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Peptides Comprising the Bulk of Rat Brain Extracts: Isolation, Amino Acid Sequences and Biological Activity

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Abstract: Chromatographic separation of rat brain extracts followed by automatic Edman sequencing of the major individual components resulted in identification of 61 endogenous peptides derived from known functional proteins (hemoglobin, myelin basic protein, cytochrome-*c* oxidase, etc.) or unknown precursors. The results are compared with the data obtained earlier for bovine brain. Although the sequences of bovine and rat hemoglobin contain about 20% of amino acid substitutions, the families of structurally related peptides are very similar in both extracts. Several other proteins also give rise to identical or closely related peptide fragments in the two mammalian species. The outlined similarity extends almost exclusively to the most abundant peptides present in the extracts. The minor components show less overlap. Four hemoglobin-derived peptides isolated from rat brain were shown to be biologically active in tumor cells. Eleven are identical to bioactive peptides from other species. Ten structurally overlap with bioactive peptides from other sources. The data obtained show similar biosynthetic pathways of pool components in different species, the resultant peptides being aimed at fulfilling related functions. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: endogenous peptides; rat brain; hemoglobin fragments

INTRODUCTION

Structural analysis of the low molecular weight components of bovine brain [1,2] and red bone marrow [3] extracts, lysate of human erythrocytes [4,5], extract of rat brain [6] and human cerebellum [7] has demonstrated that these sources mainly contain the fragments of functional proteins, in particular, hemoglobin. The number of components corresponding to chromatographic peaks, distinguishable in the chromatograms varies from 15–25 to 800–1500, i.e. each tissue contains a limited set of compounds. In other words, the composition and the content of components in the tissue show the relative specificity of proteolysis. The sets of these peptides differ significantly in different tissues, being stable and reproducible at normal conditions in the case of the same tissue and varying significantly from tissue to tissue. Proteolysis of proteins present in each tissue gives rise to a large group of peptides which was defined as a 'tissue-specific peptide pool' [8–10]. A considerable number of pool components showed pronounced activity *in vitro* and/or *in vivo* [3,5,11–17], thereby providing grounds for ascribing them an important regulatory role in the organism. The suggested function of the components of peptide pools predominantly deals with control of long-term biochemical processes, in particular, maintenance of tissue homeostasis.

In this work the analysis of peptide components of rat brain is described and the data is compared with those of bovine brain [1].

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MATERIALS AND METHODS

Preparation of Brain Specimens

Non thoroughbred white rats (12 animals) were kept in groups of three for a week at standard conditions. After this period the animals were killed by dislocation of the neck. The brains were removed immediately and placed in liquid nitrogen. The killing procedure was completed within 40–50 s after removal of each rat from the cage.

Preparation of Rat Brain Extracts

Individual specimens of rat brain were stored in liquid nitrogen. Brain preparations were homogenized in a Potter's homogenizer in 5 ml of 10% acetic acid. The mixture was centrifuged at 10 000 rev/min for 8 min. The supernatant was lyophilized. All procedures were carried out within 12–15 min at 0°C.

Size-exclusion Chromatography

The size-exclusion separation was performed using a liquid chromatography set (Pharmacia/LKB, Sweden) equipped with a Sephadex G-25 sf column (2.5 × 90 cm) equilibrated with 0.1 M acetic acid. Lyophilized preparations were dissolved in 12 ml of 0.1 M acetic acid, centrifuged and subjected to separation procedure. The fractions were then lyophilized.

Reverse-Phase HPLC Separation

Analytical separation of fractions (**I**–**IV**) obtained after size-exclusion chromatography (Figure 1) was carried out on a Nucleosil 5μ/C_8 (4.0 × 250 mm) cartridge equilibrated with 0.1% trifluoroacetic acid (TFA). Chromatographic conditions are described in the legends to Figures 2–5.

The compounds corresponding to the peaks marked with numbers were collected, lyophilized and subjected to separation on a Nucleosil C_8 120/7μ (4.0 × 250 mm) cartridge in a linear gradient of acetonitrile from 8 to 40%. The elution profiles were obtained using an integrator (Ampersend, Russia) at 226 nm. The optical density values given in Figures 2–5 correspond to the ratio: 2.56 OD = 1800 mV. Analysis of the elution profiles was performed by the Multi-Chrom V2.60 program (Ampersend, Russia).

Stability and reproducibility of the results were checked by comparing the elution profiles in three independent experiments. Both the comparative analysis of elution profiles and the sequencing data were used to verify the reproducibility of the results.

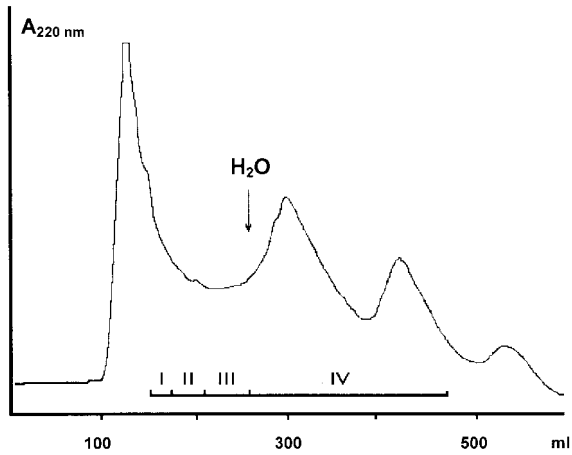

Figure 1 Size-exclusion chromatography of acidic extracts of rat brain preparation on on a Sephadex G-25 sf column (2.5 × 90 cm) equilibrated with 0.1 M acetic acid. Sample size: 200 mg. H_2O: total volume of the column. Collected fractions are marked.

Peptide Sequencing

Amino acid sequences were determined by means of a gas-phase sequencer (Model 477A, Applied Biosystems). The *C*-terminal amino acid residues of the obtained peptides were established using the data from two to three repeated sequencing procedures.

Sequence Identification

Identification of the established amino acid sequences was performed using the PIR data bank.

Cell Culturing

L929 cells (transformed murine fibroblasts) were cultured as described in [4]. Briefly, the cells were generated in RPMI 1640 medium enriched with 10% fetal calf serum (Gibco BRL) and supplemented with 2 mM of glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin.

Evaluation of Tumor Cell Number

L929 cells (5×10^4/well) were placed into 96-well plates (Flow) and co-incubated with 100 μl of serum-free culture medium containing variable concentrations of test substances. Untreated cells co-incubated with serum-free medium were used as

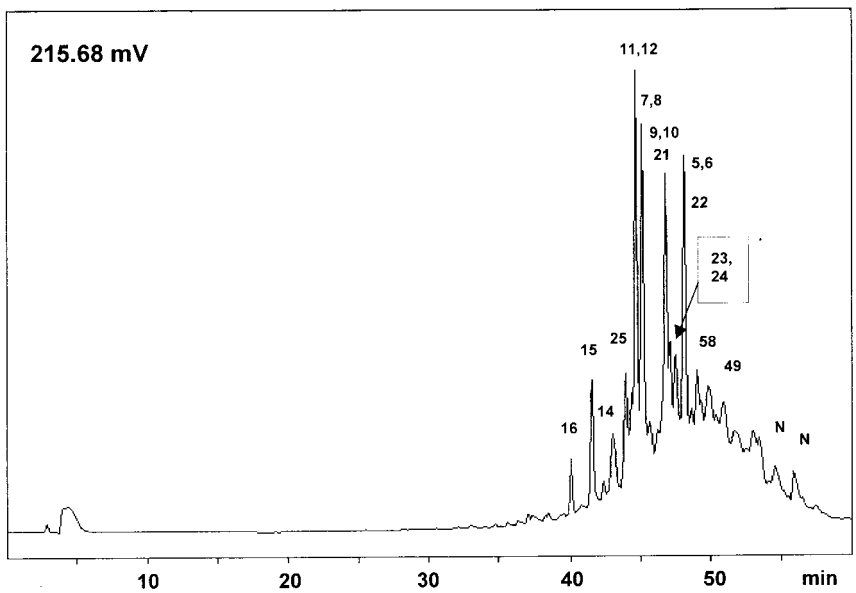

Figure 2 RP-HPLC of fraction(s) **I** (Figure 1) on a Nucleosil 5μ/C_8 (4.6 × 250 mm) cartridge equilibrated with 0.1% TFA. Elution was performed in a linear gradient of acetonitrile from 0 to 48%; duration: 60 min; flow rate: 1 ml/min; wavelength detection: 226 nm (1800 mV = 2.56 AUFS); sample size: 0.5 mg. The numbers correspond to sequences shown in Table 1. N-peaks correspond to substances for which the amino acid sequence could not be determined.

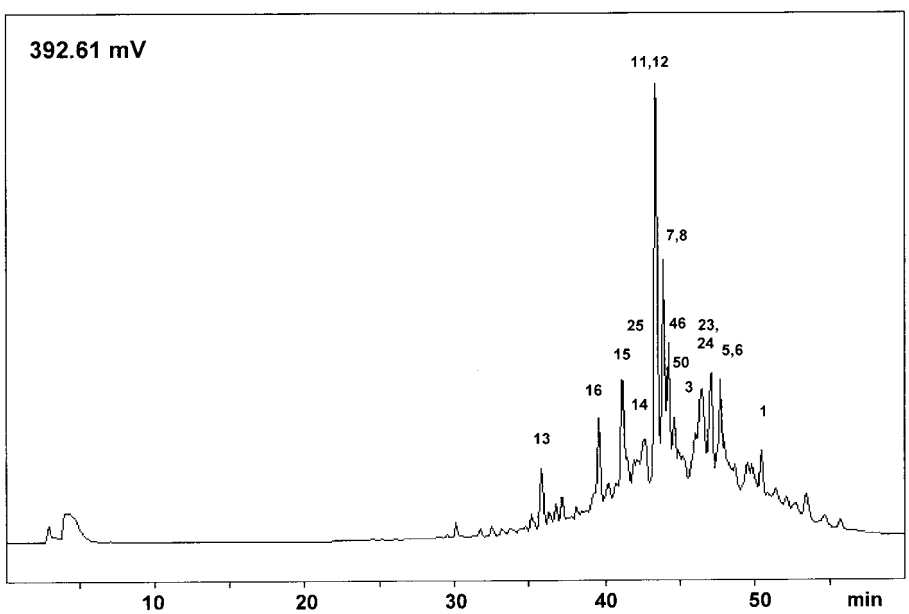

Figure 3 RP-HPLC of fraction(s) **II** (Figure 1) on a Nucleosil 5μ/C_8 (4.6 × 250 mm) cartridge equilibrated with 0.1% TFA. Elution was performed in a linear gradient of acetonitrile from 0 to 48%; duration: 60 min; flow rate: 1 ml/min; wavelength detection: 226 nm (1800 mV = 2.56 AUFS); sample size: 0.5 mg. The numbers correspond to sequences shown in Table 1. N-peaks correspond to substances for which the amino acid sequence could not be determined.

negative controls. The cells treated with epirubicin in 10^{-6} M concentration were used as a control of the standard sensitivity of the cell culture to the action of the cytostatic agent. The experimental wells containing the cells treated with the test substance were examined simultaneously with the

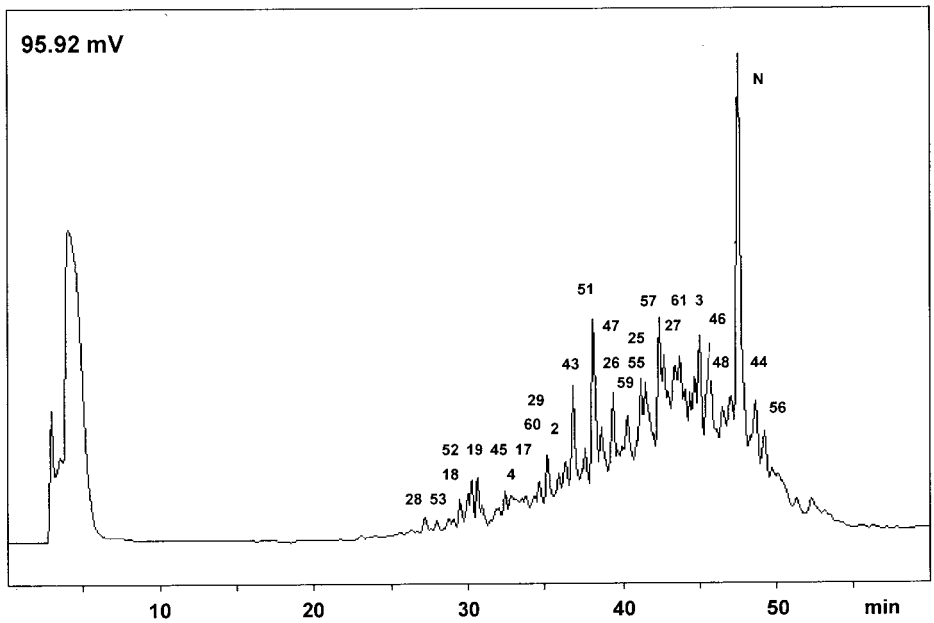

Figure 4 RP-HPLC of fraction(s) **III** (Figure 1) on a Nucleosil 5μ/C_8 (4.6 × 250 mm) cartridge equilibrated with 0.1% TFA. Elution was performed in a linear gradient of acetonitrile from 0 to 48%; duration: 60 min; flow rate: 1 ml/min; wavelength detection: 226 nm (1800 mV = 2.56 AUFS); sample size: 0.5 mg. The numbers correspond to sequences shown in Table 1. N-peaks correspond to substances for which the amino acid sequence could not be determined.

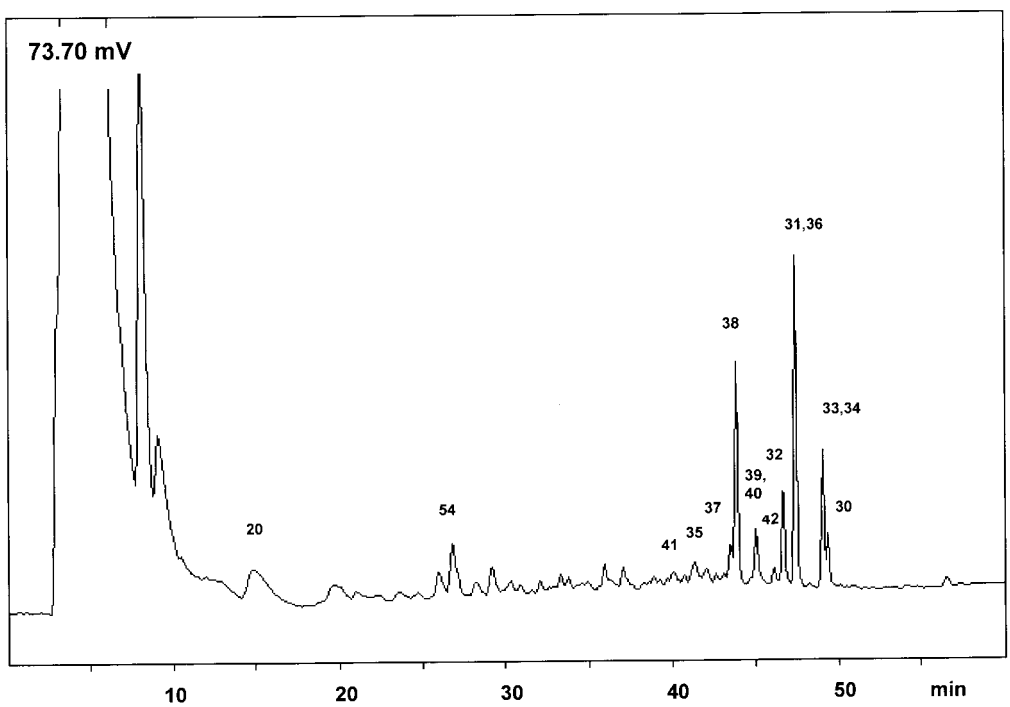

Figure 5 RP-HPLC of fraction(s) **IV** (Figure 1) on a Nucleosil 5μ/C_8 (4.6 × 250 mm) cartridge equilibrated with 0.1% TFA. Elution was performed in a linear gradient of acetonitrile from 0 to 48%; duration: 60 min; flow rate: 1 ml/min; wavelength detection: 226 nm (1800 mV = 2.56 AUFS); sample size: 0.5 mg. The numbers correspond to sequences shown in Table 1. N-peaks correspond to substances for which the amino acid sequence could not be determined.

corresponding control wells after different incubation periods. To desorb the cells from assay plates the cells were pre-treated consecutively with 100 μl of 0.25% trypsin solution (for 1 min) and 40 μl of 0.02% versene solution (for 5 min) (Gibco BRL). The cell suspension was then treated directly in versene solution with 5 μl of 0.1% Trypan blue dye solution (Sigma) in distilled water. The dead (Trypan blue-stained) and the live (non-stained) cell numbers were determined by visual detection in a Diavert microscope in the squares of a Goryaev chamber. A total of 300–400 cells were examined in each sample.

The change of live cell concentration was calculated according to formula (1):

$$[(E_x - E_o)/E_o] \times 100\% \quad (1)$$

where E_o is the live cell concentration in the negative control (mln/ml) and E_x is the live cell concentration in the sample (mln/ml).

Two replicas for each experimental data point and four replicas for controls were examined in the framework of each independent experiment. The statistical significance of the data obtained in five independent experiments was estimated. The values determined with $p < 0.05$ were assumed reliable according to Student's t-test.

RESULTS AND DISCUSSION

The task of the study was isolation and structural identification of the most abundant components of rat brain extract. The conditions applied (speed plus cooling) minimized post-mortal proteolysis of rat brain components. Extracts of individual specimens of rat brain were obtained after homogenization of tissue in 10% acidic acid at 4°C within 5–7 min. The extracts were then subjected to centrifugation and lyophilized.

The lyophilized extracts of individual rat brain specimens (0.2 g) were fractionated on a Sephadex G-25 sf column giving rise to fraction **I** (2.5–4.5 kDa, 0.3 mg), fraction **II** (1.5–2.5 kDa, 0.2 mg), fraction **III** (0.2–1.5 kDa, 0.7 mg), and fraction **IV** (< 0.2 kDa and adsorbed components, 1.2 mg). Chromatographic separation was carried out at standard conditions. A typical elution profile is given in Figure 1.

The fractions were subjected to RP-HPLC separation. The elution profiles from individual brain specimens were highly reproducible, proving the stability of the peptide composition of brain tissue, analogous to the peptide panels of bovine brain [1,2] and cerebellum [7,18,19] extracts. The typical chromatograms of the fractions **I**–**IV** are given in Figures 2–5. The substances corresponding to the peaks marked with numbers were subjected to further separation on a Nucleosil 5μ/C_8 (4.0 × 250 mm) column equilibrated with 0.05 M sodium acetate buffer (pH 4.5). Re-chromatography was carried out using the elution buffer containing 0.1% aqueous TFA and the homogenous peptides obtained were then sequenced. The amino acid sequences were compared with the sequences in the PIR data bank.

As a result, 61 amino acid sequences were established (Table 1). The overall content of the fraction containing the peptide material in the rat brain (12 mg/g tissue) was higher than in the bovine brain (7 mg/g tissue) and the number of peptide components in the rat extract was also significantly higher than in the bovine extract. At the same time, the composition of the fragments of functional proteins in both sources was very similar. As with bovine brain extract, rat brain extract was enriched with hemoglobin fragments. Fragments of several neurospecific proteins were also found.

The analogy between the peptide pools of rat and bovine brain becomes especially obvious when the most abundant components are chosen for comparison. As seen from Tables 2 and 3, 29 rat peptides present at ≥ 0.6 nmol/g of tissue and comprising approximately 75% of the total bulk of peptide material, have corresponding counterparts in the set of bovine peptides. As an example, both extracts contained neokyotorphin, hemorphins and the related peptides corresponding to (1–30) segments of α- and β-globins.

Even within the minor components, where common features are by far not so clearly observed, closely related pairs of peptides are present such as fragments of corresponding glyceraldehyde-3-phospate dehydrogenase (peptide 42 in Table 1) and cytochrome-*c* oxidase VIIA (peptide 45 in Table 1). The analogy outlined above demonstrates that the two mammalian species possess common pathways of proteolytic cleavage of hemoglobin and apparently of other functional proteins. Significantly, these pathways are not affected by considerable differences in the primary structures of degraded proteins (up to ten amino acid substitutions on relatively short peptide fragments, see Table 2).

The peptides found in rat brain, mostly hemoglobin derivatives, include several representatives, such as neokyotorphin (19) and a number of hemorphins (30, 32, 33, 36 and 38) known for their

Table 1 Fragments of Functional Proteins Identified in Rat Brain Extracts

No.	Amino acid sequence	Protein precursor (source, homology) and the position of the identified fragment		Content (nmol/g tissue)
Neurospecific proteins				
1	RFMNLIKEARWDGL	Glutamate receptor precursor GluR6 (rat, 79%)	358–371	0.3–0.5
2	IFRRQXWND	Glycine receptor α-chain 3 precursor (rat, 80%)	95–103	0.05–0.1
3	FGSDRGAPKRGSGK	Myelin basic protein (rat, 100%)	45–58	1.5–1.8
4	ELFNPY	Chromogranin B precursor (human, 100%)	520–526	0.1–0.2
Hemoglobin (rat)				
5	VLSADDKTNIKNCWGKIG-GHGGEYGEEALQRM	α_1-globin	1–32	1.5–1.8
6	VLSAADKTNIKNCWGKIG-GHGGEYGEEALQRM	α_2-globin	1–32	1.0–1.2
7	VLSADDKTNIKNCWGKIG-GHGGEYGEEALQR	α_1-globin	1–31	1.5–2.0
8	VLSAADKTNIKNCWGKIG-GHGGEYGEEALQR	α_2-globin	1–31	1.3-1.5
9	VLSADDKTNIKNCWGKIG-GHGGEYGEEALQ	α_1-globin	1–30	1.0–1.2
10	VLSAADKTNIKNCWGKIG-GHGGEYGEEALQ	α_2-globin	1–30	0.8–1.0
11	VLSADDKTNIKNCWGKIG-GHGGEY	α_1-globin	1–24	2.5–2.8
12	VLSAADKTNIKNCWGKIG-GHGGEY	α_2-globin	1–24	2.2–2.4
13	VLSADDKTNIKNCW	α_1-globin	1–14	1.5–2.0
14	IGGHGGEYGEEAL	α-globins	17–29	0.1–0.2
15	LVTLACHHPGDFTPAMHASLDK	α-globins	106–127	1.3–1.6
16	LACHHPGDFTPAMHASLDK	α-globins	109–127	1.7–2.0
17	ACHHPGDFTP	α-globins	110–119	0.4–0.6
18	TVLTSKYR	α-globins	134–141	0.3–0.4
19	TVLTSKY	α-globins	134–140	1.1–1.3
20	TSKYR	α-globins	137–141	2.4–2.6
Hemoglobin				
21	VHLTDAEKAAVNGLWGKVN-PDDVGGEALGR	β-globin	1–30	0.9–1.1
22	VHLTDAEKAAVNGLWGKVN-PDDVGGEALG	β-globin	1–29	0.8–1.0
23	VHLTDAEKAAVNGLWGKVN-PDDVGGEA	β-globin	1–27	0.5–0.6
24	VHLTDAEKAAVNGLWGKVN-PDDVGGE	β-globin	1–26	0.4–0.6
25	VHLTDAEKAAVNGL	β-globin	1–13	0.4–0.6
26	VHLTDAEK	β-globin	1–8	1.1–1.3
27	GKVNPDDVGGEA	β-globin	15–26	0.3–0.4
28	KVNPD	β-globin	16–20	1.0–1.2
29	KVNPDDVGGEA	β-globin	16–28	0.1–0.2
30	LVVYPWTQRY	β-globin	32–41	1.0–2.0
31	LVVYPWTQR	β-globin	32–40	0.3–0.4
32	LVVYPWTQ	β-globin	32–39	3.0–5.0
33	LVVYPWT	β-globin	32–38	0.7–0.9
34	LVVYPW	β-globin	32–37	0.1–0.2
35	LVVYP	β-globin	32–36	0.3–0.5
36	VVYPWTQRY	β-globin	33–41	0.6–0.8

Table 1 (continued)

No.	Amino acid sequence	Protein precursor (source, homology) and the position of the identified fragment		Content (nmol/g tissue)
37	VVYPWTQR	β-globin	33–40	0.2–0.3
38	VVYPWTQ	β-globin	33–39	0.8–1.0
39	VVYPWT	β-globin	33–38	0.1–0.2
40	VVYPW	β-globin	33–37	0.1–0.2
41	VVYP	β-globin	33–36	0.05–0.1
Other proteins				
42	ISWYDNEYGYSNRV	Glyceraldehyde-3-phosphat dehydrogenase (rat, 100%)	309–322	0.2–0.4
43	SVQCPFGG	Aldehyde dehydrogenase (rat, 100%)	461–468	0.1–0.2
44	WVAMQT	Cytochrome-*c* oxidase precursor chain IV (bovine, 86%)	115–120	0.01–0.02
45	GKNVP	Cytochrome-*c* oxidase precursor chain VIIA (bovine, 80%)	24–28	0.06–0.08
46	MQIFVKTLTGKTITL	Ubiquitin (rat, 100%)	1–15	0.5–0.7
47	ASGGIGQPL	Malate dehydrogenase precursor (rat, 100%)	32–40	0.02–0.04
48	VAYKN	Phospholipase A2 (human, 100%)	46–50	0.05–0.07
49	WTSDTQGDEAEAGEGGE	Phospholipase A2 (human, 95%)	238–255	0.04–0.05
50	MANAGPNTNGSQF	Peptidylprolylisomerase A (rat, 100%)	100–112	0.04–0.05
51	AFKDKYKQ	ATP,ADP carrier protein (human, 100%)	90–97	0.06–0.08
Unknown precursors				
52	VLNP	Sequence present in several proteins		0.01–0.02
53	AGPE	Sequence present in several proteins		0.01–0.02
54	YLE	Sequence present in several proteins		0.01–0.02
55	DVRPQVHPNY	Unknown protein (maximal homology <50%)		0.01–0.02
56	IDYSPKSTPDDN	Unknown protein (maximal homology <70%)		0.01–0.02
57	FLTAAVAQPR	Unknown protein (maximal homology <50%)		0.04–0.05
58	YYSSGSXXPTHAK(GS)GLE	Unknown protein (maximal homology <30%)		0.04–0.05
59	DRLHISPDR	Unknown protein (maximal homology <50%)		0.1–0.2
60	(YF)LTAAVAQPR	Unknown protein (maximal homology <50%)		0.01–0.02
61	(WV)EXMHP	Unknown protein (maximal homology <70%)		0.02–0.04

pronounced activity [13–17,20]. Most of the endogenous fragments of functional proteins, in particular, hemoglobin-derived peptides were shown to influence proliferation and viability of tumor cells. The authors have evaluated LVV-hemorphins (30, 31, 32) isolated from rat brain for the ability to reduce tumor cell number and compared the antiproliferative effects of the other representatives of that peptide family and have been shown to effectively inhibit growth of tumor cells [5]. No difference in the effects of the rat and the bovine LVV-hemorphin-7, differing in a single substitution of Tyr^{41} for Phe, was detected. At the same time, removal of two *C*-terminal amino acid residues from LVV-hemorphin-7 results in a twofold decrease of the activity. That is, on the one hand, substitution in the hemoglobin sequence due to species specificity does not influence the effect of LVV-hemorphin-7, at least in cell culture and on the other hand, the antiproliferative effect in the family of these peptides depends strongly on proteolytic degradation of the *C*-terminal fragment. The authors previous studies of *N*-terminally shortened hemorphins released by human erythrocytes lead to the conclusion that removal of *N*-terminal animo acid residues of LVV-hemorphins also causes significant decrease of antiproliferative activity [5].

Rat brain extract contains three overlapping peptides, neokyotorphin (α-globin-(137–140)), detected earlier in bovine brain and the peptides α-globin-(134–140) and α-globin-(134–141), not found in that source. The activities of α-globin-(134–140) and α-globin-(134–141) were compared with that of neokyotorphin, known for the ability to stimulate tumor cell growth [15]. As seen from Table 3, the peptides stimulate proliferation, the maximal effect

Table 2 Comparison of the Abundant Peptides from Rat Brain (Average Content ≥0.6 nmol/g Tissue, Table 1) with Respective Bovine Peptides [2]

Precursor and position in the sequence		Rat peptides		Bovine peptides		
		No.	Content (nmol/g tissue)	Sequence	Amino acid substitutions	Content (nmol/g tissue)
Myelin basic protein	43–56	3	1.5–1.8	FGSDRGAPKRGSGK	0	1.0–3.0
α_1-globin	1–32	5	1.5–1.8	VLSAADKGNVKAAWGKVGGHAAEYGAEALERM	10	0.1–1.0
α_2-globin	1–32	6	1.0–1.2	VLSAADKGNVKAAWGKVGGHAAEYGAEALERM	9	0.1–1.0
α_1-globin	1–31	7	1.5–2.0	ND	10	ND
α_2-globin	1–31	8	1.3–1.5	ND	9	ND
α_1-globin	1–30	9	1.0–1.5	VLSAADKGNVKAAWGKVGGHAAEYGAEALE	10	1.0–3.0
α_2-globin	1–30	10	0.8–1.0	VLSAADKGNVKAAWGKVGGHAAEYGAEALE	9	1.0–3.0
α_1-globin	1–24	11	2.5–2.8	VLSAADKGNVKAAWGKVGGHAAEY	8	1.0–3.0
α_2-globin	1–24	12	2.2–2.4	VLSAADKGNVKAAWGKVGGHAAEY	7	1.0–3.0
α_1-globin	1–14	13	1.5–2.0	ND	5	ND
α-globin	107–116	15	1.3–1.6	ND	4	ND
α-globin	110–126	16	1.7–2.0	ASHLPSDFTPAVHASLDK	4	0.1–1.0
α-globin	134–141	19	1.1–1.3	ND	0	ND
α-globin	137–141	20	2.4–2.6	TSKYR	0	0.1–1.0
β-globin	1–29	21	0.9–1.1	MLTAEEKAAVTAFWGKVKVDEVGGEALGR	9[a]	0.1–1.0
β-globin	1–28	22	0.8–1.0	MLTAEEKAAVTAFWGKVKVDEVGGEALG	9[a]	0.1–1.0
β-globin	1–8	26	1.1–1.3	MLTAEEKA	3[a,b]	0.1–1.0
β-globin	16–20	28	1.0–1.2	ND	1	ND
β-globin	31–40	30	1.0–2.0	LVVYPWTQRF	1	1.0–3.0
β-globin	31–38	32	3.0–5.0	LVVYPWTQ	0	1.0–3.0
β-globin	31–37	33	0.7–0.9	LVVYPWT	0	0.1–1.0
β-globin	32–40	36	0.6–0.8	VVYPWTQRF	1	1.0–3.0
β-globin	32–38	38	0.8–1.0	VVYPWTQ	0	0.1–1.0
Ubiqutin	1–15	46	0.5–0.7	MQIFVKTLTGKTITL	0	0.1–1.0

[a] Rat globin has an additional *N*-terminal valine residue.
[b] Bovine peptide has an additional *C*-terminal alanine residue.

Table 3 Effect of Rat Brain-Derived Peptides on L929 Cell Number (%)

No.	Peptide	Concentration (M)				
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
30	LVVYPWTQRY	-48 ± 18	-18 ± 19	-37 ± 24	-20 ± 14	-18 ± 25
*	LVVYPWTQRF	-50 ± 8	-28 ± 6	-28 ± 11	-22 ± 9	-16 ± 7
31	LVVYPWTQR	-44 ± 22	-32 ± 22	-29 ± 23	-27 ± 18	-23 ± 6
32	LVVYPWTQ	-31 ± 23	-29 ± 16	-16 ± 18	-25 ± 16	-27 ± 20
20	TSKYR	$+35 \pm 3$	$+32 \pm 5$	$+30 \pm 5$	ND	ND
19	TVLTSKYR	ND	$+38 \pm 5$	$+52 \pm 7$	$+29 \pm 6$	ND
18	TVLTSKY	ND	$+22 \pm 3$	$+41 \pm 5$	$+16 \pm 5$	$+9 \pm 3$

* Peptide isolated from bovine brain extract [2].

of α-globin-(134–141) being detected at a concentration two orders of magnitude lower than that of neokyotorphin. This difference correlates with the difference in the content of the peptides in brain tissue, i.e. the concentration of neokyotorphin in brain is more than threefold higher, as compared with that of α-(134–141). The data suggest that fragments of functional proteins contain key structural motifs responsible for manifestation of a given activity, while efficacy of action of such peptides depends strongly on the patterns of proteolytic formation. The examples given above suggest that strong similarity of patterns of proteolytic formation of hemoglobin fragments in rat and bovine brain should lead to peptides with similar biological effects. The amino acid substitutions in proteolytically related peptides should affect only the absolute value and not the nature of the biological effect.

Hemorphins and neokyotorphin-related peptides are examples of hemoglobin fragments with highly conservative sequences and their biological effects are also not species specific. The situation with other peptides, formed in a similar manner in both sources, is not so obvious because of amino acid substitutions, due to structural differences between rat and bovine hemoglobins. The summary of biological activities of related hemoglobin fragments isolated from other sources is given in Table 4. As seen from Table 4, the corresponding peptides from different species have common structural motifs and a reliable homology varying from 90% (in the

Table 4 Primary Structures and Activity of Selected Hemoglobin Derived Peptides Isolated from Mammalian Species (Variable Amino Acid Residues are Underlined)

Source	Position	Structure	Activity	Ref.
Rat	α_1-1–32	VLSADDKTNIKNCWGKIGGHGGEYGEEALQRM	Stimulation of tumor cell growth, hemopoietic activity	[12]
Rat	α_2-1–32	VLSAADKTNIKNCWGKIGGHGGEYGEEALQRM		
Bovine	α-1–32	VLSAADKGNVKAAWGKVGGHAAEYGAEALERM		
Human	α-1–32	VLSPADKTNVKAAWGKVGAHAGEYGAEALERM		
Rat	α_1-1–14	VLSADDKTNIKNCW		
Bovine	α-1–13	VLSAADKGNVKAA	Hemopoietic activity	[5,8]
Human	α-1–17	VLSPADKTNVKAAWGKV	Inhibition of tumor cell growth	
Rat	β-1–8	VHLTDAEK		
Bovine	β-1–8	MLTAEEKA	Hemopoietic	[8]
Human	β-1–8	VHLTPEEK		
Pig	β-1–10	VHLSAEEKEA	GH-releasing	[11]
Rat	β-32–41	LVVYPWTQRY	Active in classical opiod tests, antiproliferative activity in tumor cells	
Bovine	β-31–40	LVVYPWTQRF		[20]

case of hemorphins) to 40% in the family of β-globin-(1–8) fragments. It can be expected that the molecular targets of these peptides also contain certain structural differences, thereby providing conditions for specific interactions required for the manifestation of the activity of peptides in a given species. In summary, on the basis of the similar patterns of proteolytic formation on the one hand, and the reliable structural homology on the other, it is expected that the peptides derived from rat brain have effects similar to those of corresponding hemoglobin fragments in other species.

It is believed that tissue-specific peptide pools belong to the basic level of tissue regulation, mutual for all mammalian and possibly for other vertebrate species. Also, the differences between the peptide pools of rat and bovine brain could reflect unique metabolic features of the given organisms.

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