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NEUROKININ α AND β, SYNTHESIS AND PHARMACOLOGICAL PROPERTIES

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Two novel neuropeptides, neurokinin α and β, isolated from porcine spinal cord were chemically synthesized by solution method. The amino acid sequences proposed were confirmed to be correct and pharmacological properties of neurokinin peptides were studied.

Recently, we have isolated two new pharmacologically active peptides from the acetone-1 M HCl(100:3) extracts of porcine spinal cord with the aid of conventional gut-contracting assay using longitudinal muscle preparation of guinea pig ileum.[1] Amino acid sequences of the peptides were elucidated as shown below.[2]

According to the similarities of chemical structures and contraction profiles of guinea pig ileum with so called tachykinin families such as substance P and kassinin,[3] the newly isolated peptides were named neurokinin α and β, respectively.

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

To confirm the proposed amino acid sequences and to examine the pharmacological properties of neurokinin α and β, both peptides were synthesized by the solution method with maximum protecting strategy.

After the coupling of Boc-Val-Gly-OH and H-Leu-Met-NH_2 by HOBt/WSCI, the peptide chains were elongated stepwise by means of the active ester except His residue. Succinimidoester of Boc-Ser(Bzl), Boc-Thr(Bzl), Boc-Asp(OBzl) and *p*-nitrophenylester of Boc-Lys(Z) were coupled with the corresponding deacylated peptide derivatives. His was incorporated directly into chain with Boc-His(Tos) by HOBt/WSCI. The synthetic pathways of decapeptide derivatives are shown in Fig. 1. The synthesized protected peptides were treated with anhydrous hydrogen fluoride in the presence of anisole and dimethylsulfide at ice bath temperature for 1 h. The excess hydrogen fluoride was removed in vacuo and the residues were dissolved in 10% acetic acid. The solution was washed with ethylether and the water layer was passed through the column of Dowex 1x2 (AcO^-form). The crude

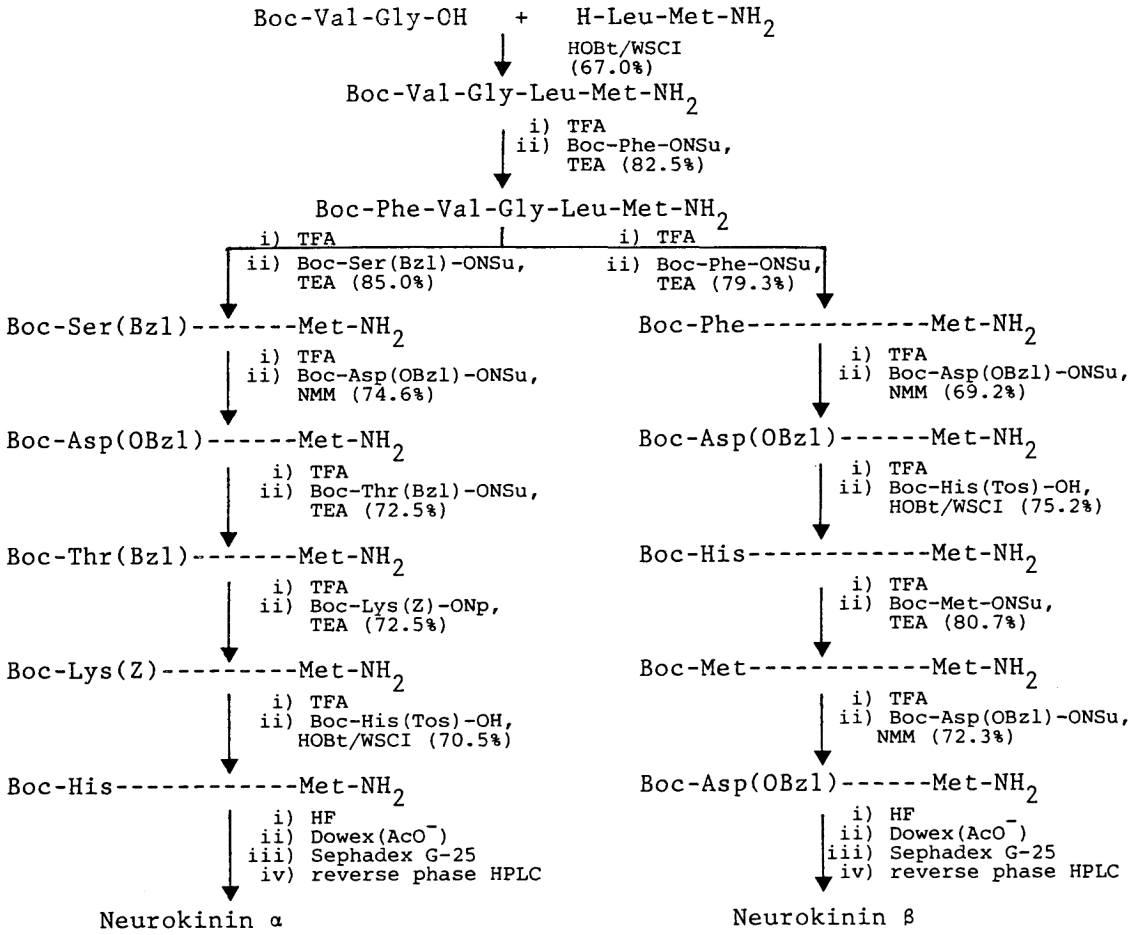

Fig. 1. Synthetic scheme of neurokinin α and β.

product of the peptides were gel-filtrated on Sephadex G-25 eluting with 0.1 M AcOH. Then peptides were purified by reverse phase high performance liquid chromatography using a column of Nucleosil 5C18 (4.6 x 300 mm) by gradient elution with CH_3CN - H_2O containing 50 mM triethylammonium phosphate. The elution diagrams of HPLC are shown in Fig. 2. Finally, the purified fractions were rechromatographed to remove phosphate salt by the column of Nucleosil 5C8 (4.6 x 300 mm) with gradient elution of CH_3CN - H_2O containing 0.1% TFA. Amino acid analysis of the synthetic products showed good agreement with the calculated values.

Neurokinin α : $Asp_{1.04}Thr_{0.95}Ser_{0.92}Gly_{\underline{1.00}}Val_{1.00}Met_{1.05}Leu_{1.02}Phe_{0.98}His_{1.05}Lys_{1.06}$
Rf=0.25*

Neurokinin β : $Asp_{1.88}Gly_{\underline{1.00}}Val_{1.02}Met_{1.79}Leu_{1.01}Phe_{2.02}His_{1.01}$
Rf=0.58*

(*TLC, Solvent: n-BuOH-AcOH-H_2O, 2-1-1)

The retention times of the synthesized neurokinin α and β on HPLC were consistent with the isolated natural peptides. Therefore proposed amino acid sequences of neurokinin α and β are proved to be correct.

The results of contracting- and salivation-test of the synthetic neurokinins

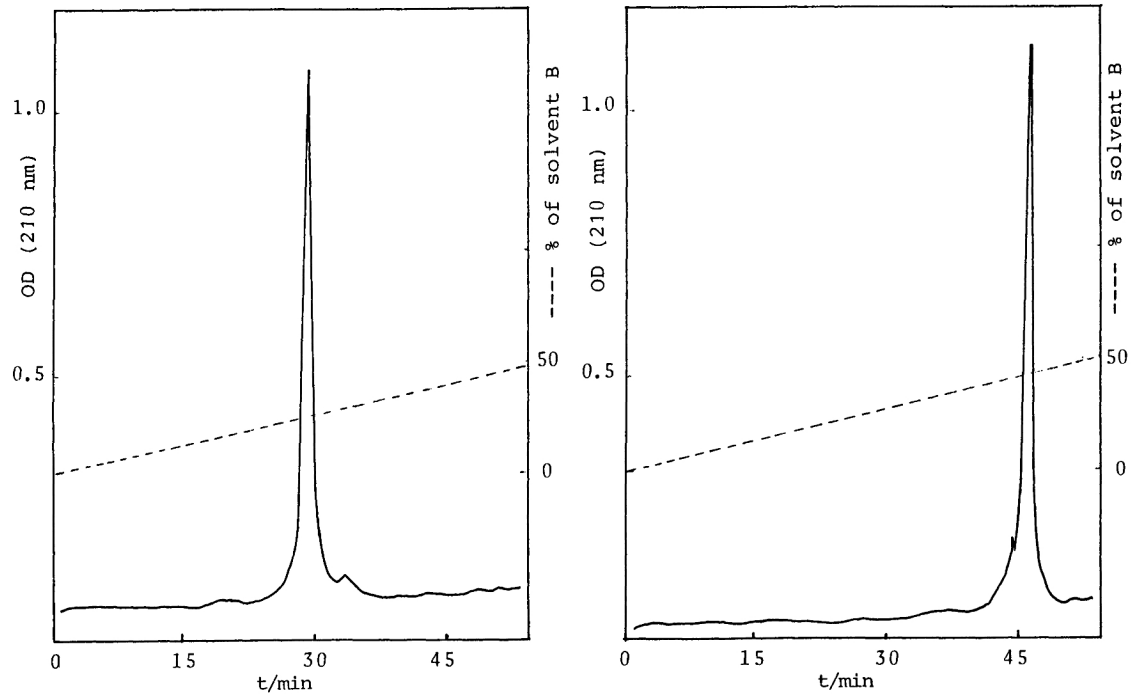

Fig. 2. Elution diagrams of neurokinin α and β on reverse phase HPLC. Column: Nucleosil 5C18(4.6x300 mm). Solvents: A = 50 mM triethylammonium phosphate in 10% CH_3CN-H_2O, B = 50 mM triethylammonium phosphate in 90% CH_3CN-H_2O. Gradient conditions are shown by dotted line. Flow rate was 1.0 ml/min.

compared with substance P are compiled in Table 1. The contracting activities of both neurokinin peptides were moderately lower than that of substance P. Neurokinin α exhibited sialogogic effect like substance P, however the effect of neurokinin β revealed to be relatively slower compared with other two peptides. In blood pressure assay with anaesthtized rats, neurokinin α possessed long lasting hypotensive effect similar to that of substance P but the rapid disappearance of the hypotension was observed in the case of neurokinin β. Both neurokinins showed comparative depolarizing effect on the spinal cord preparation of new born rat in a same manner as substance P[4] as shown in Fig. 3. Moreover, the contracting activities on guinea pig ileum and the depolarizing effects on spinal cord of new born rat were inhibited by (D-Arg^1, D-Pro^2, D-Trp^{7,9} Leu^{11},)-analog of substance P which is reported recently to act as a specific antagonist against native substance P.[5]

Table 1. Pharmacological tests of neurokinins

	Relative potencies on GPI-contraction	*Sialogogic effects*[a] *(g/ 2 min)*
Neurokinin α	0.12	++ (0.102)
Neurokinin β	0.44	+ (0.049) (0.248)[b]
Substance P	1.00	+++(0.153)

a) Administrated intravenously in anaesthetized rat.
b) Much amount of salivation was observed 5 min after the administration.

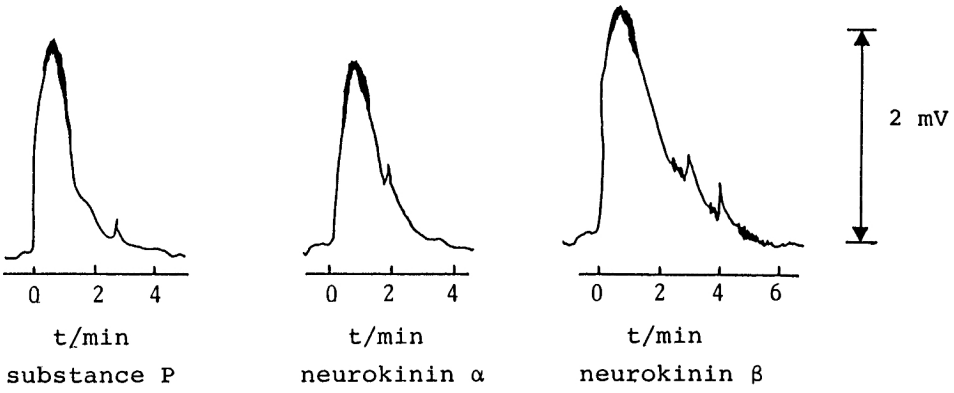

Fig. 3. Effects of substance P, neurokinin α and β on depolarization of motor neuron. The spinal cord preparation of new born rats were perfused with the media containing 3.3 x 10^{-7} M of the peptides.

As mentioned above, both neurokinin peptides possess nearly the identical pharmacological and electro-physiological properties with substance P which is already established as a neurotransmitter of primary sensory in mammalian animals,[6] although the efficiencies are not quantitatively the same.

The results of these studies suggested strongly that the newly isolated peptides, neurokinin α and β, display significant roles in central and peripheral nervous system like substance P. Detailed pharmacological and electro-physiological investigations will be described in elsewhere.

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References

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2) Abbreviations for amino acids follow the Recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (J. Biol. Chem., 247, 977(1972)). Other abbreviations used are: Boc=*t*-butyloxycarbonyl, Z=benzyloxycarbonyl, Tos=*p*-toluenesulfonyl, Bzl=benzyl, WSCI=water soluble carbodiimide (1-ethyl-3,3-dimethylaminopropyl carbodiimide), HOBt=1-hydroxybenztriazole, ONSu=succinimidoester, ONp=*p*-nitrophenylester, TFA=trifluoroacetic acid, TEA=triethylamine, NMM=N-methylmorpholine.
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