Vol. 113, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1983 Pages 30-34

RIMORPHIN (DYNORPHIN B) EXISTS TOGETHER WITH α-NEO-ENDORPHIN AND DYNORPHIN (DYNORPHIN A) IN HUMAN HYPOTHALAMUS

Kazuwa Nakao, Takaaki Yoshimasa, Mitsuaki Suda, Makoto Sakamoto, Yoshio Ikeda, Kyozo Hayashi and Hiroo Imura

Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan
Gifu College of Pharmacy (K.H.), Gifu, Japan

Received April 4, 1983

Rimorphin (dynorphin B) has been demonstrated to exist together with α-neo-endorphin and dynorphin(1-17)(dynorphin A) in the human hypothalamus. The content of rimorphin was comparable to that of α-neo-endorphin and somewhat higher than that of dynorphin. This result is quite similar to the recent observations in bovine, porcine and rat neural tissues, suggesting that rimorphin is derived from preproenkephalin B together with α-neo-endorphin and dynorphin in man.

Rimorphin, or dynorphin B, is a newly purified endogenous opioid peptide from bovine neurointermediate pituitary (1,2). This tridecapeptide having leucine-enkephalin (Leu-enkephalin) sequence at the N-terminus corresponds to amino acid residues 228-240 of preproenkephalin B from the porcine hypothalamus as determined by cDNA analysis (3). The C-terminal 29 amino acids of this precursor, called leumorphin, contains rimorphin sequence at the N-terminus. This precursor molecule contains also α- and β-neo-endorphin (4,5) and dynorphin (6,7). Rimorphin has been demonstrated to exist in association with α-neo-endorphin and dynorphin in bovine, porcine and rat posterior pituitaries and neural tissues (1,2,8).

The present study was designed to elucidate whether or not rimorphin exists together with other opioid peptides derived from preproenkephalin B in the human hypothalamus.

Address correspondence and reprint requests to Kazuwa Nakao, M.D. Second Division, Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan.

Abbreviations used: Leu-enkephalin, leucine-enkephalin; Met-enkephalin, methionine-enkephalin; RIA, radioimmunoassay; HPLC, high performance liquid chromatography; TFA, trifluoroacetic acid; LI, like immunoreactivity; TPCK, L-(tosylamide 2-phenyl) ethyl chloromethyl ketone

0006-291X/83 $1.50

MATERIALS AND METHODS

Peptides

Rimorphin (dynorphin B) was purchased from Peninsula Laboratories, Inc. (California). α- and β-neo-endorphin were donated by Dr. H. Matsuo, Department of Biochemistry, Miyazaki Medical College, Miyazaki, Japan and dynorphin(1-17)(dynorphin A) and dynorphin(1-8) were supplied by Drs. M. Fujino and M. Wakimasu, Central Research Division, Takeda Chemical Industries, Osaka, Japan. Leu-enkephalin was obtained from Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan.

Tissue and extraction procedure

A human hypothalamus obtained at autopsy within 3 hours after death from a women aged 38 with pancreatic cancer was dissected and immediately frozen and stored at -20°C until extraction. Extraction was performed by acidified methanol according to the procedure described previously (9). Briefly, the hypothalamus was weighed and homogenized immediately in 10 volumes of acidified methanol consisting of equal parts of methanol and 0.1 N HCl, incubated for 10 min at 70°C and cooled on ice. The homogenate was centrifuged at 50,000 x g for 30 min at 4°C and the supernatant was stored at -20°C.

Reverse phase high performance liquid chromatography (HPLC)

Reverse phase HPLC was carried out on a Nucleosil $_7C_{18}$ column (Macherey-Nagel, Germany). Peptides were eluted from the column with a gradient from 15% CH_3CN in 5 mM trifluoroacetic acid (TFA) to 30% CH_3CN in 5 mM TFA. The flow rate was 1.0 ml/min and the fraction volume was 0.5 ml. The retention times of synthetic α- and β-neo-endorphin, Leu-enkephalin, dynorphin(1-17), dynorphin(1-8) and rimorphin were monitored by ultraviolet absorption or radioimmunoassay (RIA).

Digestion with trypsin and carboxypeptidase B

Digestion of Leu-enkephalin containing-peptides of each fraction with trypsin and carboxypeptidase B was performed according to the method described previously by Lewis et al (10) with slight modifications. In brief, each fraction of HPLC was lyophilized, redissolved in 300 μl of 50 mM tris-HCl (pH 8.5) and digested with 1 μg of L-(tosylamido 2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin (Worthington) 5 hours at 37°C. Then the action of trypsin was inhibited by 2 μg of trypsin inhibitor (soybean trypsin inhibitor, Worthington). Tryptic digests were treated with 1 μg of carboxypeptidase B (Worthington) for 2 hours at 37°C and then for 10 min in boiling water to inactivate the enzyme. Digests with trypsin and carboxypeptidase B were assayed for Leu-enkephalin immunoreactivity.

Radioimmunoassays (RIAs)

RIA for Leu-enkephalin was performed as previously described (11,12). Leu-enkephalin antiserum (LC1-226) had a cross-reactivity of 0.7% with Met-enkephalin 0.02% with Met-enkephalin-Arg^6-Gly^7-Leu^8 and 0.09% with Met-enkephalin-Arg^6-Phe^7 (12). This antiserum showed no significant cross-reactivity with rimorphin, α- and β-neo-endorphin, dynorphin(1-17) and dynorphin(1-8)(< 0.01% on a molar basis). RIA for dynorphin was carried out according to the method described previously (9) except that dynorphin(1-17) was used instead of dynorphin(1-13).

RESULTS

Reverse phase HPLC coupled with a specific RIA for Leu-enkephalin revealed several Leu-enkephalin-containing peptides in the human hypothalamus which had Leu-enkephalin-like immunoreactivity (Leu-enkephalin-LI) after the digestion with trypsin and carboxypeptidase B as shown in Fig. 1. The highest peak of Leu-enkephalin-LI was eluted at the same retention time as that of synthetic Leu-enkephalin. Other three peaks of Leu-enkephalin-LI

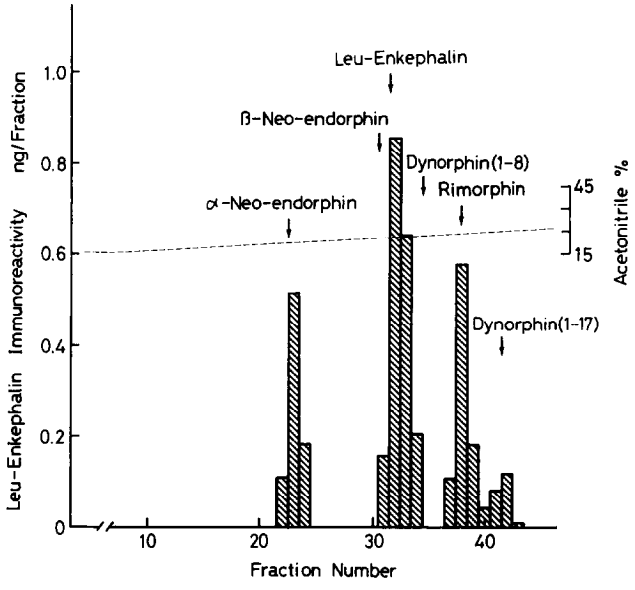

Fig. 1. A reverse phase high performance liquid chromatographic profile on a Nucleosil $_{7}C_{18}$ (0.4 x 20 cm) of human hypothalamic extract. The retention times of standard synthetic peptides are indicated by arrows.

comigrated with synthetic α-neo-endorphin, rimorphin and dynorphin(1-17), respectively. Contents of rimorphin, α-neo-endorphin, dynorphin(1-17) and Leu-enkephalin in the hypothalamus determined by HPLC coupled with RIA for Leu-enkephalin after the digestion with trypsin and caboxypeptidase B are shown in Table 1. Leu-enkephalin-LI and dynorphin(1-17)-like immunoreactivity (dynorphin-LI) determined directly by the RIAs for Leu-enkephalin and dynorphin, are also shown in Table 1. The content of rimorphin was comparable

Table 1

Contents of Rimorphin, α-Neo-Endorphin, Dynorphin(1-17) and Leu-Enkephalin in Human Hypothalamus

Peptide	Content determined after HPLC	Content determined directly by RIA
Rimorphin (Dynorphin B)	38.3	—
α-Neo-Endorphin	35.8	—
Dynorphin(1-17) (Dynorphin A)	11.3	26.4
Leu-Enkephalin	81.3	90.8

(pmole/g)

to that of α-neo-endorphin, approximately three times higher than that of dynorphin(1-17) and one and a half of that of dynorphin(1-17)-LI.

DISCUSSION

The present study demonstrates the existence of rimorphin together with α-neo-endorphin and dynorphin(1-17) in the human hypothalamus using HPLC coupled with RIA for Leu-enkephalin after the digestion with trypsin and carboxypeptidase B. The content of rimorphin was comparable with that of α-neo-endorphin and about three times higher than that of dynorphin(1-17). This finding is quite similar to the results of bovine, porcine and rat posterior pituitaries and neural tissues reported by Kilpatrick et al (1,2) and Cone et al (8). Very recently, rimorphin (dynorphin B), α-neo-endorphin, dynorphin were found in the same neuron in rat brain with immunohistochemical methods by Watson et al (13). Co-existence of rimorphin, α-neo-endorphin and dynorphin derived from preproenkephalin B is similar to that of methionine-enkephalin (Met-enkephalin), Leu-enkephalin, Met-enkephalin-Arg^6-Gly^7-Leu^8 and Met-enkephalin-Arg^6-Phe^7 derived from preproenkephalin A (14) as we and others reported previously (12,15,16,17).

The whole structure of preproenkephalin B is deduced from the nucleotide sequence of cloned DNA complementary to the porcine hypothalamic mRNA encoding it (3), but the structure of the human preproenkephalin B is not known at present. Existence of rimorphin together with α-neo-endorphin and dynorphin in the human hypothalamus demonstrated in this study indicates that the human preproenkephalin B contains at least three sets of Leu-enkephalin-containing peptides, α-neo-endorphin, dynorphin adn rimorphin. Further studies on the distribution of rimorphin in various human tissues are ongoing in our laboratory.

ACKNOWLEDGEMENTS

We are indebted to Professor H. Matsuo, Department of Biochemistry, Miyazaki Medical College, Miyazaki, Japan, for the generous supply of α- and β-neo-endorphin, to Drs. M. Fujino and M. Wakimasu, Central Research Division, Takeda Chemical Industries, Osaka, Japan, for the generous supply of dynorphin(1-17) and dynorphin(1-8), to Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan, for the gift of Leu-enkephalin. We also thank Misses K. Horii, F. Yamauchi and Y. Nakanishi for preparing the manuscript. This work was support-

ed in part by research grants from the Japanese Ministry of Health and Welfare, from the Japanese Ministry of Education, Science and Culture and from the Naito Foundation.

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