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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF NEUROKININ α AND β

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Two novel neuropeptides, neurokinin α and β isolated from porcine spinal cord, were announced. We have synthesized neurokinin α as His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2 and neurokinin β as Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2, which were 98-99% pure by HPLC. Assays on the isolated guinea pig ileum showed neurokinin α to have 81% and neurokinin β to have 65% of the activity of Substance P. Knowledge of these three related peptides having a common activity opens new considerations of their intrinsic physiological roles in neurotransmission versus pharmacological activities, and reappraisal of the diverse activities of Substance P including that in the inflammatory response of the eye.

Searching for undiscovered neuropeptides possessing significant biological activities, Kimura et al. (1) isolated two novel neuropeptides, from porcine spinal cord, which they named neurokinin α and β. During the factionation, they also identified fractions, which appeared to contain substance P and its Met^{11}-sulphoxide, according to the data of amino acid analyses.

Both neurokinin α and β are decapeptides and the establishment of the sequences revealed that both peptides are very similar in amino acid composition and sequence to the undecapeptide substance P.

As a part of our extensive study of analogs of substance P, particularly antagonists, we have synthesized neurokinin α and β and compared them in a biological assay for an activity of substance P.

MATERIALS AND METHODS

Method. The peptides were synthesized by the solid phase method, and the couplings were performed with a Beckman Model 990 Peptide Synthesizer. The benzhydrylamine (BHA) resin was used as a solid support. The α-amino functions were protected by the BOC groups. The side chain functionalities were protected by β-OBZl (ASP), Tos (His), Cl-Z (Lys), O-BZl (Ser) and O-BZl (Thr). Amino acids and the BHA resin were purchased from Beckman, Inc., Palo Alto, California,

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and from Peninsula Laboratories, Inc., San Carlos, California. All solvents (except TFA and isopropanol) were distilled before use.

The homogeneity of the peptides was demonstrated by TLC on silica gel plates. Using the following solvent systems: Rf^1 = n-BuOH: AcOH: H_2O (4:1:2); Rf^2 = n-BuOH: pyridine: AcOH: H_2O (30:30:6:24); Rf^3 = n-BuOH: EtoAC: AcOH: H_2O(2:2:1:1); Rf^4 = EtoAC: pyridine: AcOH: H_2O(5:5:1:3); Rf^5 = n-BuOH: EtoAC: AcOH: H_2O(50:33:1:40). All of the peptides showed single spots in all five systems which were detected with the ninhydrin and chlorine-O-tolidine reagents.

The amino acid analyses were performed on a Beckman Model 119 Amino Acid Analyzer.

HPLC was performed on a Water Liquid chromatography equipped with a Waters 660 Solvent programmer.

The conditions of HPLC for the peptides analysis were as follows: The column was μ-Bondapak-C_{18} (5μ) (3.9 x 300 mm); linear gradient, 20-100% / 25 min; solvent A: 0.1 M KH_2PO_4, PH 3; solvent B: 70% CH_3CN, 30% of solvent A; flow rate, 2 ml/min; detector, UV, 210 nm.

Synthesis. The first BOC amino acid was coupled to the BHA resin by the DCC method. The BHA resin, as purchased, was specified to contain 0.75 meq /g.

His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2 (Neurokinin α). - starting from 1g of BHA resin (HCl salt), 1.95g of BOC-His(Tos)-Lys(ClZ)-Thr(oBZl)-Asp(oBZl)-Ser(oBZl)-Phe-Val-Gly-Leu-Met-BHA resin was obtained. Cleavage by HF yielded 500 mg of the crude unprotected decapeptide. About 320 mg of the crude peptide was purified by Gel Filtration on Sephadex G-25 (2.5 x 100 cm) with 50% acetic acid, and 210 mg of the purified decapeptide was obtained. This product was further purified by HPLC. About 20 mg of the peptide was dissolved in 100 μl of 50% acetic acid, and 10 μl of the solution was injected. A linear gradient from 20-100% of solvent B in 20 min was used for purification (solvent A: 0.1 M KH_2PO_4, pH 3; solvent B: 70% CH_3CN; 30% of solvent A). The flow rate was 2 ml/min. A Radial-Pak liquid chromatography column was used. The peptide was detected by UV-absorbance at 210 nm. The collection of the eluted peaks of the desired peptide was lyophilized, and 10 mg of peptide which contained salt was obtained.

The desalting was carried out on a column of Sephadex G-10 (1 x 30 cm) which had been equilibrated with 50% acetic acid, and 2 mg of pure peptide was obtained; Rf values: Rf^1 = 0.37; Rf^2 = 0.61; Rf^3 = 0.03; Rf^4 = 0.57; Rf^5 = 0.37. Amino acid analysis: Asp 1.04(1); Thr 0.97(1); Ser 0.89(1); Gly 1.05(1); Val 1.06(1); Leu 0.94(1); Phe 1.01(1); His 0.96(1); Lys 1.0(1). The HPLC retention time was 9 min; purity, 99%.

Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2 (Neurokinin β). Starting with 1g of BHA resin (HCl salt), 1.8g of BOC Asp (BZl)-Met-His(Tos)-Asp(BZl)-Phe-Phe-Val-Gly-Leu-Met-BHA resin was prepared. Cleavage by HF yielded 653 mg of the crude unprotected decapeptide. A portion of 302 mg was purified by Gel Filtration on Sephadex G-25 (2.5 x 100 cm) with 50% acetic acid, and 245 mg of primary purified peptide was obtained. Then, the product was further purified by HPLC. Next, 20 mg of the peptide was dissolved in 150 µl of DMSO, and 10 µl of the DMSO solution was injected into a Radial-Pak column and a linear gradient from 30-100% of solvent B in 20 min was used (solvent A: 0.1 M NH_4OAC; solvent B: 70% CH_3CN, 30% of solvent A). The flow rate was 2 ml/min. The eluted peptide was detected by its UV-absorbance at 230 nm. The collection of eluted peaks of the desired peptide was lyophilized, and 5 mg of pure peptide was obtained. RF values: Rf^1 = 0.44; Rf^2 = 0.74; Rf^3 = 0.39; Rf^4 = 0.86; Rf^5 = 0.59. Amino acid analysis = Asp, 2.015(2); Gly, 1.055(1); Val, 0.955(1); Met, 1.982(2); Leu, 1.006(1); Phe, 1.958(2); His, 1.036(1); NH_3 1.006(1). The HPLC retention time was 14 min; purity, 98%.

RESULTS AND DISCUSSION

The sequences of the two new decapeptides, neurokinin α and β (1), and that of the well-known undecapeptide, substance P (SP), are as follows:

Neurokinin α H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2

Neurokinin β H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

Substance P H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

We synthesized neurokinin α and β by the automated and solid-phase procedure. The synthetic samples showed purities of 98-99% by HPLC.

It was expected that both of these neurokinins would show the activity of substance P on the isolated guinea pig ilium, because of the homology of the three peptides, and particularly because of the amino acids in the sequences between Phe^6 and Met^{10} of neurokinin α and β, and between Phe^7 and Met^{11} of substance P.

The basic procedure of Rosell et al. (2) was used to test for activity on the isolated guinea pig ilium.

Neurokinin α showed 81% of the activity of substance P and neurokinin β showed 65% of the activity of SP.

It is known , Folkers et al. (3), that Phe^7 is critical to the activity of SP, and it is known, Yanaihara et al. (4), that the sequence Arg^1-Pro^2-Lys^3-Pro^4 and to some extent that the N-terminal pentapeptide with Gln^5 are not essential for the high potency of SP in the guinea pig ilium assay. Consequently, the high SP-potencies in this assay of neurokinin α and β are understandable on the basis of the respective sequences of Thr^3 through Met^{10} and His^3 through Met^{10} of neurokinin α and β, respectively.

This association of neurokinin α and β and SP in the spinal cord (1), and their homology, and their common activities on the guinea pig ilium, opens new vistas of research on the neurotransmitters in the nervous system, and new concepts for biological studies of antagonists of SP, particularly as viewed by Rosell, Folkers et al. (5). The diverse biological activities of substance P, including the role of SP in the inflammatory response of the eye, Holmdahl et al. (6) and Mandahl et al. (7), can now be reappraised on the basis of the existence of neurokinin α and β toward differentiation of intrinsic physiological roles versus related pharmacological activities.

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