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BRAIN DISTRIBUTIONS OF α-NEO-ENDORPHIN AND β-NEO-ENDORPHIN: EVIDENCE FOR REGIONAL PROCESSING DIFFERENCES

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SUMMARY: The leu-enkephalin containing opioid peptides α-neo-endorphin and β-neo-endorphin [i.e., α-neo-endorphin(1-9)] were measured in rat brain region extracts with two highly specific radioimmunoassays. The molar ratio of α-neo-endorphin to β-neo-endorphin was extremely variable among brain regions. In hypothalamus and posterior pituitary β-neo-endorphin levels were almost as high as α-neo-endorphin levels. In contrast, in the striatum α-neo-endorphin was 30-fold more concentrated than β-neo-endorphin. In all other brain regions α-neo-endorphin was present in 3 to 20-fold higher concentrations than β-neo-endorphin. The β-neo-endorphin immunoreactive material was found to comigrate with authentic β-neo-endorphin on reverse phase HPLC. These findings suggest that in certain brain regions but not in others processing mechanisms exist which can generate β-neo-endorphin through processing of α-neo-endorphin or its precursors.

INTRODUCTION

α-Neo-endorphin is a ten amino acid residue opioid peptide that contains a leu-enkephalin sequence at the aminoterminus (1). In brain it is costored with dynorphin(1-17) immunoreactive material in the same neurons (2,3) and it is codistributed with the opioid peptide dynorphin(1-8) in approximately the same concentrations in all brain regions (4) indicating the two peptides are major products in the proteolytic processing of the common precursor to α-neo-endorphin and dynorphin (pro-neo-endorphin/dynorphin) (5). Hypothalamus extracts also contain β-neo-endorphin (6). β-Neo-endorphin is identical in amino acid sequence to α-neo-endorphin except that it lacks a lysine residue at the carboxyterminus. Thus β-neo-endorphin can also be called

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α-neo-endorphin (1-9). 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.

The regional distribution of β-neo-endorphin in brain is unknown. We have now developed a specific radioimmunoassay (RIA) to β-neo-endorphin. This RIA has a negligible crossreactivity with α-neo-endorphin and therefore has allowed us to compare the distribution in rat brain of β-neo-endorphin with the distribution of α-neo-endorphin which was measured with a specific RIA whose characterization was described previously (4). We found that the molar ratios of α-neo-endorphin to β-neo-endorphin were extremely variable among brain regions ranging from 1.5:1 in the posterior pituitary to 30:1 in striatum. These findings indicate the existence of brain region specific processing enzymes which convert α-neo-endorphin or its precursor(s) extensively into β-neo-endorphin in some brain regions, whereas in other regions α-neo-endorphin is not significantly converted into the smaller opiate peptide.

METHODS

Antibodies and Radioimmunoassays

The antiserum to β-neo-endorphin was generated in rabbits by immunization with a carbodiimide treated peptide-thyroglobulin mixture as described for the production of specific antibodies to α-N-acetyl β-endorphin (7). The generation and characterization of the α-neo-endorphin antiserum has been described (4). Radioimmunoassays were carried out as described (4,7). Separation of antibody-bound from free antigen was performed by double antibody immunoprecipitation.

The specificity of the α- and β-neo-endorphin RIAs is shown in Table 1. As can be seen in Table 1, the two RIAs did not significantly crossreact with related peptides or with the peptide which was detected by the respective other RIA.

Tissue Extraction

Male Sprague Dawley rats (200-260 g) were killed by decapitation and brain regions were dissected by the method of Glowinsky and Iversen (8). Tissue samples were immediately frozen on dry ice. After weighing in the frozen state each sample was extracted by sonication in 1 ml of acid acetone (acetone:water:12 N HCl, 40:6:1). The extracts were spun at 12,000 g for 5 min and supernatants were evaporated under a stream of air. The residues were taken up in RIA buffer and aliquots from appropriately diluted samples

Table 1.

SPECIFICITY OF RIAs USED FOR MEASURING α-NEO-ENDORPHIN AND β-NEO-ENDORPHIN IN RAT BRAIN REGION EXTRACTS

Synthetic Peptide	Percent Crossreactivity in RIA for α-Neo-endorphin	β-Neo-endorphin
α-neo-endorphin	100	<0.01
β-neo-endorphin	<0.01	100
Dynorphin(1-8)	0	0
Dynorphin(1-17)	0	0
Leu-enkephalin	0	0
Met-enkephalin	0	0

The crossreactivity was calculated based on the amount of unlabelled peptide needed to obtain a 50 percent displacement of ^{125}I-labelled peptide from the antisera. The highest concentration of unlabelled peptide tested was 1 μM. All synthetic peptides used in this study were solid phase synthesized and purified by standard procedures.

were assayed in the two RIAs. Technical details including buffers and incubation protocols of our RIA procedures have been published in previous papers (4,7).

Chromatographic Characterization of Immunoreactive β-Neo-Endorphin

The β-neo-endorphin immunoreactive material from 3 brain regions (midbrain, hypothalamus, posterior pituitary) was characterized by gel filtration and reverse phase high performance liquid chromatography (RP-HPLC). For gel filtration chromatography, brain regions from 10 animals each were pooled and extracted in acid acetone as described above. The acid acetone extract was delipidized with heptane as described (4), evaporated and taken up in 1 ml 50 percent acetic acid. The sample was subjected to gel filtration chromatography on a 120 x 0.9 cm column packed with Sephadex G50 in 50 percent acetic acid. Elution was with 50 percent acetic acid. Fractions, 1.8 ml each, were collected and 50 μl aliquots were evaporated under reduced pressure and the residues assayed with the β-neo-endorphin RIA. Aliquots of the peak fractions were subjected to RP-HPLC using a separation system that has been described elsewhere (4). Aliquots of HPLC fractions were evaporated under reduced pressure and residues analyzed with the β-neo-endorphin RIA.

RESULTS

Table 2 shows the concentrations of α-neo-endorphin and β-neo-endorphin immunoreactive material in 8 brain regions and in the posterior intermediate lobe of the pituitary. The regional distribution of α-neo-endorphin and the amounts are in agreement with previous results reported by us (4) and they are similar to results published by others (9,10). The α-neo-endorphin concentrations measured in the hippocampus were slightly higher than the ones reported in our previous study (4). This was due to the fact that we modified our dissection protocol resulting in a more complete removal of hippocampus. As shown in our previous paper (4), more than 95 percent of

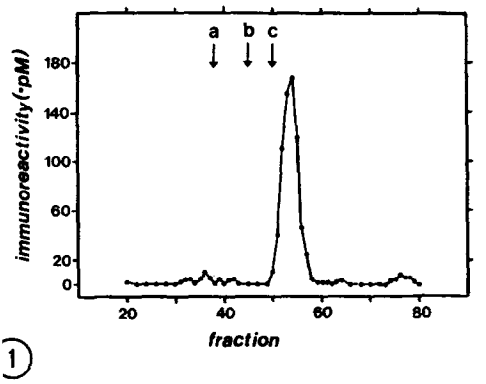
Table 2.

CONCENTRATION AND MOLAR RATIOS OF α-NEO-ENDORPHIN AND β-NEO-ENDORPHIN IN RAT BRAIN REGIONS

Brain Region	Immunoreactivity (pmol/g tissue, n=10)		Molar Ratio
	α-Neo-endorphin	β-Neo-endorphin	α-Neo:β-Neo
Spinal cord	29.7 ± 2.3	9.9 ± 1.0	3.0
Pons/medulla	36.6 ± 1.6	12.9 ± 0.6	2.8
Midbrain	73.8 ± 2.9	12.8 ± 0.6	5.7
Cerebellum	< 0.5	< 0.2	
Hypothalamus	79.2 ± 3.9	32.3 ± 1.8	2.5
Striatum	74.6 ± 2.7	2.4 ± 0.4	31.0
Hippocampus	49.0 ± 4.3	2.7 ± 0.3	18.1
Cortex	25.7 ± 0.9	3.3 ± 0.9	7.8
Posterior pituitary	1600 ± 78	1032 ± 85	1.5

the α-neo-endorphin immunoreactive material found in various brain regions corresponds to authentic α-neo-endorphin as determined by gel-filtration chromatography and RP-HPLC.

When β-neo-endorphin immunoreactive material was measured in the same samples in which α-neo-endorphin had been measured, it was found that both the hypothalamus and the posterior/intermediate pituitary contained rather large amounts of β-neo-endorphin compared to α-neo-endorphin (Table 2). Thus, the posterior pituitary was found to contain almost as much β-neo-endorphin as α-neo-endorphin and the hypothalamus contained almost half as much β-neo-endorphin as α-neo-endorphin. The molar ratios of α-neo-endorphin to β-neo-endorphin in all other brain regions were highly variable ranging from 3:1 in spinal cord and pons/medulla to 30:1 in striatum. α-Neo-endorphin was always present in higher concentrations than β-neo-endorphin (Table 2). The β-neo-endorphin immunoreactive material from 3 brain regions (midbrain, hypothalamus, posterior pituitary) was further characterized by gel filtration chromatography and RP-HPLC. As shown in Figure 1, the β-neo-endorphin immunoreactive material eluted on Sephadex G50 shortly after α-neo-endorphin which has a slightly higher molecular weight than β-neo-endorphin. On RP-HPLC the immunoreactivity comigrated exactly with synthetic β-neo-endorphin (Fig. 2) suggesting the material measured corresponded to authentic β-neo-endorphin.



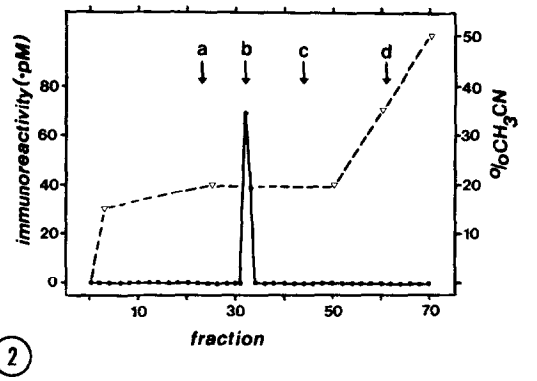


Figure 1. Sephadex G50 chromatography (elution with 50 percent acetic acid) of β-neo-endorphin immunoreactivity from rat hypothalamus. Tissue was extracted and chromatographed as described in the text. Fifty μl aliquots from the fractions were evaporated to dryness under reduced pressure and assayed with the β-neo-endorphin RIA. The vast majority of the immunoreactive material eluted shortly after ^{125}I-α-neo-endorphin from the column. α-Neo-endorphin is identical in amino acid sequence to β-neo-endorphin with an additional lysine residue at the C-terminus. Markers: a, ^{125}I-α-N-acetyl β-endorphin. b, ^{125}I-dynorphin(1-17). c, ^{125}I-α-neo-endorphin.

Figure 2. Reverse phase high performance liquid chromatography (RP-HPLC) of posterior/intermediate pituitary extract. Tissue was extracted and chromatographed on Sephadex G50 in 50 percent acetic acid as described in the text. An aliquot from the β-neo-endorphin immunoreactive peak fraction was evaporated and rechromatographed by RP-HPLC as described in reference 4. Aliquots (300 μl) of the fractions were evaporated to dryness under reduced pressure and the residues were assayed with the β-neo-endorphin RIA. All the immunoreactivity eluted at exactly the same position as synthetic β-neo-endorphin.
Markers: a, α-neo-endorphin. b, β-neo-endorphin. c, dynorphin(1-8). d, dynorphin(1-17).

DISCUSSION

The results presented here show that β-neo-endorphin and α-neo-endorphin are differentially distributed in brain. The neo-endorphins and dynorphin (1-17) (11,12) are derived from a common precursor (pro-neo-endorphin/dynorphin) (5) with α-neo-endorphin and dynorphin(1-8) being major processing products of this precursor in rat brain (4). The elucidation of the primary structure of pro-neo-endorphin/dynorphin together with our recent results of the distribution of some of the major processing products of this precursor in brain (4) has begun to reveal some remarkable proteolytic processing patterns which have not been recognized previously in other peptide hormone precursors.

Cleavage of peptide hormone precursors into their final secretory products classically is thought to occur at the carboxyterminus of paired

basic amino acid residues followed by removal of the dibasic residue by a carboxypeptidase B-like enzyme (13-17). In pro-neo-endorphin/dynorphin the dibasic amino acid residues do not seem to be the only signals for processing. For example, dynorphin(1-8), the major dynorphin related peptide in brain (4), seems to be cleaved from its precursor at a single arginine residue. Similarly dynorphin-B (18) [also referred to as rimorphin (19), a naturally occurring (19) thirteen amino acid residue opioid peptide], which is a fragment of the third leu-enkephalin containing opioid peptide in pro-neo-endorphin/dynorphin (5) seems to be generated by cleavage at a single arginine residue.

In the case of α-neo-endorphin and β-neo-endorphin, the processing does occur at a double basic lys-arg residue. However, the fact that α-neo-endorphin is present in much higher concentrations in most brain regions than β-neo-endorphin suggests that the carboxypeptidase B-like enzyme thought to be involved in removal of the basic residues does not readily remove the C-terminal lysine in α-neo-endorphin - a step which is necessary to generate β-neo-endorphin. In this respect, it is important to note that the amino acid residue preceding the carboxyterminal lysine in α-neo-endorphin is proline. Although peptide bonds where proline participates are not attacked by most of the common proteases (20), both exo- and endo-peptidases have been described that are capable to cleave peptide bonds in which proline is involved (21,22). One of these has been shown to occur in brain (23). As shown in Table 2, there are indeed brain regions where β-neo-endorphin is generated in substantial quantities: in the hypothalamus and in posterior/intermediate pituitary β-neo-endorphin concentrations are almost as high as those of α-neo-endorphin. In striatum, on the other hand, only negligible conversion of α-neo-endorphin into β-neo-endorphin seems to occur. Thus, certain brain regions - perhaps certain neuronal populations - seem to contain (an) enzyme(s) which is(are) capable of generating β-neo-endorphin, whereas in other brain regions these enzymes may be inactivated or not present at all (Table 2). The question whether these

enzymes are similar to or different from the above mentioned proline specific peptidases should be addressed by further studies.

While recent results suggest that both α-neo-endorphin and β-neo-endorphin are agonists of the kappa subtype of the opiate receptor (24), it would be of interest to determine the precise receptor selectivity and(or) potency of the two peptides in various assay systems since the region specific processing may be a reflection of functional requirements leading to generation of β-neo-endorphin in some brain regions but not in others.

The present results are further evidence that the proteolytic processing of pro-neo-endorphin/dynorphin seems to follow pathways (4) that are not always seen in other opiate peptide hormone precursors (14-17). Further studies of the processing of pro-neo-endorphin/dynorphin promise interesting insights into previously unrecognized processing signals and may reveal peptide products with unique opiate receptor binding properties (25,26).

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