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DISTRIBUTION OF IMMUNOREACTIVE β-NEO-ENDORPHIN IN DISCRETE AREAS OF THE RAT BRAIN AND PITUITARY GLAND: COMPARISON WITH α-NEO-ENDORPHIN

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Abstract

The distribution of immunoreactive (ir)-β-neo-endorphin in 101 miscrodissected rat brain and spinal cord regions as well as in the neurointermediate lobe of pituitary gland was determined using a highly specific radioimmunoassay. The highest concentration of β-neo-endorphin in brain was found in the median eminence (341.4 fmol/mg of protein). High concentrations of ir-β-neo-endorphin (>250 fmol/mg of protein) were found in 11 nuclei, including dorsomedial nucleus, substantia nigra, parabrachial nuclei, periaqueductal gray matter, anterior hypothalamic nucleus, and lateral preoptic areas. Moderate concentrations of the peptide (between 100 and 250 fmol/mg of protein) were found in 66 brain nuclei such as the amygdaloid and septal nuclei, most of the diencephalic structures (not including the hypothalamus), and the majority of the medulla oblongata nuclei and others. Low concentrations of ir-β-neo-endorphin (<100 fmol/mg of protein) were found in 21 nuclei, e.g., cortical structures (frontal., cingulate, piriform, parietal, entorhinal, occipital), olfactory tubercle, and cerebellum (nuclei and cortex). The olfactory bulb has the lowest β-neo-endorphin concentration (21.3 fmol/mg of protein). Spinal cord segments exhibit low peptide concentrations. The neurointermediate lobe of the pituitary gland is extremely rich in ir-β-neo-endorphin.

β-Neo-endorphin is an endogenous opioid peptide which was originally isolated from extracts of porcine hypothalami (Minamino et al., 1981). It is a potent opiate agonist in the *in vitro* guinea pig ileum assay (Minamino et al., 1981; Oka et al., 1982). β-Neo-endorphin is identical to α-neo-endorphin except that it lacks a lysine residue at the carboxyl terminal (Kangawa et al., 1981, Minamino et al., 1981). The structural relationship of the two peptides is illustrated below:

α-neo-endorphin: Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys
β-neo-endorphin: Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro

α- and β-Neo-endorphin are parts of the same precursor that gives rise to dynorphin A (Goldstein et al., 1981) and dynorphin B (Fischli et al., 1982; Kilpatrick et al., 1982). The amino acid sequence of this precursor has been deduced from the nucleotide sequence of cloned DNA complementary to the porcine hypothalamic mRNA encoding it (Kakidani et al., 1982). Immunohistochemical studies have shown that perikarya, nerve fibers, and terminals containing α-neo-endorphin are widely distributed throughout the central nervous system (Weber et al., 1982a). The distribution of β-neo-endorphin measured by radioimmunoassay (RIA) in gross brain areas has been reported (Kitamura et al., 1982). The hypothalamus is the region richest in the peptide, followed by the pons-medulla, midbrain, spinal cord, cortex, hippocampus, and striatum. The cerebellum and olfactory bulb contain relatively little β-neo-endorphin. The posterior pituitary has a high β-neo-endorphin concentration (Weber et al., 1982a, b). In this paper we describe the topographical distribution of ir-β-neo-endorphin among 101 microdissected brain and spinal cord areas as well as the neurointermediate lobe of the pituitary gland.

Materials and Methods

Animals. Male Sprague-Dawley rats (Zivic Miller Laboratories, Inc., Allison Park, PA), weighing 220 to 250 gm, were housed under alternate 12-hour periods of dark and light (lights on from 6:00 A.M. to 6:00 P.M.) and were given standard rat chow and tap water ad libitum.

Tissue preparation and extraction. The animals were killed by decapitation between 8:00 and 10:00 A.M. The brains and spinal cords were quickly removed and frozen on dry ice. The spinal cords were grossly dissected into cervical, thoracic, and lumbar segments. Brain nuclei

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were removed by the micropunch method (Palkovits, 1973) from 300-μm-thick coronal frozen sections cut in a cryostat at −10°C. Tissue from each microdissected area was pooled from two animals. β-Neo-endorphin and α-neo-endorphin were assayed in the same tissue samples. (The distribution of the latter has been presented in the preceding report by Zamir et al., 1984). The pituitary gland was immediately removed and dissected into neurointermediate and anterior lobes.

Tissue samples were placed in 1.5-ml conical Eppendorf tubes containing 200 μl of 0.1 N HCl and transferred to a boiling water bath for 10 min. The tissue samples were then homogenized by sonication, and 20-μl aliquots of the homogenates were removed for protein determination (Lowry et al., 1951). The extracts were centrifuged at 2000 × g for 10 min at 4°C. The supernatants were transferred to 12 × 75 mm polypropylene tubes and evaporated to dryness in a vacuum centrifuge. The efficiency of the extraction was determined to be 80 to 90% by measuring the recovery of [^{125}I]-β-neo-endorphin internal standards from tissue homogenates.

To determine whether conversion of α-neo-endorphin to β-neo-endorphin occurs in the course of tissue preparation, we measured the concentrations of these peptides in samples of median eminence, supraoptic nucleus, and paraventricular nucleus dissected from frozen slices and slices of unfrozen brain cut with a razor blade. Neither the concentrations nor the ratio of α-neo-endorphin to β-neo-endorphin changed detectably due to the one brief freeze-thaw cycle required for mounting frozen sections in the microdissection technique used.

Radioimmunoassay (RIA). Samples were rehydrated in phosphate-buffered saline (pH 7.6) containing 0.1% gelatin, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% merthiolate. The antiserum was used at final dilution of 1:10,000 at which dilution 30 to 40% of the ^{125}I-labeled β-neo-endorphin was bound. Each sample was incubated at 4°C in a 500-μl volume that contained 300 μl of sample in assay buffer, 100 μl of ^{125}I-labeled β-neo-endorphin, and 100 μl of β-neo-endorphin antiserum in assay buffer. After 16 to 24 hr, 1 ml of dextran-coated charcoal in phosphate-buffered saline (2.5 gm of charcoal and 0.25 gm of dextran/liter) was added to each tube, and the tubes were incubated at 4°C for 10 min and centrifuged at 2000 × g for 20 min. The radioactivity of the supernatant was measured in a Micromedic 4/200 gamma counter. The RIA sensitivity (i.e., the amount of peptide that displaced 20% of the label) was 8 to 10 pg.

Properties of the antiserum. The antiserum was a gift from E. Weber (Stanford University, Palo Alto, CA). The specificity of the β-neo-endorphin antiserum has been described (Weber et al., 1982a). The antiserum shows less than 0.01% cross-reactivity with a α-neo-endorphin. It does not cross-react with Leu-enkephalin, Met-enkephalin, dynorphin A, or dynorphin A(1–8).

Peptides. β-Neo-endorphin was purchased from Peninsula Laboratories (San Carlos, CA).

Iodination procedure. Synthetic β-neo-endorphin was iodinated with Na^{125}I, using chloramine T (Hunter et al., 1962). The reaction was stopped with sodium metabisulfite. The radiolabeled peptide was purified by chromatography on octadecylsilyl-silica cartridges (ODS silica, Sep-Pak C_{18}, Waters Associates), using an increasing gradient of methanol in 0.01 M HCl/0.1 M acetic acid solution.

Results

ir-β-Neo-endorphin was detectable in all brain and spinal cord areas investigated. The peptide is unevenly distributed. A 16.0-fold difference in β-neo-endorphin concentration was measured between the richest (median eminence, 341.4 fmol/mg of protein) and the poorest (olfactory bulb, 21.3 fmol/mg of protein) brain regions. The spinal cord contains low levels of the peptide, whereas the neurointermediate lobe is extremely rich in it (9407.3 fmol/mg of protein).

Telencephalon (Table I). The telencephalic structures contain low to moderate concentrations of β-neo-endorphin. The cerebral cortical areas contain low levels of the peptide (<100 fmol/mg of protein); limbic system structures (hippocampus, dentate gyrus, amygdaloid nuclei, and septal nuclei) have moderate levels (between 100 and 250 fmol/mg of protein). The central and lateral amygdaloid nuclei have higher β-neo-endorphin concentrations than the other telencephalic structures (205.9 and 226.1 fmol/mg of protein, respectively). The basal ganglia (rostral component of extrapyramidal system) contain moderate (nucleus accumbens, globus pallidus, and claustrum) to low (caudate and caudate-putamen) levels of the peptide.

Diencephalon (not including the hypothalamus) (Table

TABLE I

ir-β-Neo-endorphin concentrations in telencephalic nuclei of the rat

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio[a] (α-neo:β-neo)
		fmol/mg of protein	
1	Frontal cortex	72.2 ± 4.6 (5)[b]	0.8
2	Cingulate cortex	64.5 ± 14.8 (6)	0.9
3	Piriform cortex	87.8 ± 15.4 (5)	1.1
4	Parietal cortex	65.5 ± 7.1 (5)	0.9
5	Entorhinal cortex	96.2 ± 22.9 (6)	1.2
6	Occipital cortex	71.7 ± 14.5 (6)	1.4
7	Claustrum	113.1 ± 22.6 (6)	0.8
8	Hippocampus	110.4 ± 17.2 (6)	3.7
9	Dentate gyrus	126.2 ± 26.6 (6)	5.4
10	Olfactory bulb	21.3 ± 3.5 (6)	2.0
11	Olfactory tubercle	95.0 ± 14.0 (5)	1.9
12	Nucleus of the diagonal band	129.8 ± 18.2 (6)	2.6
13	Nucleus accumbens	124.7 ± 23.2 (6)	5.1
14	Bed nucleus of the stria terminalis	160.5 ± 25.5 (6)	2.8
15	Globus pallidus	104.7 ± 12.8 (6)	6.0
16	Caudate nucleus	67.0 ± 7.8 (5)	2.6
17	Caudate-putamen	70.4 ± 12.3 (6)	3.4
18	Lateral septal nucleus	138.9 ± 35.9 (6)	2.1
19	Medial septal nucleus	115.1 ± 14.9 (5)	1.2
20	Dorsal septal nucleus	101.9 ± 22.0 (5)	1.4
21	Cortical amygdaloid nucleus	146.8 ± 32.8 (5)	0.7
22	Basal amygdaloid nucleus	171.7 ± 34.9 (6)	0.8
23	Medial amygdaloid nucleus	179.4 ± 40.8 (6)	1.0
24	Lateral amygdaloid nucleus	205.9 ± 31.8 (6)	0.7
25	Central amygdaloid nucleus	226.1 ± 28.9 (6)	1.9

[a] Based on Zamir et al., 1984.

[b] Numbers in parentheses, number of samples.

II). The thalamic (with the exception of the ventral thalamic nucleus, which is poor in peptide), epithalamic (habenula), metathalamic (geniculates), and subthalamic (zona incerta) structures all contain moderate levels of β-neo-endorphin. The nucleus reuniens is the richest thalamic nucleus (171.6 fmol/mg of protein).

Hypothalamus. In general the hypothalamic nuclei are relatively rich in ir-β-neo-endorphin (Table III). The median eminence contains the highest β-neo-endorphin concentration in the brain (341.1 fmol/mg of protein). High levels of the peptide (above 250 fmol/mg of protein) were measured in the dorsomedial, anterior hypothalamic, arcuate, paraventricular, ventromedial, and suprachiasmatic nuclei, and in the lateral preoptic area. All of the other hypothalamic nuclei investigated exhibited moderate levels of the peptide with lowest concentrations in mammilary body, medial forebrain bundle (preoptic), and supraoptic nucleus.

Mesencephalon (Table IV). The substantia nigra and the periaqueductal gray (SGC) have the highest concentrations of β-neo-endorphin in the midbrain (312.9 and 288.6 fmol/mg of protein, respectively). Cell groups surrounding the substantia nigra (red nucleus, ventral tegmental area, and interpeduncular nucleus) have much lower levels than does the substantia nigra itself. The rest of the mesencephalic nuclei, except the inferior colliculus which is poor in the peptide, contain moderate amounts of the peptide.

Pons (Table V). In general, the tegmentum (parabrachial nuclei, nucleus locus ceruleus, and tegmental nuclei) is richer in β-neo-endorphin than is the basal pons (pontine nuclei, pontine reticular nuclei, and superior olive). The parabrachial nuceli are among the richest nuclei in the brain.

Cerebellum (Table V). Both the cerebellar cortex (samples from the vermis and hemispheres and cerebellar nuclei (all three nuclei were pooled) contain low levels of β-neo-endorphin.

TABLE II

ir-β-Neo-endorphin concentrations in the diencephalic (except hypothalamic) nuclei of the rat

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio (α-neo:β-neo)
		fmol/mg of protein	
26	Anterior ventral thalamic nucleus	112.6 ± 17.6 (5)[a]	0.5
27	Periventricular thalamic nucleus	166.3 ± 13.1 (6)	1.4
28	Ventral thalamic nucleus	92.1 ± 12.7 (5)	1.0
29	Medial thalamic nucleus	110.6 ± 11.0 (5)	0.6
30	Nucleus reuniens	176.1 ± 19.6 (5)	0.7
31	Rhomboid nucleus	112.9 ± 21.2 (6)	1.0
32	Lateral thalamic nucleus	114.0 ± 27.4 (5)	0.5
33	Parafascicular nucleus	118.6 ± 10.0 (5)	1.2
34	Posterior thalamic nucleus	122.5 ± 16.2 (6)	0.5
35	Lateral geniculate body	140.7 ± 37.6 (5)	0.5
36	Medial geniculate body	116.1 ± 22.5 (5)	0.9
37	Habenular nuclei (med. & lat.)	133.9 ± 13.8 (4)	0.6
38	Zona incerta	155.9 ± 23.5 (6)	2.3

[a] Numbers in parentheses, number of samples.

TABLE III

ir-β-Neo-endorphin content in nuclei of the hypothalamus

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio (α-neo:β-neo)
		fmol/mg of protein	
39	Medial preoptic nucleus	217.2 ± 31.3 (5)[a]	1.7
40	Lateral preoptic area	278.8 ± 49.4 (6)	3.6
41	Medial forebrain bundle (preoptic)	161.5 ± 30.8 (5)	2.0
42	Periventricular nucleus	230.6 ± 21.2 (6)	1.8
43	Supraoptic nucleus	176.2 ± 21.4 (6)	1.1
44	Paraventricular nucleus	266.3 ± 29.9 (6)	1.7
45	Suprachiasmatic nucleus	256.2 ± 30.1 (6)	1.6
46	Anterior hypothalamic nucleus	282.3 ± 34.9 (6)	1.8
47	Median eminence	341.4 ± 30.7 (5)	1.5
48	Arcuate nucleus	276.2 ± 41.8 (6)	1.4
49	Ventromedial nucleus	262.5 ± 44.9 (6)	1.5
50	Dorsomedial nucleus	320.1 ± 38.4 (6)	1.3
51	Perifornical nucleus	249.8 ± 38.2 (6)	0.8
52	Posterior hypothalamic nucleus	202.2 ± 24.4 (6)	1.2
53	Medial forebrain bundle (hypothalamic)	204.8 ± 44.7 (5)	1.7
54	Dorsal premammillary nucleus	248.1 ± 28.3 (6)	1.4
55	Ventral premammillary nucleus	214.5 ± 23.2 (6)	1.2
56	Mammillary body	125.7 ± 32.8 (6)	1.1

[a] Numbers in parentheses, number of samples.

Medulla oblongata (Table V). A wide range of ir-β-neo-endorphin concentrations was found in the medulla oblongata. The richest area (nucleus of the solitary tract, medial part) contains 4.6 times as much peptide as the poorest (cochlear nuclei). The prepositus hypoglossal nucleus also contains a high level of the peptide. The remainder of medullary nuclei investigated exhibit moderate concentrations of the peptide (with the exception of the motor facial nucleus which has a low concentration of the peptide).

Circumventricular organs (Table VI). Moderate concentrations of ir-β-neo-endorphin were detected in the circumventricular organs. The highest concentration was measured in the subcommissural organ followed by organum vasculosum laminae terminalis. The subfornical organ has a lower ir-β-neo-endorphin concentration than the other circumventricular organs.

Spinal cord. Low levels of ir-β-neo-endorphin were measured in the cervical, thoracic, and lumbar segments of the spinal cord (Table VII).

Pituitary gland. The neurointermediate lobe of the pituitary gland contains very high levels of β-neo-endorphin (9407.3 fmol/mg of protein) (Table VIII).

Discussion

Using a sensitive and specific RIA, we have studied the distribution of the opioid peptide, β-neo-endorphin, in discrete areas of the rat brain, in the spinal cord, and in the neurointermediate lobe of the pituitary gland. Our data show (*1*) that ir-β-neo-endorphin is present at all levels of the neuraxis, (*2*) that it is unevenly distributed,

TABLE IV

ir-β-Neo-endorphin content in the mesencephalon and pons

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio (α-neo:β-neo)
		fmol/mg of protein	
Mesencephalon			
57	Substantia nigra	312.9 ± 47.1 (6)[a]	5.4
58	Ventral tegmental area	133.5 ± 26.7 (6)	1.2
59	Interpeduncular nucleus	110.3 ± 24.1 (6)	1.2
60	Red nucleus	152.2 ± 21.6 (5)	0.5
61	Superior colliculus	113.2 ± 21.0 (6)	0.8
62	Inferior colliculus	72.9 ± 10.9 (6)	1.2
63	Periaqueductal gray matter (SGC)	288.6 ± 30.5 (6)	1.5
64	Dorsal raphe nucleus	154.6 ± 19.7 (6)	1.6
65	Nucleus cuneiformis (ret. form.)	119.4 ± 17.6 (5)	1.4
Pons			
66	Locus ceruleus	170.5 ± 22.7 (6)	1.8
67	Parabrachial nuclei (dors. & vent.)	294.3 ± 34.0 (6)	2.3
68	Pontine tegmental nuclei	107.0 ± 13.8 (6)	1.4
69	Pontine reticular nuclei (oral & caud.)	84.3 ± 18.4 (6)	1.5
70	Pontine nuclei	91.3 ± 22.8 (5)	0.8
71	Superior olive	120.1 ± 13.7 (4)	0.6
72	Motor trigeminal nucleus	107.4 ± 23.5 (5)	1.0
73	Sensory trigeminal nucleus	143.5 ± 19.0 (6)	1.5

[a] Numbers in parentheses, number of samples.

TABLE V

ir-β-Neo-endorphin concentrations in medulla oblongata and cerebellum

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio α-neo:β-neo)
		fmol/mg of protein	
Medulla oblongata			
74	Nucl. tract. spin. Vth	107.8 ± 16.6 (6)[a]	0.9
75	Gracilis nucleus	118.9 ± 20.4 (5)	1.1
76	Cuneate nucleus	139.4 ± 17.1 (6)	1.3
77	Medullary reticular nuclei (vent. & dors.)	117.2 ± 16.6 (4)	1.3
78	Paramedian reticular nucleus	118.9 ± 23.5 (5)	0.7
79	Nucleus of solitary tract (NTS) (medial)	278.5 ± 60.8 (6)	1.6
80	NTS (commissural part)	228.1 ± 23.4 (6)	1.7
81	Motor hypoglossal nucleus	185.5 ± 26.0 (5)	1.3
82	Prepositus hypoglossal nucleus	252.9 ± 31.8 (6)	1.6
83	Nucleus ambiguus	164.6 ± 21.5 (6)	1.7
84	Lateral reticular nucleus	137.0 ± 10.4 (5)	1.1
85	Cochlear nuclei (dors. & vent.)	59.0 ± 13.0 (6)	1.1
86	Medial vestibular nucleus	128.7 ± 19.5 (5)	0.6
87	Lateral vestibular nucleus	116.8 ± 13.8 (5)	0.6
88	Inferior olive	102.2 ± 15.7 (6)	1.3
89	Nucleus raphe magnus	114.0 ± 19.1 (6)	1.2
90	Motor facial nucleus	97.3 ± 18.0 (5)	0.9
91	Gigantocellular reticular nucleus	125.7 ± 14.3 (4)	0.7
92	Parvicellular reticular nucleus	143.2 ± 18.6 (6)	1.6
Cerebellum			
93	Cortex	82.2 ± 13.5 (5)	0.5
94	Nuclei	90.1 ± 17.5 (5)	0.5

[a] Numbers in parentheses, number of samples.

TABLE VI

Concentrations of ir-β-neo-endorphin in the circumventricular organs

	Regions	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio α-neo:β-neo)
		fmol/mg of protein	
95	Organum vasculosum laminae terminalis	202.4 ± 31.1 (6)[a]	0.8
96	Subfornical organ	108.1 ± 19.8 (5)	0.7
97	Subcommissural organ	205.0 ± 28.1 (5)	1.1
98	Area postrema	190.7 ± 20.7 (5)	1.0

[a] Numbers in parentheses, number of samples.

and (*3*) that the ratio of α-neo-endorphin to β-neo-endorphin in different brain areas is variable.

Our results are in good agreement with reports describing the distribution of ir-β-neo-endorphin in gross anatomical brain regions (Kitamura et al., 1982; Weber et al., 1982a). No immunohistochemical data on the distribution of β-neo-endorphin in the rat central nervous system have been published to date, but it seems that areas with high concentrations of ir-β-neo-endorphin contain large numbers of ir-α-neo-endorphin fibers and terminals. Thus, the most dense α-neo-endorphin-positive fiber systems were found in the median eminence and substantia nigra, which contain substantial amounts of ir-β-neo-endorphin. Significant amounts of β-neo-endorphin were also found in areas containing α-neo-endorphin-positive cell bodies such as the dorsomedial, anterior hypothalamic, paraventricular, and parabrachial nuclei, central gray, and nucleus of the solitary tract. All of the above-mentioned nuceli also contain α-neo-endorphin-positive nerve fibers and/or terminals.

The abundance of β-neo-endorphin in median eminence (the richest brain area investigated), in several hypothalamic nuclei, and in the posterior pituitary suggests a role for this peptide in neuroendocrine regulation.

TABLE VII
Distribution of ir-β-neo-endorphin in the spinal cord

Areas	ir-β-Neo-endorphin Concentrations (mean ± SEM)	Molar Ratio (α-neo:β-neo)
Cervical spinal cord	84.0 ± 7.8 (6)[a]	1.6
Thoracic spinal cord	50.2 ± 9.8 (5)	1.7
Lumbar spinal cord	39.2 ± 9.6 (6)	1.5

[a] Numbers in parentheses, number of samples.

TABLE VIII
ir-β-Neo-endorphin content in the neurointermediate lobe of the pituitary gland

Lobe	ir-β-Neo-endorphin Concentration (mean ± SEM)	Molar Ratio (α-neo:β-neo)
	fmol/mg of protein	
Neurointermediate	9407.3 ± 776.6 (18)	1.1

As mentioned earlier, α-neo-endorphin and β-neo-endorphin are derived from a larger precursor molecule. They are separated from dynorphin A and dynorphin B by two basic amino acids—lysine and arginine (Kakidani et al., 1982). It appears that, when the precursor is processed proteolytically, it is cleaved either (*1*) between the lysine and arginine or (*2*) on the carboxy terminal side of the Arg, and then the Arg is efficiently removed, generating α-neo-endorphin. The C-terminal lysine of α-neo-endorphin could, in principle, be trimmed off by a carboxypeptidase B-like enzyme (one that removes basic residues from a peptide's C-terminus) or by a post-proline cleaving enzyme. Both enzyme activities have been demonstraed in brain homogenates (Hersh and McKelvy, 1979; Docherty et al., 1982), but the fact that removal of basic residues by pancreatic carboxypeptidase B is inhibited by adjacent prolyl residues (Folk and Gladner, 1958) suggests that a post-proline cleaving enzyme may be the better candidate.

Our data show that the molar ratio of α-neo-endorphin to β-neo-endorphin is inconstant; in two-thirds of brain regions α-neo-endorphin predominates, suggesting that the trimming enzyme, no matter what its type, does not remove α-neo-endorphin's C-terminal lysine readily. Surprisingly, though, in about one-third of the brain areas examined, β-neo-endorphin concentrations were greater than those of α-neo-endorphin. The physiological importance of α-neo-endorphin versus β-neo-endorphin remains to be determined.

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