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# Structural Characterization of β-Endorphin through the Design, Synthesis, and Study of Model Peptides

JOHN W. TAYLOR,[1] RICHARD J. MILLER,[2] AND E. T. KAISER[1,3]

*Department of Chemistry and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, Illinois 60637*

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SUMMARY

Analysis of the amino acid sequence of β-endorphin reveals a potential amphiphilic α- or π-helix in the COOH-terminal region, residues 13–29. We have proposed that the biological properties of β-endorphin are determined by the connection of a highly specific opiate receptor recognition sequence at the $NH_2$ terminus, having the same sequence as (Met[5])enkephalin, to this amphiphilic helical structure at the COOH terminus, via a hydrophilic peptide link [Taylor *et al. J. Am. Chem. Soc.* **103**:6965 (1981)]. This proposal was investigated by studying peptides 1 and 2, two 31-amino acid analogues of β-endorphin designed to retain the general characteristics of the amphiphilic helix postulated for β-endorphin, but using amino acid sequences with minimal homology to the natural sequence in that region. In addition, peptide 2 has minimal homology to β-endorphin in the proposed hydrophilic linking region, so that peptide 2 residues 6–31 have only four residues homologous to the corresponding residues in the natural sequence. Both peptide 1 and peptide 2 had 50% α-helical structure in 50% aqueous 2,2,2-trifluoroethanol, compared with 40% found for β-endorphin, and both model peptides exhibited an even greater resistance to proteolysis in rat brain homogenates than did β-endorphin. Peptide 1 had higher affinities for δ- and μ-receptors than did β-endorphin, while retaining the same δ/μ selectivity, and had a greater potency than did β-endorphin in opiate assays on guinea pig ileum. In opiate assays on rat vas deferens, which are highly specific for β-endorphin, peptide 1 had the same activity ($IC_{50}$ = 61 ± 12 nM) as did β-endorphin ($IC_{50}$ = 52 ± 3 nM). These results strongly support the importance of an amphiphilic helical structure in β-endorphin residues 13–29 which contributes to the opiate receptor interactions and determines the resistance to proteolysis of the whole molecule. Peptide 2 showed δ-receptor binding and guinea pig ileum activities similar to those of β-endorphin, but behaved quite differently in μ-receptor binding assays (in Tris buffer at 25°) and displayed a somewhat weaker activity on rat vas deferens ($IC_{50}$ = 450 ± 15 nM). This suggests that the hydrophilic linking region of β-endorphin (residues 6–12) plays a role in determining its opiate receptor selectivity.

## INTRODUCTION

Amphiphilic secondary structural features have been hypothesized to play a major role in determining the biological and physical properties of a variety of peptides which bind to lipid or membrane surfaces (1, 2). Plasma apo A-I,[4] for instance, is thought to contain 6 highly homologous 22-amino acid domains which have a high potential to form amphiphilic α-helices (3, 4). In a stringent test of the importance of these amphiphilic α-helical segments, a model amphiphilic helical docosapeptide having minimal homology to apo A-I was synthesized and shown to possess the fundamental characteristics of apo A-I, including the ability to activate lecithin:cholesterol acyltransferase (5, 6). Following related considerations, it was shown that the 20 $NH_2$-terminal amino acid residues of melittin, a cytotoxic peptide from bee venom, have a purely structural role which can be duplicated by a nonhomologous sequence of high α-

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[1] Department of Chemistry.

[2] Department of Pharmacological and Physiological Sciences.

[3] Present address, The Rockefeller University, New York, N. Y. 10021.

[4] The abbreviations used are: apo A-I, apolipoprotein A-I; CPK, Corey-Pauling-Koltun; [$^3$H]DADL, ([3,5-$^3$H]Tyr[1], D-Ala[2], D-Leu[5])-enkephalin; [$^3$H]DHM, [1,7,8-$^3$H]dihydromorphine; HPLC, high-pressure liquid chromatography; Boc, $N^{\alpha}$-*tert*-butyloxycarbonyl; TFE, 2,2,2-trifluoroethanol; GPI, guinea pig ileum; RVD, rat vas deferens.

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helix-forming ability and of the proper hydrophobic-hydrophilic balance. In view of these findings and the limited possibilities of well-defined secondary structures for peptides, it was suggested that amphiphilic helical segments could be of importance in hormones as well (7). In considering which hormones might contain amphiphilic helical regions, we have focused our attention on peptides that are at least 20 amino acids in length and that do not contain multiple disulfide bridges. Human β-endorphin (Fig. 1) is a 31-amino acid peptide hormone with potent opiate activities (8, 9) where the structural approach earlier employed for the design of models for apo A-I and melittin appeared attractive.

The first five residues at the $NH_2$ terminus of β-endorphin are identical with (Met[5])enkephalin, which has opiate activities of its own (10, 11). Studies of β-endorphin analogues having single amino acid residue deletions indicate that the (Met[5])enkephalin region has highly specific interactions with opiate receptors, whereas residues 6 through 31 do not (12). An examination of the β-endorphin sequences from a variety of species leads to the same conclusion, since residues 1 through 5 are conserved, but the remainder of the peptide shows a number of variations (13). Several types of structures have been suggested for residues 6 through 31 of β-endorphin (12, 14, 15), and this region of the molecule has been implicated in the specific recognition of receptors (12, 16), the modification of the receptor selectivity of the enkephalin region (17, 18), and the protection of the enkephalin region from enzymatic degradation (14). Thus, we have undertaken an examination of structure-activity relationships in β-endorphin by designing, preparing, and characterizing model peptides containing the (Met[5])enkephalin sequence at their $NH_2$ termini and retaining the general characteristics of structural units hypothesized for residues 6 through 31 of the naturally occurring peptide, using nonhomologous sequences.

By inspection of CPK models of β-endorphin arranged with the residues between the helix breakers (19) Pro[13] and Gly[30] in either an α-helical or a π-helical form (20), we have previously identified a potential hydrophobic domain in the β-endorphin structure which is not readily apparent from simple examination of the primary sequence (15). These structures are represented in 2-dimensional form on an α-helical and a π-helical net (21) in Fig. 2. In the α-helical arrangement, residues 13 through 29 form an amphiphilic structure, where the hydrophobic domain covers one-half of the surface of the helix and twists around the length of the helix, and the hydrophilic residues are either neutral or basic. In a π-helix, these residues form a similar amphiphilic structure, except that the hydrophobic domain now runs straight along the length of the helix. In view of our observations employing peptide models of apo A-I and melittin, showing that similar amphiphilic helical structures can interact with protein or phospholipid surfaces, these features of the β-endorphin structure are of considerable interest. In β-endorphin, the importance of lipid interactions in stabilizing helical structure on the opiate receptor has been suggested (22, 23), and opiate activities have been correlated to α-helicity in COOH-terminal deletion analogues (18). Furthermore, it is likely that an amphiphilic

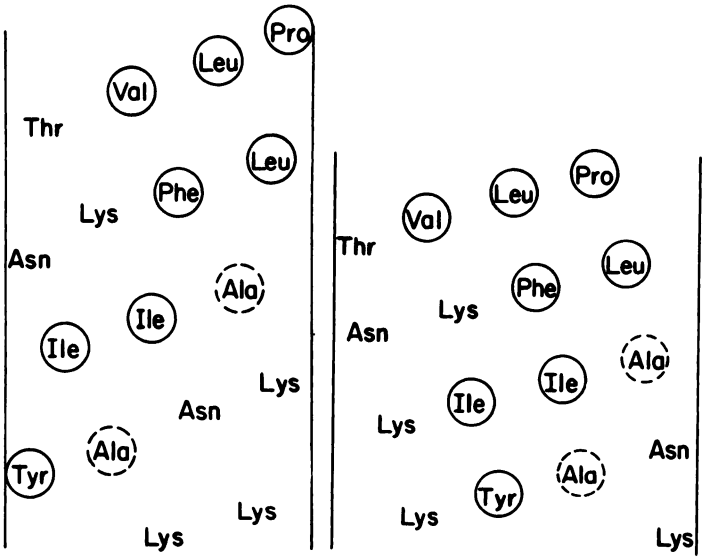


FIG. 2. *Residues 13 through 29 of human β-endorphin represented in an α-helical net (left) and a π-helical net (right) (21)*

Hydrophobic residues are *circled*. The flexibility of the lysine side chains allows formation of a continuous hydrophobic domain, which twists around the length of an α-helix and lies straight along the length of a π-helix.

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                                    5                   10
β-Endorphin H2N-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-

Peptide 1   H2N-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-

Peptide 2   H2N-Tyr-Gly-Gly-Phe-Met-Ser-Gly-Ser-Gly-Ser-Gly-Ser-Pro-

     15                  20                  25                  30
Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH

Leu-Val-Thr-Leu-Phe-Lys-Gln-Leu-Leu-Lys-Gln-Leu-Gln-Lys-Leu-Leu-Gln-Lys-OH

Leu-Leu-Gln-Leu-Trp-Gln-Lys-Leu-Leu-Lys-Gln-Leu-Gln-Lys-Leu-Leu-Gln-Lys-OH
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FIG. 1. *Amino acid sequences of human β-endorphin, peptide 1, and peptide 2*

helical structure in β-endorphin could protect the molecule from proteolytic degradation, either through intramolecular hydrophobic interactions with the enkephalin region or by intermolecular interactions.

Residues 6 through 12 of human β-endorphin are not hydrophobic and have little propensity for formation of secondary structure (19). The length and the hydrophilic nature of the peptide chain between the enkephalin residues and Pro[13] are conserved in a variety of species for which β-endorphin has been sequenced, although several of these residues are variant (13). On this basis, we have suggested that residues 6 through 12 serve as a spacing link between the specific enkephalin sequence and the putative amphiphilic helical region (15). However, little information is available on exactly how the character of this region might affect the properties of β-endorphin, even though some β-endorphin analogues with variations in the sequences of residues 6 through 12 have been studied (12, 24).

We present here the design, synthesis, and properties of peptides 1 and 2, two peptide models of β-endorphin possessing sequences designed to test the importance of the structural considerations outlined above. Some initial studies on peptide 1 have already been reported which strongly support a role for the amphiphilic helical structure that we have described in determining the receptor binding properties and resistance to proteolysis exhibited by β-endorphin (15). The conclusions drawn by us have more recently been corroborated by receptor binding studies of COOH-terminal deletion analogues of β-endorphin (25) and are extended to other opiate activities of β-endorphin in the work we now describe. The properties of peptide 2 also support our analysis of the results obtained for peptide 1 and, in addition, provide information on the role of the proposed spacer region in determining the receptor selectivity of β-endorphin.

## MATERIALS AND METHODS

[$^3$H]DADL (25–35 Ci/mmole) was obtained from New England Nuclear Corporation (Boston, Mass.), and [$^3$H]DHM (73 Ci/mmole) was obtained from Amersham Corporation (Arlington Heights, Ill.). Human β-endorphin was purchased from Peninsula Laboratories (San Carlos, Calif.) or Beckman Instruments (Schiller Park, Ill.) and was homogeneous by reversed-phase HPLC. (Met$^5$)Enkephalin was purchased from United States Biochemical Corporation (Cleveland, Ohio), and (D-Ala$^2$,D-Leu$^5$)enkephalin was a gift of the Wellcome Research Laboratories (Research Triangle Park, N. C.). Morphine and naloxone were gifts of Bruce Wainer (Department of Pediatrics, University of Chicago), and bacitracin was obtained from Sigma Chemical Company (St. Louis, Mo.).

*Peptide synthesis and purification.* The solvents and reagents used for synthesis of peptides 1 and 2 were purified by the methods described previously (26). Boc derivatives of amino acids used in the syntheses were purchased from Bachem (Torrance, Calif.), United States Biochemical Corporation, or Chemical Dynamics (South Plainfield, N. J.). These were as follows: L-glutamic acid, γ-benzyl ester; L-glutamine; glycine; L-leucine; $N^{\epsilon}$-2-chlorobenzyloxycarbonyl-L-lysine; L-methionine; L-phenylalanine; L-proline; *O*-benzyl-L-serine; *O*-benzyl-L-threonine; $N^{i}$-formyltryptophan; 2,6-dichloro-*O*-benzyl-L-tyrosine; and L-valine.

Chloromethylated styrene-divinyl benzene copolymer (0.2 mmole/g) was prepared (27) using Bio-Beads SX-1 (200–400 mesh) purchased from Bio-Rad Laboratories (Richmond, Calif.), and the chloromethylated resin was then esterified (0.08 mmole/g) by reaction with the lysine derivative in the presence of anhydrous KF (28). Synthesis of the fully protected peptide 1 from this esterified resin (1.99 g) then proceeded according to published methods (29). In the synthesis of peptide 2, 1% cross-linked chloromethylated styrene-divinyl benzene copolymer (0.67 mmole/g) was purchased from United States Biochemical Corporation and esterified (0.23 mmole/g) using the lysine derivative and anhydrous KF. The synthesis of peptide 2 then proceeded by the same methods as for peptide 1, using 4.00 g of this esterified resin.

Both peptides were cleaved from their polymeric supports and deprotected by reaction with anhydrous HF in the presence of anisole at 0° (30). The peptides were then extracted from the peptide and resin mixtures with aqueous acetic acid, and the extracts were lyophilized.

Peptide 1 was purified by gel permeation and ion-exchange and partition chromatographies as previously described (31). The overall yield of peptide 1 was 8% on the basis of the initial substitution level of the esterified resin. After extraction from the resin, the crude ($N^{i}$-formyl-Trp$^{18}$) peptide 2 was eluted from a Sephadex G-15 column with 0.2 M acetic acid. The peptide-containing fractions that eluted near the void volume were lyophilized and then treated with 5% (w/v) dithiothreitol in 0.02 M potassium phosphate buffer (pH 6.8) for 6 hr at room temperature. This material was subjected to ion-exchange chromatography on CM-Sephadex C-25 [0.05 M sodium borate buffer (pH 9.0) containing 1 mM dithiothreitol], using a linear gradient of 0.1–0.4 M NaCl. A single major peak which eluted near the end of the gradient was collected and desalted. The protected peptide (2 mg/ml) was then deformylated in 0.2 M aqueous piperidine at 0° (31). This reaction was quenched after 15 min by the addition of 5 ml of glacial acetic acid. Further purification of peptide 2 was achieved by reversed-phase HPLC on a DuPont Zorbax $C_{18}$ semi-preparative column. The deformylated peptide (12 mg) was loaded onto the column in 1 ml of 0.2 M sodium phosphate buffer (pH 2.5) at a flow rate of 2.5 ml/min. Peptide 2 was then eluted as a major peak in the absorbance at 280 nm, near the middle of a gradient of 40–43% (v/v) acetonitrile over 1 hr in the same buffer at the same flow rate. Under these conditions, baseline separation of the formylated and deformylated peptide 2 was achieved. After desalting on Sephadex G-15, the highly purified peptide 2 was obtained in 2% over-all yield based on the initial Boc-Lys-resin.

Thin-layer chromatographs on cellulose, developed with 1-butanol/acetic acid/water/pyridine (15:3:12:10) and visualized by ninhydrin, showed single spots for both peptide 1 ($R_F$ = 0.57) and peptide 2 ($R_F$ = 0.53). Analytical HPLC of peptide 1 on a DuPont Zorbax $C_{18}$ column, eluting with a mixture of 60% (v/v) acetonitrile in 0.1 M

sodium perchlorate containing 0.1% (v/v) phosphoric acid, showed a major symmetrical peak at 210 nm ($K'$ = 6.3) and minor impurities, none of which gave greater than 2% of the total absorbance. Analytical HPLC of peptide 2, on the same column, showed a symmetrical peak in the absorbance at 210 nm ($K'$ = 8.3) with no detectable impurities, when the peptide was eluted with 45% (v/v) acetonitrile in 0.2 M sodium phosphate buffer (pH 2.5). Both peptides were analyzed for their amino acid content after hydrolysis in 5.5 M HCl at 110° for 24 hr. The results were, for peptide 1, Gly (2) 1.90, Glu (6) 6.02, Leu (7) 7.00, Lys (5) 5.02, Met (1) 0.76, Phe (2) 1.99, Pro (1) 0.94, Ser (2) 1.35, Thr (3) 2.45, Tyr (1) 0.82, Val (1) 1.01; and for peptide 2, Gly (5) 4.83, Glu (5) 5.23, Leu (8) 8.00, Lys (4) 4.06, Met (1) 0.90, Phe (1) 0.85, Pro (1) 1.15, Ser (4) 3.29, Trp (1) small quantity, Tyr (1) 0.95. Both peptide 1 and peptide 2 gave the expected sequences by Edman degradation, and no previewed residues were detected.

*Conformation studies in solution.* Apparent molecular weights were determined for peptide 1 and peptide 2 by the method of Pollet *et al.* (32), using a Beckman Spinco Airfuge. Peptide solutions ($5.0 \times 10^{-5}$ M) in 0.02 M sodium phosphate buffer (pH 7.4) containing 0.16 M KCl and 5 mg/ml Dextran T40 (Pharmacia Fine Chemicals, Piscataway, N. J.) were centrifuged at 84,800 rpm for 20 hr. Peptide concentrations were determined by fluorescamine assay.

CD spectra of solutions of peptide 1, peptide 2, and β-endorphin in 0.02 M sodium phosphate buffer (pH 7.4) containing 0.16 M KCl, or in the same buffered salt solutions containing 50% (v/v) TFE, were measured from 200 nm to 250 nm using a Cary 60 spectropolarimeter.

*Opiate receptor binding.* The affinities of peptides 1 and 2 for brain opiate receptors were compared with those of β-endorphin by determining their abilities to inhibit the specific binding of [$^3$H]DADL (0.5–1.0 nM) or [$^3$H]DHM (0.2–0.4 nM) to guinea pig whole-membrane preparations. The procedure was essentially the same as previously described (33). The tritiated opiates, peptides, and membranes were incubated in 1 ml of 0.05 M Tris-HCl (pH 7.4 at 25°) for 50 min. The activities of peptide 1 or peptide 2 were compared with those of β-endorphin in the same experiments, and all measurements were made in triplicate. Nonspecific binding of [$^3$H]DADL was determined in the presence of $8.0 \times 10^{-5}$ M (D-Ala$^2$,D-Leu$^5$)enkephalin, and that of [$^3$H]DHM was determined in the presence of $8.0 \times 10^{-5}$ M morphine.

In additional binding assays, the inhibitory effect of each peptide on [$^3$H]DHM binding in 0.05 M Tris-HCl (pH 7.4 at 25°) was compared directly with its inhibitory effect in Krebs-Ringer solution with added glucose (11 mM) at 37°, using the same membrane preparation and [$^3$H]DHM concentration.

*Opiate assays on the guinea pig ileum and rat vas deferens.* GPI (34) and RVD (35) opiate assays were performed according to established procedures, using white female Hartley guinea pigs (400–500 g) purchased from Lock Erickson, and white male Lewis rats (250–300 g) purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Assays were performed in 10-ml or 25-ml organ baths containing Krebs-Ringer solution with added glucose (11 mM) and choline chloride (20 μM), bubbled slowly with 95% $O_2$-5% $CO_2$, and kept at 37.0 ± 0.5°. A platinum ring anode at the top of the bath and a platinum hook cathode at the bottom of the bath were used for electrical stimulation. In the GPI assays, 3-cm portions of ileum were isolated from the small intestine between 10 and 20 cm proximal to the ileocecal valve and washed. These whole segments were then suspended between the electrodes at 0.3 g of tension and subjected to electrical pulses of 0.6-msec duration at 80 V and 0.1 Hz. In the RVD assays, the isolated tissues were gently pressed to remove the semen, then suspended between the electrodes at 0.2 g of tension and stimulated with 0.6-msec electrical pulses at 60 V and 0.1 Hz. After 30–60 min of stimulation with frequent washings, followed by a period of 30 min to allow the tissues to equilibrate, peptides 1 and 2 and β-endorphin were tested for their abilities to inhibit the electrically stimulated contractions. Because of the difficulty of washing out the added peptides completely, each tissue preparation was used for only one assay. Peptides were added in increasing doses, allowing the tissue contractions to reach a new equilibrium after each addition. The time allowed for re-equilibration was kept to a minimum in assays of β-endorphin to reduce possible enzymatic degradation (35), and was always less than 5 min. In contrast, tissues were much slower to respond fully to additions of peptide 1 or peptide 2, and the time allowed for re-equilibration was frequently greater than 20 min, since no indication of a loss of potency due to degradation of these peptides was observed. After the maximal inhibitory effect of each peptide had been determined, tissues were tested for their ability to recover upon addition of $1 \times 10^{-5}$ M naloxone.

*Resistance to proteolysis.* The relative resistances of peptide 1, peptide 2, β-endorphin, and [Met$^5$]enkephalin toward degradation by proteolytic enzymes endogenous to rat brain were determined by incubating the peptides separately with portions of whole rat brain homogenate in 0.05 M Tris-HCl (pH 7.4 at 37°). In these suspensions, the peptide concentrations were $1.0 \times 10^{-5}$ M, and the rat brain homogenate was 8-fold diluted from one complete brain homogenized in 10 ml of the Tris buffer. After various incubation times from 0 to 90 min, 10-μl aliquots were removed from mixtures duplicated for each peptide and immediately frozen and stored at −80°. These aliquots were subsequently assayed for opiate receptor binding activity by the method described above, using [$^3$H]DADL in the presence of 0.05% (w/v) bacitracin as an inhibitor of proteolytic activity.

Alternatively, the rat brain homogenates were analyzed specifically for peptide 1, peptide 2, or β-endorphin by HPLC. In these experiments, 1-ml aliquots were removed from the incubation mixtures and placed in capped tubes in a boiling water bath for 10 min. These samples were then centrifuged briefly to remove most of the particulate matter, and the supernatants were frozen. The supernatants were analyzed by loading 200 to 700-μl aliquots onto a DuPont Zorbax $C_{18}$ column, fitted with a guard column and equilibrated with 0.02 M sodium phosphate buffer (pH 2.5) containing 20% (v/v) acetonitrile. Peptides were eluted with a gradient of 20–50% (v/

v) acetonitrile over 60 min, followed by isocratic elution at 50% (v/v) acetonitrile, in the same buffer at a flow rate of 1.2 ml/min. Elution times after the start of the gradient were as follows: β-endorphin, 28 min; peptide 2, 60 min; peptide 1, 64 min. Peptides were quantitated by the height of their absorbance peaks at 210 nm, relative to those of standard samples of the same peptide in water.

## RESULTS

*Design of the model peptides.* At the outset of our work, because the principles for design of a π-helix and the properties of such a helix had not yet been determined, we began our β-endorphin modeling studies by synthesizing two 31-amino acid peptides, each of which was designed so that residues 14 through 31 might form an amphiphilic α-helix which had a hydrophobic domain lying straight along one side of the helix (Fig. 3).

Peptide 1 has the same amino acid sequence as β-endorphin from residue 1 through residue 19, but has no sequence homology from residue 20 through residue 31 (Fig. 1). In the latter region, peptide 1 employs leucines as hydrophobic residues, glutamines as neutral hydrophilic residues, and lysines as basic hydrophilic residues. These residues were chosen to optimize the potential for α-helix formation (19) while retaining the basicity, the approximate hydrophobic-hydrophilic balance, and the potential amphiphilicity of β-endorphin residues 14 through 31.

Peptide 2 was designed to be a complete structural model of β-endorphin. Only the (Met$^5$)enkephalin region (residues 1 through 5) as the specific opiate receptor recognition site, and Pro$^{13}$ as a helix-breaking residue, are retained from the natural sequence of β-endorphin (Fig. 1). The three additional sequence homologies (Ser$^{10}$, Leu$^{14}$, and Leu$^{17}$) are coincidental to the design of peptide 2. Residues 6 through 12 consist of alternate serine and glycine residues, chosen to mimic the hydrophilic spacer region proposed for the corresponding β-endorphin residues. This sequence should form a flexible hydrophilic peptide chain with little propensity for secondary structure formation (19). Residues 14 through 31 were selected to allow formation of an amphiphilic α-helix in peptide 2 as in peptide 1 (Fig. 3). Again, the basicity of the hydrophilic domain and the size of the hydrophobic domain in the amphiphilic helical region postulated for β-endorphin (Fig. 2) are retained in peptide 2, using leucine, glutamine, and lysine residues for their high α-helix-forming potential. In addition, a single tryptophan residue was placed in position 18 to allow the possibility of intrinsic fluorescence studies in the future. Peptide 2 has an amino acid sequence identical with that of peptide 1 for residues 21 through 31, and considerable similarity in residues 13 through 20.

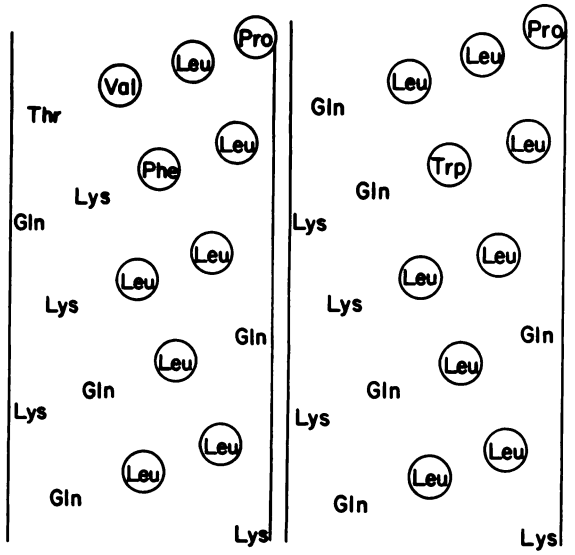


FIG. 3. *Residues 13 through 31 of peptide 1 (left) and peptide 2 (right), represented on an α-helical net (21)*

The hydrophobic residues are *circled* and form a hydrophobic domain lying straight along the length of the α-helix in each case.

*Peptide synthesis and purification.* Peptides 1 and 2 were synthesized by standard solid-phase methods. Both peptides were initially purified by gel permeation and ion-exchange chromatography. Peptide 1 was further purified by partition chromatography, yielding a final product that showed a single spot upon thin-layer chromatography, had the expected amino acid composition, and showed the correct sequence by Edman degradation with no previewed residues. Analytical reversed-phase HPLC of peptide 1 revealed one major symmetrical peak and several minor impurities, none of which had greater than 2% of the total absorbance at 210 nm. Peptide 2 was further purified by reversed-phase HPLC. The final product was homogeneous upon thin-layer chromatography and analytical HPLC, and gave the amino acid composition expected for peptide 2 and the correct sequence without previews by Edman degradation.

*Conformation studies in solution.* Molecular weight determinations were made for $5.0 \times 10^{-5}$ M solutions of peptide 1 and peptide 2, in buffered salt solution at pH 7.4, by the method of Pollet *et al.* (32). Peptide 1 yielded a molecular weight of 17,030 ± 1,180, which corresponds to 4.73 ± 0.33 times the monomer molecular weight. This result suggests a higher state of aggregation for peptide 1 than the trimerization reported previously (15). Peptide 2 gave a molecular weight of 13,580 ± 360, or 3.95 ± 0.25 times the monomer molecular weight, indicating that this peptide also exists in a self-associated form under these conditions. In contrast, β-endorphin is monomeric at $4.0 \times 10^{-5}$ M (15).

In the same aqueous buffered salt solution, the CD spectra of both peptide 1 and peptide 2 showed minima at 210 and 222 nm, indicative of α-helical structure (36). A concentration-dependent increase in the mean residue ellipticity, $\theta$ at 222 nm, consistent with self-association and a concomitant stabilization of the α-helical structure, was observed at concentrations greater than $2.0 \times 10^{-7}$ M for peptide 1 and $3.0 \times 10^{-6}$ M for peptide 2. Below these concentrations, $[\theta]_{222}$ was approximately −9,000 deg cm$^2$/dmole for peptide 1 and − 11,000 deg cm$^2$/dmole for peptide 2. From these values, we calculate about 30% α-helix for monomeric peptide 1 and about 35% α-helix for monomeric peptide 2 (37).

The CD spectrum of β-endorphin under these conditions is more typical of a random coil structure (36), although a helix content of 12% can be calculated from $[\theta]_{222} = -1{,}800$ deg cm$^2$/dmole. However, helical struc-

ture is readily induced in β-endorphin by the addition of TFE, and distinct minima appear in the CD spectra at 208 and 222 nm (14). In 50% TFE, we have found the CD spectra of β-endorphin and peptides 1 and 2 to be very similar, and from $[\theta]_{222}$ we calculate 40% α-helix for β-endorphin and 50% α-helix for the model peptides.

*Opiate receptor binding.* The affinities of peptides 1 and 2 for different opiate receptors were investigated using guinea pig brain whole-membrane preparations in Tris buffer (pH 7.4 at 25°). The abilities of the model peptides to inhibit binding of [$^3$H]DADL or [$^3$H]DHM were compared directly with those of β-endorphin in the same assays. At the concentrations used in these studies, [$^3$H]DADL should label δ-receptors selectively and [$^3$H]DHM should label μ-receptors selectively (38, 39).

The binding potencies relative to β-endorphin are listed in Table 1. Peptide 1 shows stronger binding to each type of receptor than does β-endorphin, but the μ/δ receptor binding selectivity is similar. Peptide 2 was 60 times more potent than β-endorphin in its affinity for μ-receptors, but was slightly less potent in the δ-receptor binding assay.

*Opiate activities on GPI and RVD.* The opiate activities of β-endorphin, peptide 1, and peptide 2 on isolated GPI and RVD preparations in Krebs-Ringer solution at 37° were compared. All three peptides were capable of inhibiting greater than 80% of the electrically stimulated contractions in both muscle preparations. Furthermore, these effects were completely reversed by addition of the opiate antagonist naloxone, indicating that all of the peptides were acting directly on opiate receptors. However, the model peptides differed from β-endorphin in the time courses of their actions on these muscles. GPI and RVD muscle contractions reached new minima approximately 20 min after the addition of peptide 1 or peptide 2 in amounts near the $IC_{50}$ values of these peptides. In contrast, the full inhibitory effect of adding any amount of β-endorphin was achieved within 5 min on either type of muscle. Furthermore, on RVD the effects of β-endorphin concentrations near its $IC_{50}$ last a relatively short time, and contractions return to normal within 1 hr. This behavior has been attributed to degradation of β-endorphin by enzymes present in RVD tissue (35). No such reversal behavior was detected for peptide 1 or peptide 2, even at low concentrations, over periods of up to 2 hr. These effects are illustrated in Fig. 4.

TABLE 1

*Relative potencies of β-endorphin, peptide 1, and peptide 2 in radioreceptor binding assays using guinea pig brain whole membrane preparations in Tris buffer at 25°*

| | Relative potency[a] | |
|---|---|---|
| | Displacement of [$^3$H DADL] (δ-receptor ligand)[b] | Displacement of [$^3$H DHM] (μ-receptor ligand)[b] |
| β-Endorphin | 1[c] | 1[d] |
| Peptide 1 | 2.2 | 2.9 |
| Peptide 2 | 0.6 | 60 |

[a] Potencies are relative to that determined for β-endorphin in the same assay, using the relationship: potency = $IC_{50}$ (β-endorphin)/$IC_{50}$ (peptide).

[b] At the concentrations used, [$^3$H]DADL selectively labels δ-receptors and [$^3$H]DHM selectively labels μ-receptors.

[c] In the presence of 0.47 nM [$^3$H]DADL, $IC_{50}$ (β-endorphin) = 35 nM.

[d] In the presence of 0.29 nM [$^3$H]DHM, $IC_{50}$ (β-endorphin) = 200 nM.

$IC_{50}$ values for all three peptides in both assays are presented in Table 2. In the GPI assay, peptide 1 was 5 times more potent than β-endorphin, and peptide 2 was about one-half as potent as β-endorphin. Since GPI activities of opiate agonists usually correspond well to their affinities for μ-receptors, these results were surprising. In particular, the potency of peptide 2 was 60 times that of β-endorphin in the μ-receptor binding assay, but only one-half that of β-endorphin in the GPI assay. However, Tris buffer at 25° had been employed in the μ-receptor binding assays, and the GPI assay was performed in Krebs-Ringer solution at 37°. Therefore, these findings led us to investigate the effect of buffer and temperature on the binding data (*vide infra*).

On RVD, the opiate activity of peptide 1 was very similar to that of β-endorphin, and peptide 2 was about 9 times less potent. No correlation of RVD activities to a receptor binding assay has yet been established.

*Effect of buffer and temperature on μ-receptor binding.* The potencies of peptides 1 and 2 and β-endorphin for displacing [$^3$H]DHM from guinea pig brain whole membranes in Tris buffer at 25° were compared with their potencies in the same assay conducted in the modified Krebs-Ringer solution at 37°. The $IC_{50}$ values are shown in Table 3 together with the potency ratios of each peptide under the different conditions. The $IC_{50}$ of peptide 1 in Krebs-Ringer solution at 37° is essentially the same as that in Tris buffer at 25°, whereas β-endorphin and peptide 2 are 4 and 60 times less potent, respectively, in Krebs-Ringer solution at 37°. Thus, the potencies of these three peptides in the GPI assay correspond more closely to their binding to μ-receptors if identical buffer and temperature conditions are employed (Krebs-Ringer solution at 37°).

*Resistance to proteolysis.* The resistances of peptides 1 and 2 to the actions of proteolytic enzymes endogenous to rat brain were compared with those of β-endorphin and (Met$^5$)enkephalin. Equal concentrations ($1.0 \times 10^{-5}$ M) of peptide 2, β-endorphin, or (Met$^5$)enkephalin were incubated in rat brain homogenate at 37°. Aliquots of these mixtures were assayed after various incubation times for their opiate receptor binding activities. After 90 min, the incubation mixtures containing peptide 2 still retained full binding activity. β-Endorphin lost all receptor binding activity after 70 min, and (Met$^5$)enkephalin was completely degraded in less than 5 min. These results are shown in Fig. 5. In an identical experiment previously reported (15), peptide 1 gave the same results as peptide 2.

In another experiment, mixtures of rat brain homogenate containing $1.0 \times 10^{-5}$ M β-endorphin, peptide 1, or peptide 2 were incubated at 37° for 0, 30, or 60 min, then boiled for 10 min and centrifuged. After centrifugation, the supernatants were analyzed by HPLC for β-endorphin, peptide 1, or peptide 2. The results, shown in Fig. 6, are qualitatively the same as those found on analysis of the mixtures by receptor binding activities, and show that the concentrations of peptides 1 and 2 are constant, whereas β-endorphin is slowly degraded. This rules out the possibility that peptides 1 and 2 are degraded to form

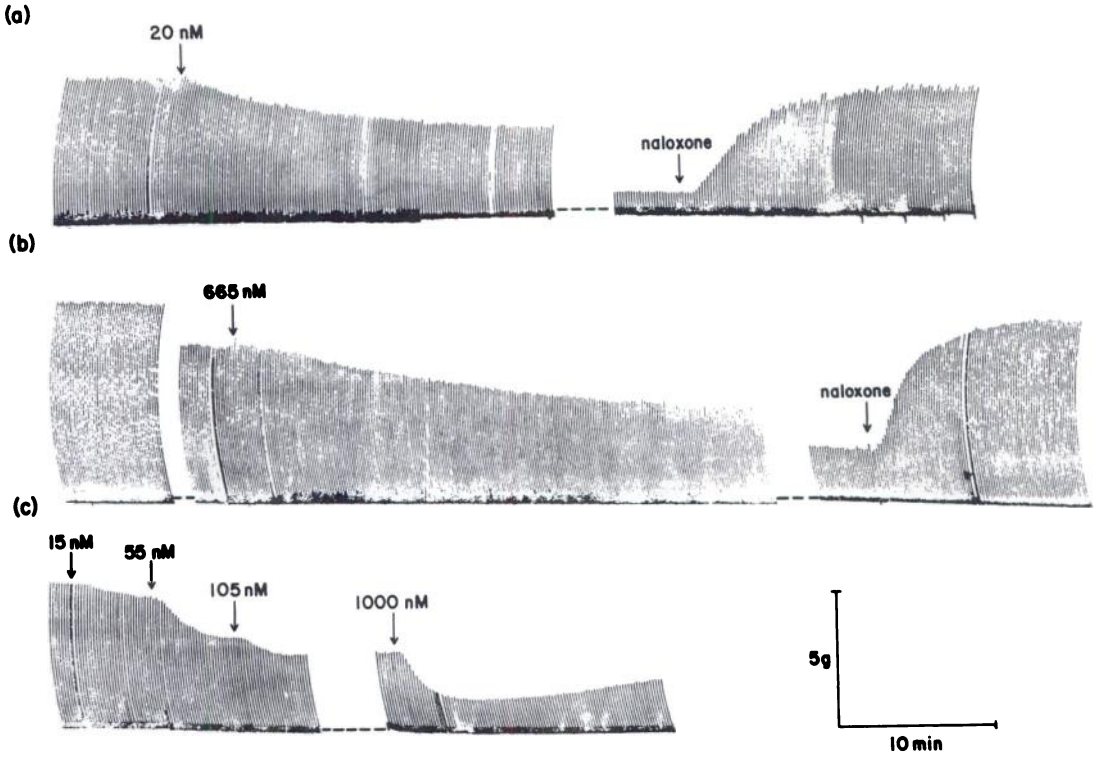


FIG. 4. *Rates of inhibitory actions of peptides on RVD*

a, Initial application of peptide 1 (20 nM) and subsequent naloxone reversal of the effect of peptide 1 (1680 nM) on the same tissue. b, Initial equilibrated contractions, followed by the effect of an increase in peptide 2 concentration (165 nM to 665 nM) near the $IC_{50}$ and subsequent naloxone reversal of the effect of peptide 2 (4165 nM) on the same tissue. c, Effect of stepwise increases in β-endorphin concentration, followed by the later application of 1000 nM β-endorphin to the same tissue demonstrating subsequent degradation of β-endorphin (35).

products which also have opiate receptor binding activities.

Finally, β-endorphin ($1.0 \times 10^{-5}$ M) was incubated in rat brain homogenate as before, in the absence or presence of both peptide 1 ($4.0 \times 10^{-5}$ M) and peptide 2 ($4.0 \times 10^{-5}$ M), and the mixtures were analyzed by HPLC for β-endorphin after 0 and 30 min of incubation. Within experimental error, the model peptides had no effect on the degradation of β-endorphin, showing that the stability of the model peptides is not due to any inhibitory action that they or their degradation products may have on the proteolytic enzymes responsible for degrading β-endorphin (results not shown.)

## DISCUSSION

We have previously proposed (15) that three separate structural regions can be identified in the linear sequence of β-endorphin: a specific opiate receptor recognition site having the same sequence as (Met[5])enkephalin in residues 1 through 5; a hydrophilic spacer region in residues 6 through 12; and a 16-residue sequence between helix-breaker residues (19) Pro[13] and Gly[30], which is capable of forming an amphiphilic α- or π-helix with one-half of its surface hydrophobic and the hydrophilic residues being neutral or basic.

We have designed and synthesized two model peptides, peptide 1 and peptide 2 (Fig. 1), in order to investigate the possible roles of the proposed spacer region and amphiphilic helix in determining the properties of β-endorphin. As a first principle, homology to the natural sequence of β-endorphin was minimized in the regions that are modeled in peptides 1 and 2, while the general characteristics were retained.

TABLE 2

*Inhibitory concentrations of β-endorphin, peptide 1, and peptide 2 on GPI and RVD assays*

| | $IC_{50}$[a] | |
|---|---|---|
| | GPI[b] | RVD[b] |
| | nM | nM |
| β-Endorphin | 78 ± 16 ($n = 3$) | 52 ± 3 ($n = 3$) |
| Peptide 1 | 15 ± 1 ($n = 3$) | 61 ± 12 ($n = 4$) |
| Peptide 2 | 151 ± 21 ($n = 5$) | 450 ± 15 ($n = 3$) |

[a] Values are means ± standard error of the mean.

[b] Assays were conducted in modified Krebs-Ringer solution at 37°.

TABLE 3

*Effect of buffer conditions and temperature on the abilities of β-endorphin, peptide 1, and peptide 2 to inhibit the specific binding of 0.46 nM [$^3$H]DHM to guinea pig brain whole membrane preparations*

| | Concentration required for 50% inhibition | | $IC_{50}$ ratio[a] |
|---|---|---|---|
| | In Tris buffer at 25° | In Krebs-Ringer solution at 37° | |
| | nM | nM | |
| β-Endorphin | 230 | 940 | 4.1 |
| Peptide 1 | 70 | 63 | 0.9 |
| Peptide 2 | 6 | 350 | 58 |

[a] $IC_{50}$ in Krebs-Ringer solution at 37°/$IC_{50}$ in Tris at 25°.

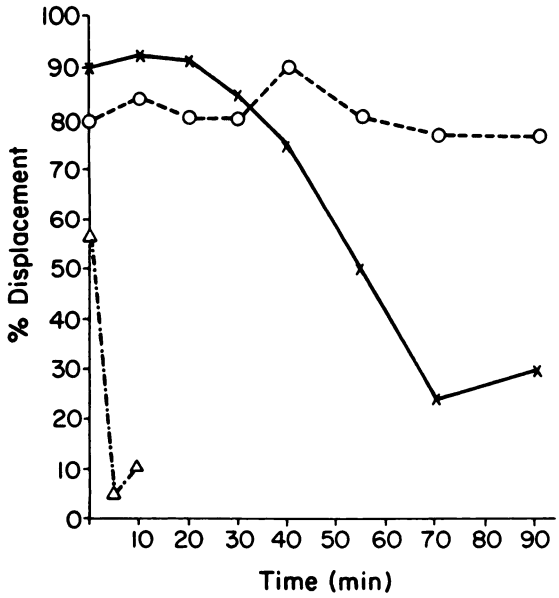


FIG. 5. *Relative resistances of peptide 2* (○), *β-endorphin* (×), *and* (*Met*$^5$)*enkephalin* (△) *toward degradation by proteolytic enzymes endogenous to rat brain*

Peptide solutions ($1.0 \times 10^{-5}$ M) in a suspension of whole rat brain homogenate in 0.05 M Tris-HCl buffer (pH 7.4) were incubated at 37°. The opiate receptor binding activities of these solutions after 100-fold dilution were then determined as a function of the time of incubation by the method described in the text.

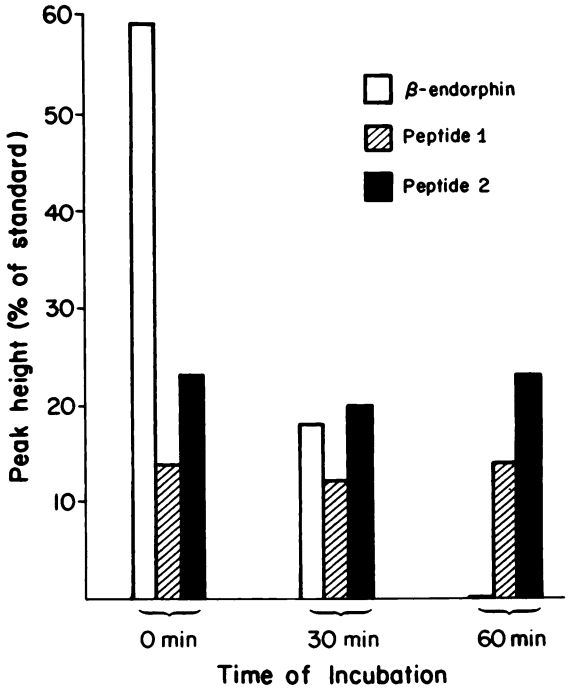


FIG. 6. *Relative resistances of β-endorphin, peptide 1, and peptide 2 toward degradation by proteolytic enzymes endogenous to rat brain*

Peptide solutions ($1.0 \times 10^{-5}$ M) in a suspension of whole rat brain homogenate in 0.05 M Tris-HCl buffer (pH 7.4) were incubated at 37°. After various incubation times, these suspensions were boiled for 10 min and centrifuged. The supernatants were then analyzed by HPLC for their content of β-endorphin, peptide 1, or peptide 2 as a function of the incubation time by the method described in the text.

In aqueous solution, peptides 1 and 2 self-associate readily, and have considerable α-helical structure even in the monomeric form, whereas β-endorphin does not self-associate and exhibits little helical character. The concentrations at which peptides 1 and 2 self-associate, as indicated by increasing molar ellipticities at 222 nm, are too high to affect their receptor binding and opiate activities, but may be important in determining their resistances to proteolytic degradation. Self-association is a property typical of peptides designed to form amphiphilic α-helices of the type shown in Fig. 3 (5, 7). The lack of self-association of β-endorphin at the concentrations studied is probably due to the lack of a suitable hydrophobic domain lying straight along one side of an α-helix in the COOH-terminal region, and/or the inherent instability of a π-helix (40) which would have a hydrophobic surface suitable for self-association.

In CPK models of β-endorphin the side chains of Ile$^{22}$ and Ile$^{23}$ have very restricted mobilities in a helical conformation. This could contribute to the lack of helical structure in aqueous solutions of β-endorphin as compared with monomeric peptide 1 or peptide 2. However, helical structure is readily induced in β-endorphin by the addition of TFE (41), methanol (41), or certain lipids chosen to reproduce the environment of the opiate receptor (22). Furthermore, the helicity induced in β-endorphin by TFE lies in the COOH-terminal region of the molecule (18, 25). Therefore, the similarity we have observed in the CD spectra of peptide 1, peptide 2, and β-endorphin in 50% TFE solutions and the high helicity calculated for each suggest that the model peptides can reproduce the conformational characteristics of β-endorphin that are potentially important for its biological activities.

Peptide 1 has a higher affinity for both δ- and μ-receptors than does β-endorphin, but the binding selectivity is similar. The importance of the COOH-terminal region of β-endorphin in determining its binding to these receptors is well illustrated by the relatively weak binding of α-endorphin [β-endorphin 1–17 (42)]. Therefore, our results indicate a necessary but relatively nonspecific role for this region of β-endorphin and peptide 1 on the receptors, because these peptides have no sequence homology in residues 20 through 31. Furthermore, studies of β-endorphin analogues with single-residue deletions or substitutions in this region show differences in receptor binding potencies that are similar in magnitude to those found for peptide 1 relative to β-endorphin (12). This strongly suggests that the basicity, amphiphilicity, and hydrophobic-hydrophilic balance of the α- or π-helix postulated for residues 14 through 29 of β-endorphin are important in determining its receptor binding properties, since all of these characteristics are retained in the α-helix designed for peptide 1.

In contrast, peptide 2 is slightly less potent than β-endorphin in δ-receptor binding, but 60 times more potent (in Tris buffer at 25°) in its affinity for μ-receptors. Peptide 2 differs from peptide 1 most markedly in the proposed hydrophilic spacer region (residues 6 through 12). Thus, it is likely that this region is important in determining the affinity of β-endorphin for μ-receptors. Since all three peptides have similar affinities for δ-receptors, these receptors are probably less sensitive to the nature of the residues linking the enkephalin sequence to the proposed helical regions of these substrates.

The opiate activities of peptide 1 and peptide 2 in the GPI assay are very similar to those of β-endorphin, peptide 1 being somewhat more potent and peptide 2 slightly less potent. The GPI assay is relatively insensitive to the nature of β-endorphin analogues, provided that the enkephalin region at the $NH_2$ terminus is not altered (12). Nevertheless, the activity of peptide 2, in particular, provides strong support for our structural hypothesis, since peptide 2 only has four residues homologous to β-endorphin in its sequence from residues 6 through 31 (Fig. 1). In contrast to the GPI assay, opiate activity in the RVD assay is related to a distinct class of opiate receptors called ε-receptors, which appear to be highly specific for β-endorphin (35) and show only very low sensitivity towards (Met[5])enkephalin or the enzyme-resistant δ-agonist (D-Ala[2],D-Leu[5])enkephalin. No agonist activity at all has been detected for the μ-agonist morphine. Studies of COOH-terminal deletion analogues of β-endorphin show a considerable loss of activity in peptides containing the $NH_2$-terminal 21 amino acid residues of β-endorphin or less, indicating that an interaction of the COOH terminus of β-endorphin with the ε-receptor is necessary for expression of opiate activity in this system (43). Therefore, the high activities of peptide 1 ($IC_{50}$ = 61 ± 12 nM) and peptide 2 ($IC_{50}$ = 450 ± 15 nM) in this assay, compared with β-endorphin (residues 1–21) ($IC_{50}$ > 2000 nM) and the shorter $NH_2$-terminal fragments of β-endorphin ($IC_{50}$ > 50,000 nM), demonstrate that all of the major structural features of β-endorphin that are necessary for this activity have been retained in the design of both model peptides. Once again, when the activities of peptide 1, peptide 2, and β-endorphin in the RVD assay are correlated to their sequence differences (Fig. 1), it is reasonable to conclude that the hydrophilic spacer region proposed for residues 6 through 12 of β-endorphin serves as an address sequence which moderates its receptor selectivity. Thus, residues 6 through 12 of peptide 2 may be less suited to ε-receptor agonist activity than are their β-endorphin counterparts. In this context, the conservation of charged residues in positions 8 and 9 of β-endorphin sequences from a variety of natural sources may be significant (13).

(Met[5])Enkephalin is rapidly degraded in rat brain homogenates by a number of enzymes, including an aminopeptidase which cleaves the Tyr[1]—Gly[2] bond (44), and a dipeptidyl carboxypeptidase which cleaves the Gly[3]—Phe[4] bond and has also been shown to have endopeptidase action on other substrates (45). In contrast, β-endorphin is relatively resistant to proteolysis and is initially cleaved on the $NH_2$-terminal side of Leu[17] or Phe[18], yielding α- and γ-endorphin, respectively, which are then more rapidly degraded by aminopeptidase action (46). Furthermore, there is evidence that a tertiary or quaternary structure exists in β-endorphin at high concentrations in aqueous solution which is lost upon enzymatic cleavage in the COOH-terminal region (47). Therefore, it has been suggested that the $NH_2$-terminal enkephalin segment of β-endorphin is protected from degradation by interaction with the COOH-terminal region (14).

Both peptide 1 and peptide 2 display an increased resistance toward proteolytic degradation in rat brain homogenates as compared with β-endorphin (Fig. 5 and 6). This property is not a result of any inhibitory action of the model peptides on the enzymes which degrade β-endorphin. Peptide 1 has the same primary sequence as β-endorphin for residues 1 through 19, which includes all of the potential cleavage sites implicated in studies of the degradation of β-endorphin in rat brain homogenates, and peptide 2 contains the (Met[5])enkephalin sequence as its first five residues.

These considerations suggest that the strong resistance to proteolysis exhibited by peptide 1 and peptide 2 is related to the presence of stable structure in their solution conformations. The formation of an amphiphilic α-helix from residues 14 through 31 in peptide 1 or peptide 2 could allow a tertiary structure to form, where the hydrophobic residues Tyr[1], Phe[4], and Met[5] in the $NH_2$ terminus bind to the hydrophobic face of the α-helix. Our construction of CPK models suggests that a similar interaction can occur in β-endorphin, where Phe[18] and Tyr[27] can readily interact with Phe[4] and Tyr[1], respectively, if residues 14 through 29 are in a helical conformation as illustrated in Fig. 2. Alternatively, peptides 1 and 2 may owe their resistance to proteolytic degradation to their abilities to self-associate, or to bind nonspecifically to membranes or other substances present in rat brain homogenates.

The importance of nonspecific binding to membranes in the studies in rat brain homogenates is suggested by the low recovery yields of both of the model peptides in the HPLC experiments, where all particulate matter is removed before assaying for the peptides (Fig. 4). Peptide modeling studies of melittin indicate that amphiphilic α-helical structures of the type designed in peptides 1 and 2 (Fig. 3) might be expected to interact very strongly with membrane surfaces (7). This type of interaction could also explain the slower onset of inhibition observed for the model peptides in the GPI and RVD assays as compared with β-endorphin (Fig. 4). If the model peptides first bind nonspecifically to the tissue surfaces and then migrate to the opiate receptors, the rate of onset of inhibition would depend on the rate of migration on the tissue surfaces or the rate of dissociation from these surfaces. This type of behavior has been postulated to be of importance for β-endorphin also (25), but the high yield obtained for β-endorphin in our HPLC analysis of the rat brain homogenate (Fig. 6, time of incubation = 0 min) and the rapid onset of its actions on GPI and RVD suggest that relatively little nonspecific binding of β-endorphin occurs.

The results reported in this paper clearly illustrate the utility of the structural approach we have adopted. Further investigations on model peptides with the objective of refining the hypothesis presented for β-endorphin structure are being pursued by us.

## REFERENCES


1. Segrest, J. P., R. L. Jackson, J. D. Morrisett, and A. M. Gotto, Jr. A molecular theory of lipid-protein interactions in the plasma lipoproteins. *F. E. B. S. Lett.* **38**:247–253 (1974).
2. Edelstein, C., F. J. Kézdy, A. M. Scanu, and B. W. Shen. Apolipoproteins and the structural organization of plasma lipoproteins: human plasma high density lipoprotein-3. *J. Lipid Res.* **20**:143–153 (1979).
3. Fitch, W. M. Phylogenics constrained by the crossover process as illustrated by human hemoglobins and a thirteen-cycle, eleven-amino acid repeat in human apolipoprotein A-I. *Genetics* **86**:623–644 (1977).
4. McLachlan, A. D. Repeat helical pattern in apolipoprotein A-I. *Nature (Lond.)* **267**:465–466 (1977).
5. Fukushima, D., J. P. Kupferberg, S. Yokoyama, D. J. Kroon, E. T. Kaiser, and F. J. Kézdy. A synthetic amphiphilic helical docosapeptide with the

surface properties of plasma apolipoprotein A-I. *J. Am. Chem. Soc.* **101**:3703–3704 (1979).
6. Yokoyama, S., D. Fukushima, J. P. Kupferberg, F. J. Kézdy, and E. T. Kaiser. The mechanism of activation of lechithin:cholesterol acyltransferase by apolipoprotein A-I and an amphiphilic peptide. *J. Biol. Chem.* **255**:7333–7337 (1980).
7. DeGrado, W. F., F. J. Kézdy and E. T. Kaiser. Design, synthesis and characterization of a cytotoxic peptide with melittin-like activity. *J. Am. Chem. Soc.* **103**:679–681 (1981).
8. Bradbury, A. F., D. G. Smyth, C. R. Snell, N. J. M. Birdsall, and E. C. Hulme. C-fragment of lipotropin has a high affinity for brain opiate receptors. *Nature* (*Lond.*) **260**:793–794 (1976).
9. Cox, B. M., A. Goldstein, and C. H. Li. Opioid activity of a peptide, β-lipotropin-(61–91), derived from β-lipotropin. *Proc. Natl. Acad. Sci. U. S. A.* **73**:1821–1823 (1976).
10. Waterfield, A., R. W. J. Smokcum, J. Hughes, H. W. Kosterlitz, and G. Henderson. In vitro pharmacology of the opioid peptides, enkephalins and endorphins. *Eur. J. Pharmacol.* **43**:107–116 (1977).
11. Brownstein, M. J. Opioid peptides: search for the precursors. *Nature* (*Lond.*) **287**:678–679 (1980).
12. Li, C. H., D. Yamashiro, L.-F. Tseng, W.-C. Chang, and P. Ferrara. β-Endorphin omission analogs: dissociation of immunoreactivity from other biological activities. *Proc. Natl. Acad. Sci. U. S. A.* **77**:3211–3214 (1980).
13. Li, C. H., D. Yamashiro, and P. Nicolas. β-Endorphin: replacement of tyrosine in position 27 by tryptophan increases analgesic potency—preparation and properties of the 2-nitrophenylsulphenyl derivative. *Proc. Natl. Acad. Sci. U. S. A.* **79**: 1042–1044 (1982).
14. Graf, L., G. Cseh, E. Barat, A. Z. Ronai, J. I. Szekely, A. Kennesey, and S. Bajusz. Structure-function relationships in lipotropins. *Ann. N. Y. Acad. Sci.* **297**:63–83 (1977).
15. Taylor, J. W., D. G. Osterman, R. J. Miller, and E. T. Kaiser. Design and synthesis of a model peptide with β-endorphin-like properties. *J. Am. Chem. Soc.* **103**:6965–6966 (1981).
16. Hammonds, R. G., Jr., and C. H. Li. Human β-endorphin: specific binding in neuroblastoma N-18-TG2 cells. *Proc. Natl. Acad. Sci. U. S. A.* **78**:6764– 6765 (1981).
17. Lord, J. A. H., A. A. Waterfield, J. Hughes, and H. W. Kosterlitz. Endogenous opioid peptides: multiple agonists and receptors. *Nature* (*Lond.*) **267**:495–499 (1977).
18. Graf, L., M. Hollosi, I. Barna, I. Hermann, J. Borvendeg, and N. Ling. Probing the biologically and immunologically active conformation of β-endorphin: studies on C-terminal deletion analogs. *Biochem. Biophys. Res. Commun.* **95**:1623–1627 (1980).
19. Chou, P. Y., and G. D. Fasman. Empirical predictions of protein conformation. *Annu. Rev. Biochem.* **47**:251–276 (1978).
20. Dickerson, R. E., and I. Geis. *The Structure and Action of Proteins*. Benjamin/Cummings Publishing Company, London, 28–29 (1969).
21. Dunnill, P. The use of helical net diagrams to represent protein structures. *Biophys J.* **8**:865–875 (1968).
22. Wu, C.-S. C., N. M. Lee, H. H. Loh, J. T. Yang, and C. H. Li. β-Endorphin: formation of α-helix in lipid solutions. *Proc. Natl. Acad. Sci. U. S. A.* **76**:3656–3659 (1979).
23. Lee, N. M., and A. P. Smith. A protein-lipid model of the opiate receptor. *Life Sci.* **26**:1459–1464 (1980).
24. Li, C. H., D. Yamashiro, R. G. Hammonds, Jr., P. Nicolas, and L.-F. Tseng. β-Endorphin: replacement of glutamic acid in position 8 by glutamine increases analgesic potency and opiate receptor-binding activity. *Biochem. Biophys. Res. Commun.* **101**:118–123 (1981).
25. Hammonds, R. G., Jr., A. S. Hammonds, N. Ling, and D. Puett. β-Endorphin and deletion peptides: a correlation of opiate receptor affinity with helix potential. *J. Biol. Chem.* **257**:2990–2995 (1982).
26. Kroon, D. J., and E. T. Kaiser. Studies on the solid-phase synthesis of peptide fragments of apolipoproteins A-I and A-II. *J. Org. Chem.* **43**:2107–2113 (1978).
27. Feinberg, R. S., and R. B. Merrifield. Zinc chloride-catalyzed chloromethylation of resins for solid phase peptide synthesis. *Tetrahedron* **30**:3209–3212 (1974).
28. Horiki, K., K. Igano, and K. Inouye. Synthesis of the Merrifield resin esters of N-protected amino acids with the aid of hydrogen bonding. *Chem. Lett.* 165–168 (1978).
29. Yamashiro, D., and C. H. Li. Total synthesis of ovine β-lipotropin by the solid-phase method. *J. Am. Chem. Soc.* **100**:5174–5179 (1978).
30. Scotchler, J., R. Lozier, and A. B. Robinson. Cleavage of single amino acid residues from Merrifield resin with hydrogen chloride and hydrogen fluoride. *J. Org. Chem.* **35**:3151–3152 (1970).
31. Ohno, M., S. Tsukamoto, S. Makisumi, and N. Izumiza. Improved solid-phase synthesis of tryptophan-containing peptides. *Bull. Chem. Soc. Jpn.* **45**:2852–2855 (1972).
32. Pollet, R. J., B. A. Haase, and M. L. Standaert. Macromolecular characterization by sedimentation equilibrium in the preparative ultracentrifuge. *J. Biol. Chem.* **254**:30–33 (1979).
33. Pasternak, G. W., and S. H. Snyder. Opiate receptor binding effects: effects of enzymatic treatments. *Mol. Pharmacol.* **10**:183–193 (1974).
34. Kosterlitz, H. W., R. T. Lydon, and A. F. Watt. The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α- and β-adrenoreceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmacol.* **39**:398–413 (1970).
35. Schultz, R., E. Faase, M. Wuster, and A. Herz. Selective receptors for β-endorphin on the rat vas deferens. *Life Sci.* **24**:843–850 (1979).
36. Greenfield, N., and G. D. Fasman. Computed circular dichroism spectra for the evaluation of protein conformation. *Biochemistry* **8**:4108–4116 (1969).
37. Morrisett, J. D., J. S. K. David, H. J. Pownall, and A. M. Gotto, Jr. Interaction of an apolipoprotein (Apo LP-Alanine) with phosphatidylcholine. *Biochemistry* **12**:1290–1299 (1973).
38. Chang, K.-J., and P. Cautrecasas. Multiple opiate receptors. *J. Biol. Chem.* **254**:2610–2618 (1979).
39. Leslie, F. M., C. Chavkin, and B. M. Cox. Opioid binding properties of brain and peripheral tissues: evidence for heterogeneity in opioid ligand binding sites. *J. Pharmacol. Exp. Ther.* **214**:395–402 (1980).
40. Sasaki, S., Y. Yasumoto, and I. Uematsu. π-Helical conformation of poly(β-phenethyl L-aspartate). *Marcromolecules* **14**:1797–1801 (1981).
41. Yang, J. T., T. A. Bewley, G. C. Chen, and C. H. Li. Conformation of β-endorphin and β-lipotropin: formation of helical structure in methanol and sodium dodecyl sulfate solutions. *Proc. Natl. Acad. Sci. U. S. A.* **74**:3235–3238 (1977).
42. Law, P.-Y., H. H. Loh, and C. H. Li. Properties and localization of β-endorphin receptor in rat brain. *Proc. Natl. Acad. Sci. U. S. A.* **76**:5455–5459 (1979).
43. Schulz, R., M. Wuster, and A. Herz. Pharmacological characterization of the ϵ-opiate receptor. *J. Pharmacol. Exp. Ther.* 216:604–606 (1981).
44. Wagner, G. W., M. A. Tavianini, K. M. Hermann, and J. E. Dixon. Purification and characterization of an enkephalin aminopeptidase from rat brain. *Biochemistry* **20**:3884–3890 (1981).
45. Almenoff, J., S. Wilk, and M. Orlowski. Membrane bound pituitary metalloendopeptidase: apparent identity to enkephalinase. *Biochem. Biophys. Res. Commun.* **102**:206–214 (1981).
46. Burbach, J. P. H., E. R. DeKloet, P. Schotman, and D. DeWied. Proteolytic conversion of β-endorphin by brain synaptic membranes. *J. Biol. Chem.* **256**:12463–12469 (1981).
47. Nicolas, P., T. A. Bewley, L. Graf, and C. H. Li. β-Endorphin: demonstration of a tertiary structure in aqueous solution. *Proc. Natl. Acad. Sci. U. S. A.* **78**:7290–7293 (1981).

**Send reprint requests to:** Dr. E. T. Kaiser, The Rockefeller University, 1230 York Avenue, New York, N. Y. 10021.