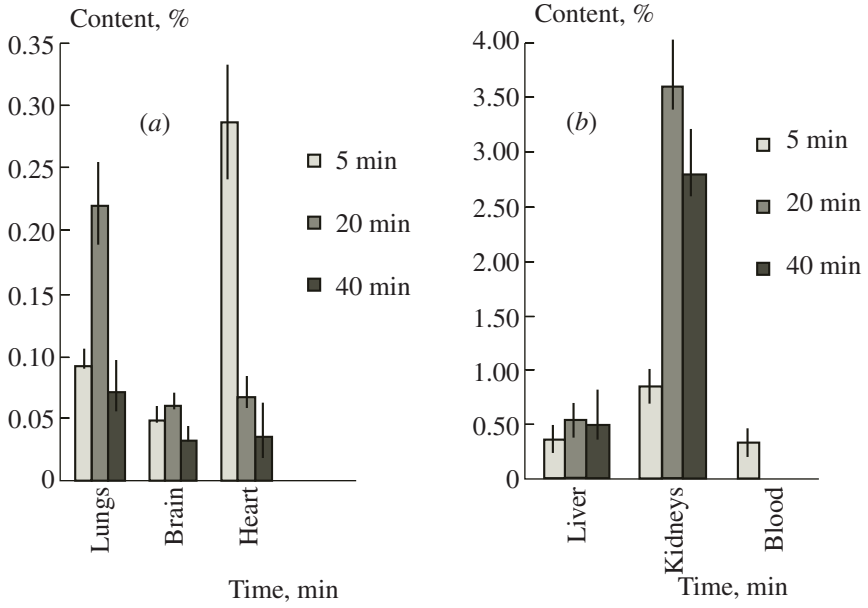


Fig. 1. Time changes in the content of (*a*) H-Tyr-[3,4-^{3}H]Pro-Ser-NH_2 [3,4-^{3}H](**IVa**), in lungs, brain, heart and (*b*) liver, kidneys, and blood.

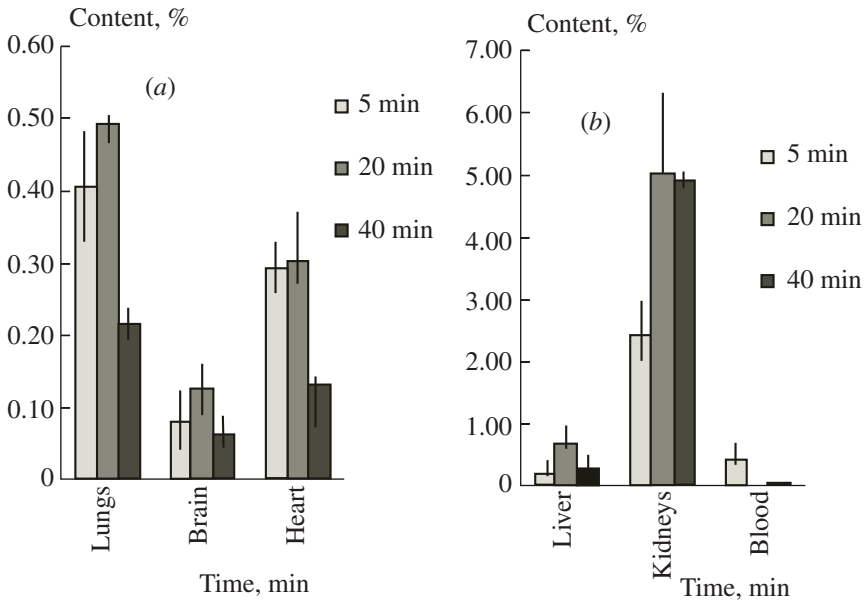


Fig. 2. Time changes in the content of H-Tyr-*D*-[3,4-^{3}H]Pro-Ser-NH_2 [3,4-^{3}H](**IVb**), in lungs, brain, (*a*) heart and liver, (*b*) kidneys and blood.

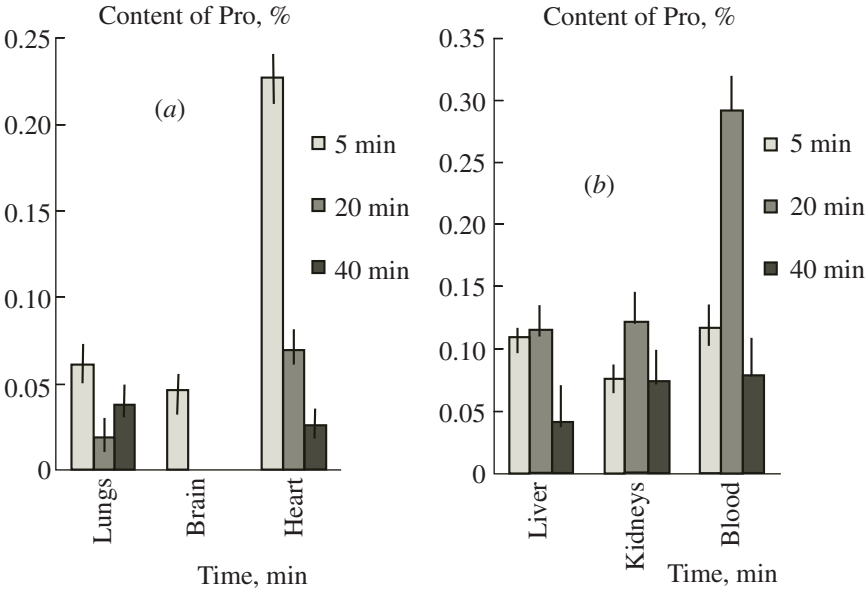


Fig. 3. Time changes in the content of Pro in lungs, brain, (*a*) heart and liver, (*b*) kidneys and blood.

Our results show that the labeled proline is more quickly formed from [3,4-^{3}H](**IVa**) than from the corresponding *D*-isomer in all the rat tissues. This fact allows assign a decreased content of [3,4-^{3}H](**IVa**) in the rat brain precisely to its faster metabolism. Note that the total amounts of label we measured in the rat brain are practically equal for both isomers of the tripeptide; i.e., they do not depend on the proline configuration. One can conclude from this fact that, first, both forms of tripeptide (**IV**) penetrate into the brain within the first five minutes after their intramuscular administration with equal ease and, second, the double level of the *D*-proline isomer in comparison with the *L*-proline isomer registered already five minutes after the administration is associated with the known enzymatic stability of the peptides containing *D*-amino acid residues.

EXPERIMENTAL

Commercially available amino acids, reagents, and solvents were used in this study. Mps were determined

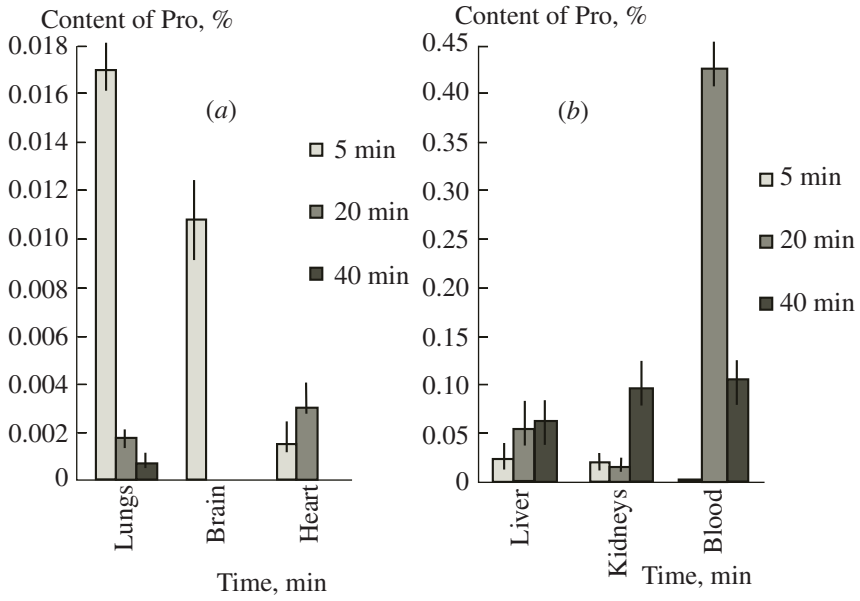


Fig. 4. Time changes in the content of *D*-Pro in (*a*) lungs, brain, and heart and (*b*) liver, kidneys, and blood.

on a Boetius device (Rapido, Germany) and are given without correction.

The homogeneity of the synthesized compounds was determined by TLC on Silufol plates (Czech Republic) and by HPLC. The substances on TLC plates were detected by the treatment with ninhydrin solution.

The chromatographic mobilities (R_f) in the following chromatographic systems are given: (A) 2 : 1 : 1 acetone–benzene–acetic acid and (B) 8 : 1.75 : 0.25 chloroform–methanol–concentrated ammonia.

The labeled preparation was analyzed and purified by HPLC on a Gilson chromatograph (France) equipped with a Kromasil SGX C_{18} column (5 μm, 3 × 150 mm) (Eka Chemicals AB, Sweden). The fractionation was carried out in an isocratic regime at a flow rate of 1 ml/min in 5% methanol in $NH_4H_2PO_4 + H_3PO_4$ buffer (50 mM, pH 2.8).

The MultiKhrom system (OOO Ampersend, Russia) on the basis of an IBM PC/AT was used for collection and processing the data. Radioactivity was measured on a Berthold LB 506 scintillation counter (Berthold, Germany) at a 30% efficiency of the tritium registration using the dioxane scintillator. Mass spectra were recorded on an LCQ Finnigan MAT ESI/MS spectrometer (United States).

Boc-Tyr(Boc)-*D*-Pro-OH (Ib). Boc-Tyr(Boc)-OH (13.8 g, 36.2 mmol) was dissolved in acetonitrile (125 ml), triethylamine (5.6 ml, 39.8 mmol) and pivaloyl chloride (4.9 ml, 39.8 mmol) were added to the solution at –20°C, and the reaction mixture was kept at –20°C for 20 min. *D*-Proline (5 g, 43.4 mmol) was dissolved in water (20 ml) and diluted with acetonitrile (60 ml). Triethylamine (6.08 ml, 43.8 mmol) was added, and the solution was cooled to –20°C, and a cooled solution of the mixed anhydride was added. The reaction mixture was stirred for 20 min at –20°C and 1 h at –10°C and monitored by TLC in chromatographic system A. When the process was completed, the reaction mixture was evaporated in a vacuum. The residue was dissolved in water (20 ml), acidified with $NaHSO_4 \cdot 3H_2O$ (29.9 g) to pH 3, and extracted with ethyl acetate (5 × 50 ml). The combined organic extracts were washed with water, 10% solution of $KHSO_4$, water, and the saturated solution of NaCl, dried with $MgSO_4$, and evaporated. The residue was reprecipitated from ethyl acetate with hexane two times. The precipitate powder was filtered and dried in a vacuum. The yield of (**Ib**) was 13.9 g (81%); R_f 0.52 (A); mp 75–77°C.

Boc-Tyr(Boc)-Pro-OH (**Ia**) (yield of 95%; R_f 0.26, mp 79–81°C) and Boc-Tyr(Boc)-*DL*-3,4-ΔPro-OH (**Ic**) (yield of 99%; R_f 0.38 (A); mp 93–95°C) were similarly prepared.

Boc-Tyr(Boc)-*D*-Pro-Ser-OCH_3 (IIb). *N*-Hydroxysuccinimide (4.07 g, 35.4 mmol) was added to a solution of (**Ib**) (13.02 g, 27.2 mmol) in acetonitrile (100 ml). The reaction mixture was cooled to 0°C, treated with DCC (7.30 g, 35.4 mmol), and stirred for 1 h at 0°C. Then $HCl \cdot Ser\text{-}OCH_3$ (5.50 g, 35.4 mmol) and triethylamine (5.0 ml, 35.4 mmol) were added, and stirring was continued for 1 h at 0°C and 2 days at room temperature. The reaction mixture was filtered, evaporated, diluted with ethyl acetate (250 ml), and washed with water (20 ml), 10% solution of $KHSO_4$ (45 ml), water (30 ml), 5% solution of $NaHCO_3$ (30 ml), water (30 ml), and saturated solution of NaCl. The ethyl acetate layer was dried with $MgSO_4$, filtered, and evaporated. The oily residue was washed with ether and dried in a vac-

uum. The yield of dried residue was 10.8 g (68%); R_f 0.27 (A); mp 81–83°C.

Boc-Tyr(Boc)-Pro-Ser-OCH_3 (**IIa**) (yield 96%; R_f 0.18 (B); mp 86–88°C), Boc-Tyr(Boc)-*DL*-3,4-ΔPro-Ser-OCH_3 (**IIc**) (yield 87%, R_f 0.24 (A) and 0.81 (B), mp 85–87°C), Boc-Pro-Ser-OCH_3 (**Va**), and Boc-*D*-Pro-Ser-OCH_3 (**Vb**) were similarly prepared.

Boc-Tyr(Boc)-*D*-Pro-Ser-NH_2 (IIIb). A solution of tripeptide (**IIb**) (5.55 g, 9.6 mmol) in methanol (200 ml) was saturated with gaseous ammonia for 60 min at 0°C and kept for 2 days at room temperature. The reaction mixture was coevaporated with ethyl acetate (2 × 10 ml) and with anhydrous methanol (2 × 10 ml). The product was precipitated with ether from absolute methanol; yield of (**IIIb**) 5.28 g (95%); R_f 0.70 (B); mp 115–117°C.

Boc-Tyr(Boc)-Pro-Ser-NH_2 (**IIIa**) (yield 98%, R_f 0.72 (B), mp 127–129°C), Boc-Tyr(Boc)-*DL*-3,4-ΔPro-Ser-NH_2 (**IIIc**) (yield 95%, R_f 0.69 (A), mp 123–125°C), Boc-Pro-Ser-NH_2 (**VIa**) from (**Va**), and Boc-*D*-Pro-Ser-NH_2 (**VIb**) from (**Vb**) were similarly prepared.

HCl · H-Tyr-*D*-Pro-Ser-NH_2 (IVb). A solution of (**IIIb**) (2.50 g, 4.4 mmol) in 1 N HCl in acetic acid (13.5 ml) was kept for 45 min and evaporated in a vacuum. The residue was washed with hexane two times and precipitated from anhydrous methanol with ether. The precipitate was filtered and dried in a vacuum; yield of tripeptide (**IVb**) was 1.8 g (97%); R_f 0.15 (B); mp 151–155°C; ESI MS: 365.0 $[M + H]^+$.

HCl · H-Tyr-Pro-Ser-NH_2 (**IVa**) (yield 96%, R_f 0.36 (B), mp 157–159°C; ESI MS: 365.0 $[M + H]^+$), HCl · H-Pro-Ser-NH_2 (**VIIa**) from (**VIa**), HCl · H-*D*-Pro-Ser-NH_2 (**VIIb**) from (**VIb**), HCl · H-Tyr-Pro-OH (**VIIIa**) from (**Ia**), and HCl · H-Tyr-*D*-Pro-OH from (**Ib**) were similarly prepared.

HCl · H-Tyr-[3,4-^{3}H]Pro-Ser-NH_2 ([3,4-^{3}H] (IVa)) and **HCl · H-Tyr-*D*-[3,4-^{3}H]Pro-Ser-NH_2 ([3,4-^{3}H](IVb)).** A solution of (**IIIc**) (4 mg) in dioxane (0.5 ml) was placed in a reaction ampule and 5% PdO/$BaSO_4$ (20 mg) was added. The ampule with the reaction mixture was cooled in liquid nitrogen, evacuated, saturated with the protium–tritium mixture (with the tritium content of 60%) to the pressure of 400 gPa, thawed, and stirred for 3 h at room temperature. The reaction mixture in an ampule was again freezed in liquid nitrogen, and the tritium gas was removed in a vacuum. The catalyst was filtered off and washed with methanol (3 × 1 ml), and easily exchangable tritium was removed by evaporation with methanol (3 × 1 ml). The labeled protected tripeptide [3,4-^{3}H](**IIIc**) (Boc-Tyr(Boc)-*DL*-[3,4-^{3}H]-Pro-Ser-NH_2) was isolated by HPLC in yield of 40–45% (molar radioactivity was 35 Ci/mmol). The protecting groups were removed by the treatment with HCl in dioxane (0.5 ml) for 1 h, subsequent evaporation, and purification by HPLC. The yields of [3,4-^{3}H](**IVa**) and [3,4-^{3}H](**IVb**) were 20 and 18%, respectively. Their molar radioactivities were 35 Ci/mmol.

Pharmacokinetics of the labeled tripeptides [3,4-^{3}H](IVa) and [3,4-^{3}H](IVb) in rat organism. An aqueous solution (200 μl) of the mixture of labeled [3,4-^{3}H](**IVa**) or [3,4-^{3}H](**IVb**) (200 μCi, 0.06 μmol) and nonradioactive (**IVa**) or (**IVb**) (198 μg, 0.54 μmol) was intramuscularly administered in outbred male rats (body mass of 200 ± 20 g). After the necessary time interval, the rats were decapitated, and their organs and blood samples were taken. The samples were stored at –70°C.

Isolation of the peptide fraction, preliminary purification by the solid phase extraction, and HPLC analysis were carried out according to the method described in [11]. For the preparation of tissue extracts, the samples of organs and blood were weighted, placed in a porcelain mortar, freezed in liquid nitrogen, crushed to a powder, and quantitatively transferred into centrifuge tubes. The mortar and pestle were washed with a acetonitrile–water mixture adjusted with HCl to pH 2.0 (4 : 1, 10 ml). Solutions (100 μl) of the corresponding unlabeled peptides (**IVa**) (11 μg), (**VIIa**) (41 μg), (**VIIIa**) (11 μg), and Pro (15 μg) were added to the homogenate and extracted with the same mixture (2 × 15 ml) by centrifugation at 8000 rpm for 40 min. The supernatants were combined, evaporated, and analyzed for the labeled peptides. The data of three experiments averaged for every time point are given in Table 2.

ACKNOWLEDGMENTS

This study was supported by the Program Molecular and Cellular Biology of Fundamental Studies of Presidium of the Russian Academy of Sciences, by the Program of Leading Scientific Schools (project nos. NSh-2150.2003.4 and NSh-5638.2006.4), and by the grants of the State Contract no. 2006-RN-112.0/001/156 and no. 02.245.11.7317 09.06.2006.

REFERENCES

1. *Montecucchi P.C., de Castiglione R., Erspamer V.* // Int. J. Pept. Protein Res. 1981. V. 17. P. 316–321.
2. *Broccardo M., Erspamer V., Falconieri-Erspamer G., Improta G., Linari G., Melchiorri P., Montecucci P.C.* // Br. J. Pharmacol. 1981. V. 73. P. 625–631.
3. *Emel'yanova T.G., Usenko A.B., Deigin V.I., Yarova E.P., Kamensky A.A.* // Peptides. 1996. V. 17. P. 241–245.
4. *Melchiorri P., Negri L.* // Gen. Pharmacol. 1996. V. 27. P. 9469–9474.
5. *Darlak K., Benovitz D., Spatola A., Grzonka Z.* // Biochem. Biophys. Res. Commun. 1988. V. 156. P. 125–130.
6. *Broccardo M., Usenko A.B., Uranova M.G., Guzevatykh L.S., Kamensky A.A., Andreeva L.A., Alfeeva L.Y., Myasoedov N.F., Giannini E., Improta G., Emel'yanova T.G.* // Peptides. 2003. V. 24. P. 419–428.

7. *Andreeva L.A., Alfeeva L.Y., Potaman V.N., Nezavibat'ko V.N.* // Int. J. Peptide Res. 1992. № 39. P. 493–496.

8. *Gershkovich A.A., Kibirev V.K.* Sintez peptidov. Reagenty i metody. Kiev: Naukova dumka, 1992.

9. *Shevchenko K.V., Nagaev I.Yu., Myasoedov N.F.* Mechennye tritiem lipofil'nye soedineniya. Moscow: Nauka, 2003.

10. *Potaman V.N., Antonova L.V., Dubynin V.A., Zaitzev D.A., Kamensky A.A., Myasoedov N.F., Nezavibatko V.N.* // Neurosci. Lett. 1991. V. 127. P. 133–136.

11. *Shevchenko K.V., Nagaev I.Yu., Alfeeva L.Yu., Andreeva L.A., Kamenskii A.A., Levitskaya N.G., Shevchenko V.P., Grivennikova I.A., Myasoedov N.F.* // Russ. J. of Bioorganic Chemistry. 2006. V. 32. P. 57–62.