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SYNTHESIS, CONFORMATION AND OPIOID ACTIVITY OF DELTORPHINS

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Summary: A series of deltorphin analogues was prepared by solid-phase peptide synthesis. Their opioid activity was evaluated in rat opiatic assay and their conformation was determined by two-dimension Nuclear Magnetic Resonance Spectroscopy. The analogues containing D-alanine acid at position 2 were much more potent in the assay than their corresponding isomers containing L-alanine acid at this position. The conformational analysis on NMR study in DMSO showed that C-terminal tetrapeptides of both deltorphin II and its L-alanine analog might form a 3_{10} helix, which confirms that the substitution of D-amino acid at position 2 decreased the opioid activity.

Deltorphins are natural opioid peptides of dermorphin family (1,2,3), a potent υ-selective peptide isolated from frog skin (4) . They share with dermorphin the same tripeptide message sequence (Tyr-D-Ala-Phe). Dermorphin possess the highest affinity and selectivity for the υ -receptor but deltorphins for the δ -receptor. Its opioid activity, as inducing long lasting analgesia, particularly on the central nervous system, is up to 1000 folds greater than that of morphin (5,6). Deltorphins show higher affinity for δ-receptor than other opioid peptides (7), the affinity is from 10 to 200 folds higher than that of the synthetic enkephalin analogs (8).The presence of a D -amino acid has not been found yet in the peptides from animal except these peptides mentioned above. The study with clone technique suggested that the D-alanine in this series is converted from L-alanine in their precursor(1). It was reported that the main cause of υ or δ selectivity is conformational. In particular, receptor's selectivity can be traced to the conformation of the C-terminal part (9).The conformational analysis of dermorphin and deltorphins based on NMR studies in DMSO and cryoprotective mixtures had been made. From internal energy calculations show that deltorphin and dermorphin have the enormous difference in receptor selectivity interpreted on the basis of opiatic receptor models for δ and υ but they recognize the same β-turn in the N-terminal part (10).

We synthesized deltorphin I and II as well as their L-alanine analogues at position 2 with SPPS and Boc strategy. A 2D NMR study for deltorphin II and [L -Ala^2] deltorphin II in DMSO solution revealed that the conformations of C-terminal tetrapeptide of these two peptides might form a 3_{10} helix . The molecular models were established by computer treatment. The opioid activity study showed that deltorphins containing D-alanine at position 2 possessed higher potency than their corresponding isomers containing L-isomers.

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MATERIALS AND METHODS

Solid-phase peptide synthesis : All of the N^{α}-Boc protected amino acids, side chain functional groups of aspartic and glutamic acid protected by benzyl, were purchased from Bachem Inc.USA. , DCC was from Aldrich Co. . Sephadex G -10 was produced by Phamacia and MBHA resin by Bio Rad Lab.(the substitution value is 0.62 mmol per gram).These four peptides were synthesized with Merrifield's solid phase methodology.The synthesis was started from MBHA resin and each step of the synthesis was followed by Tam·s procedures (11). After completion of the synthesis, the one gram protected peptide-resin was treated with a mixture consisting of 1.5ml anisole and 10ml liquid anhydrous hydrogen fluoride at 0°C for two hours to cleave the peptide from the resin anchor as well as to deprotect the side chain protecting groups. The resulting crude peptide product was purified on Sephadex G-10. The purified peptides were characterized by analytical HPLC, amino acid analysis, sequence analysis and FAB MS.

Opioid activity :Male Wistar rats weighing 200 to 250g were anesthetized with chloral hydrate (3mg /kg). Catheters were inserted into the myelocele for injections. 24 hours after the operation, pain threshold was measured by radiation tail flick method.

2D NMR study: We got the ^{1}H -^{1}H COSY and 2D NOESY spectra by using Brucker models AM 500 spectrometer. Samples for NMR study were dissolved in DMSO-d_6. All chemical shifts are reffered to interal tetramethylane. NOESY spectra were acquired using a 800-ms mixing time.

RESULTS AND DISCUSSION

The purity of these four peptides was analysized by reverse HPLC (Figure 1). The molecular ion peaks (M+1)+ (770 and 784) of them were found from their FAB mass spectrum. Acid hydrolysis in 6N HCl followed by amino acid analysis gave the following ratio of amino acids: deltorphin I : Tyr 1.00 (1.0) Ala 1.09 (1.0) Phe 1.18 (1.0) Asp 1.07 (1.0) Val 1.54 (2.0) Gly 1.18 (1.0). [L-Ala^2]

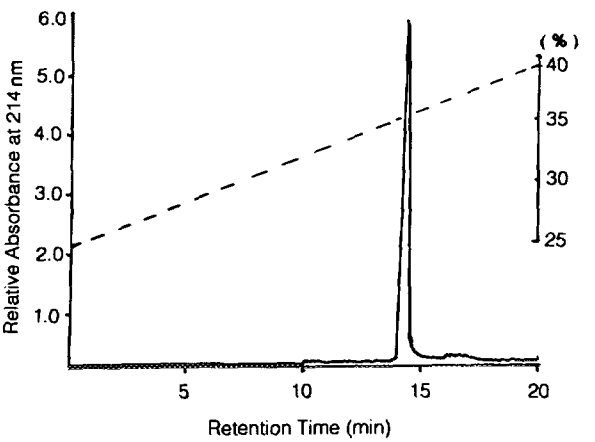

Fig. 1. RP-HPLC of deltorphin II on a cosmisil C_{18} column. Buffer A: 0.1% trifluoroacetic acid in water; Buffer B: 0.1% trifluoroacetic acid in acetonitrile.

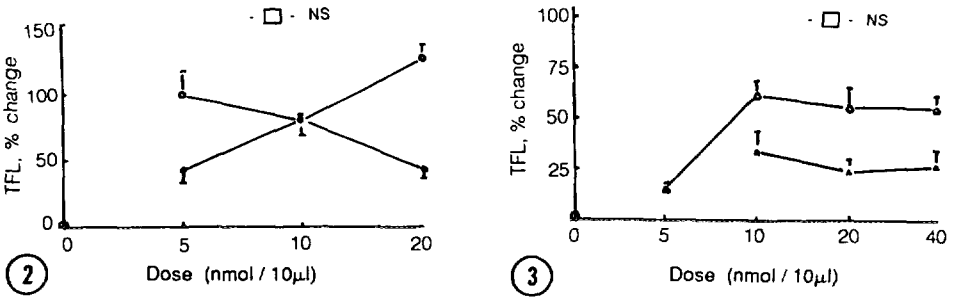

Fig. 2. The antinociceptive effect of deltorphin I (o) and [L-Ala^2] deltorphin I (•) in rats. Each point represents the mean with the SD in six to eight rats. $P<0.05$; significant difference from the solvent.

Fig. 3. The antinociceptive effect of deltorphin II (Δ) and [L-Ala^2] deltorphin II (▲) in rats. $P<0.05$; significant difference from the solvent.

deltorphin I: Tyr 1.00 (1.0) Ala 1.09 (1.0) Phe 1.07 (1.0) Asp 1.01 (1.0) Val 1.34 (2.0) Gly 1.08(1.0) Deltorphin II: Tyr 1.00 (1.0) Ala 1.09 (1.0) Phe 1.07 (1.0) Glu 1.05 (1.0) Val 1.53 (2.0) Gly 1.18 (1.0). [L-Ala^2] deltorphin II: Tyr 1.00 (1.0) Ala 1.09 (1.0) Phe 1.08 (1.0) Glu 1.47 (1.0) Val 1.91 (2.0) Gly 1.45 (1.0). The sequences of deltorphin II and [L-Ala ^{2}] deltorphin II were determined by 470A amino acid sequence analysis instrument, which were Y-A-F-E-V-V-G.

Normal saline and different doses of deltorphins were injected to the cavitas subarachnoidealis spinalis of rats respectively, tail flick latency was measured every other 10 minutes in 50 minutes. Data were obtained as the meas of five times. Deltorphins containing D-alanines at position 2 were more potency than their L-alanine containing isomers (Fig. 2, Fig. 3).

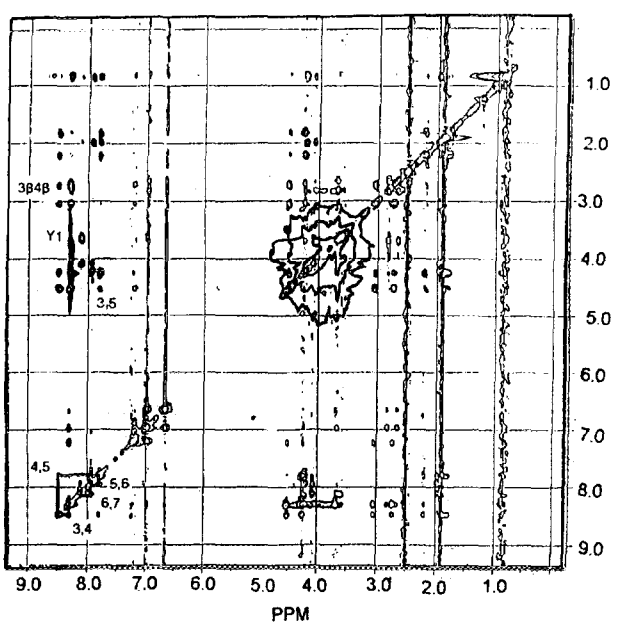

Fig. 4. NOESY spectra (acquired at 500 MHz, with mixing time 1.5 s) of deltorphin II in DMSO-d_6. Assignments are labeled by standard one-letter codes and numbers for amino acid residues.

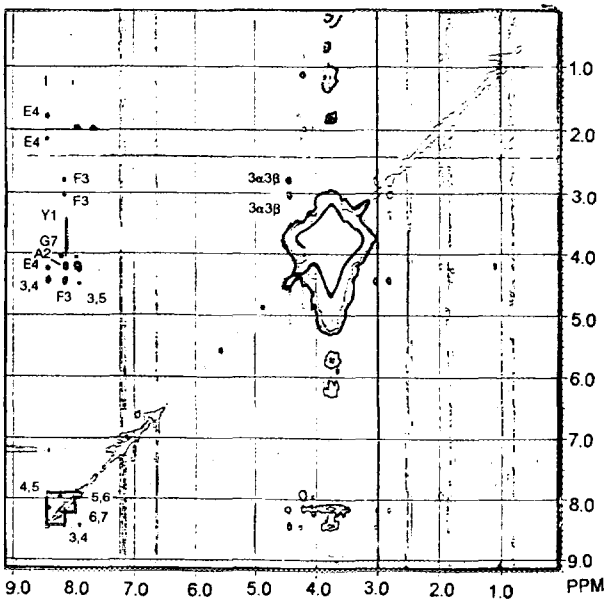

Fig. 5. NOESY spectra (acquired at 500 MHz, with mixing time 800 ms) of [L-Ala^2] deltorphin II in DMSO-d_6. Assignments are labeled by standard one-letter codes and numbers for amino acid residues.

Deltorphin II and [L-Ala^2] deltorphin II were studied in DMSO-d_6. Residue types and sequential assignments were performed by means of standard 2D techniques: ^{1}H- ^{1}H COSY and 2D NOESY (Fig. 4, 5). They are similar in NH-NH effects: F^3-E^4, E^4-V^5,V^5-V^6, V^6-G^7 **in deltorphin II and the [L-Ala^2] deltorphin II**, which suggested that folded conformations were characterized in C-terminal. Morever, the effects are probably even more numerous, since the close proximity of the diagonal peaks make it impossible to identify other significant NH-NH NOEs particularly for the N-terminal region. In

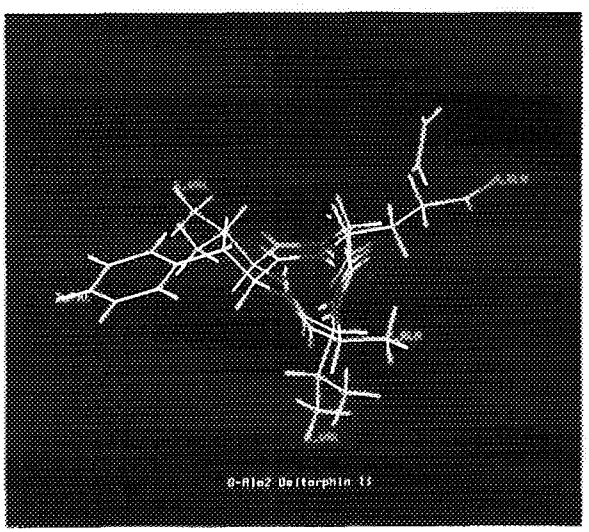

Fig. 6. Molecular models of deltorphin II by computer treatment.

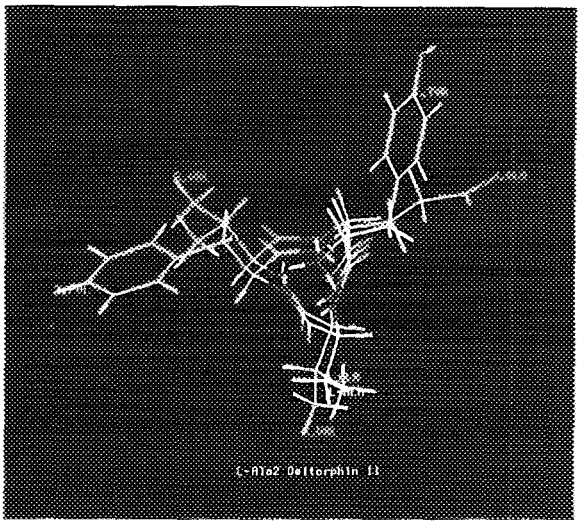

Fig. 7. Molecular model of [L-Ala^2] deltorphin II by computer treatment.

NOESY spectra, the interaction of Phe^3 C$^\alpha$-H and Val^5 NH were also found which is the characterization of 3_{10} helix. So that, the C-fragment tetrapeptide of deltorphins might form 3_{10} helix structure. The molecular models of these peptides constructed by conmputer treatment were shown in Fig. 6 and 7.

The bioactive study demonstrates that these four peptides all have opiatic activity conmparing with morphine (12). [D-Ala^2]deltorphin I and [D-Ala^2] deltorphin II are more potent than L-alanine containing isomers. However, the conformations of deltorphin II and [L-Ala^2]deltorphin II are almost the same at the C-terminal, both of which form 3_{10} helix structures at the C-terminal tetrapeptide. The only difference is the configuration of alanines at position 2. This fact reveals that N terminal of the peptide might be the active part in bioactivity and the configuration of alanine at position 2 is very important for the opiatic activity.

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