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Elucidation of the Receptor-Bound Conformation of the Enkephalins

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The biologically relevant conformers of enkephalin predicted by solid state, solution state, and theoretical energy studies have been compared with the published structure-activity data on these compounds. No conformational technique proposes a model consistent with all the pharmacological data; the shortcomings of each approach are evaluated. An alternative approach, which correlates the structure-activity data of opiate compounds with that of the enkephalins, is described and shown to produce a model consistent with the available structure-activity data.

Key words: enkephalin, receptor, conformation, opiate, X-ray, NMR

The enkephalins are the smallest of the opioid-like neuropeptides, having the sequence tyrosyl-glycyl-glycyl-phenylalanyl-X, where X is either methionine (MENK) or leucine (LENK). Their relatively small size has permitted conformational studies using theoretical energy calculations, x-ray crystallography, and several different spectroscopic methods (Table I). In many cases, the authors have attempted to infer from such studies the biologically active conformation of receptor-bound enkephalin. The different conformational studies have assessed the biological relevance of the proposed structures by examining whether their structures can sterically accommodate the substitution of different amino acids known to produce biologically active analogs. This mode of analysis assumes that biologically active analogs of enkephalin share a common peptide backbone conformation with the native enkephalins when bound to the receptor.*

*"Receptor-bound conformation" of small, linear, flexible peptides is meant to imply a time-averaged conformation with kT amounts of vibrational and torsional degrees of freedom.

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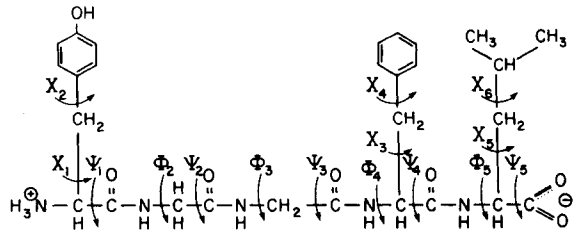
TABLE I. Proposed Conformations of Enkephalin



CRYSTAL STRUCTURES:	X_1	X_2	Ψ_1	Φ_2	Ψ_2	Φ_3	Ψ_3	Φ_4	Ψ_4	X_3	X_4	Φ_5	Ψ_5	X_5	X_6
Tyr-Gly-Gly-Phe-Leu	-43 -86	-89 -30	126	59	25	97	- 7	-136	145	-62	90	-105	- 4	- 69	178
Tyr-Gly-Gly-Phe	63	70	170	74	11	90	0	- 71	-35	168	70	—	—	—	—
— Gly-Gly-Phe-Leu	—	—	—	—	172	-69	161	- 57	-41	71	65	-127	- 12	61	60
CALCULATED STRUCTURES:															
Isogai et. al. (MENK)	-174	82	155	-159	100	74	-100	- 85	-41	-179	74	-165	124	-174	57
Momany (VI C)	-61	-75	81	69	-126	-65	- 55	- 64	114	177	-109	49	56	- 62	-176
De Coen et. al.	180	90	140	80	-80	-80	80	-80	80	-60	-60	-140	140	- 60	180
Balodis et. al.	180	90	140	80	-80	-60	-60	-140	140	180	90	-140	140	-80	80
SOLUTION STRUCTURES:															
Garbay-Jaureguiberry et. al. (DMSO-d_6)	-60	—	±60	180	60-180	±65	±30	-150	-60	-60	—	-155	-60	- 60	—
Jones et. al. (DMSO-d_6)	—	—	—	—	—	-60	-30	- 90	0	—	—	—	—	—	—
RECEPTOR-BOUND STRUCTURES:															
Gorin & Marshall	-106	-163	129	160	-87	-118	98	-87	—	-87	-56	—	—	—	—

The pharmacology of the opiates and enkephalins is complex, and it is likely that these compounds act in vivo at several species of receptors. The conformational studies cited in this article utilize structure-activity data obtained from the in vitro systems of mouse vas deferens, guinea pig ileum, and rat brain binding assays. There is no assurance that the opiate receptors are equivalent in these systems, although the relative potencies of opiates and analogs of enkephalin are reported to correlate reasonably well between the different assay systems [1, 2]. Analogs of enkephalin tested in several in vitro systems and found to be active in one type of bioassay have been reported to be biologically active in the other in vitro systems although absolute potencies of an individual compound in the different in vitro bioassays might differ significantly [3]. Thus, the extensive structure-activity data on the enkephalins in these systems can be used to assess the validity of the biologically relevant conformers predicted by these conformational studies.

D-, or L-, N-methyl, and particularly α-methyl amino acids locally restrict backbone rotational freedom when substituted into a peptide. The allowed regions of torsional space for each of these substitutions have been calculated [4], and the results correspond very well with published protein crystal data (Fig. 1). The one reported value which conflicts

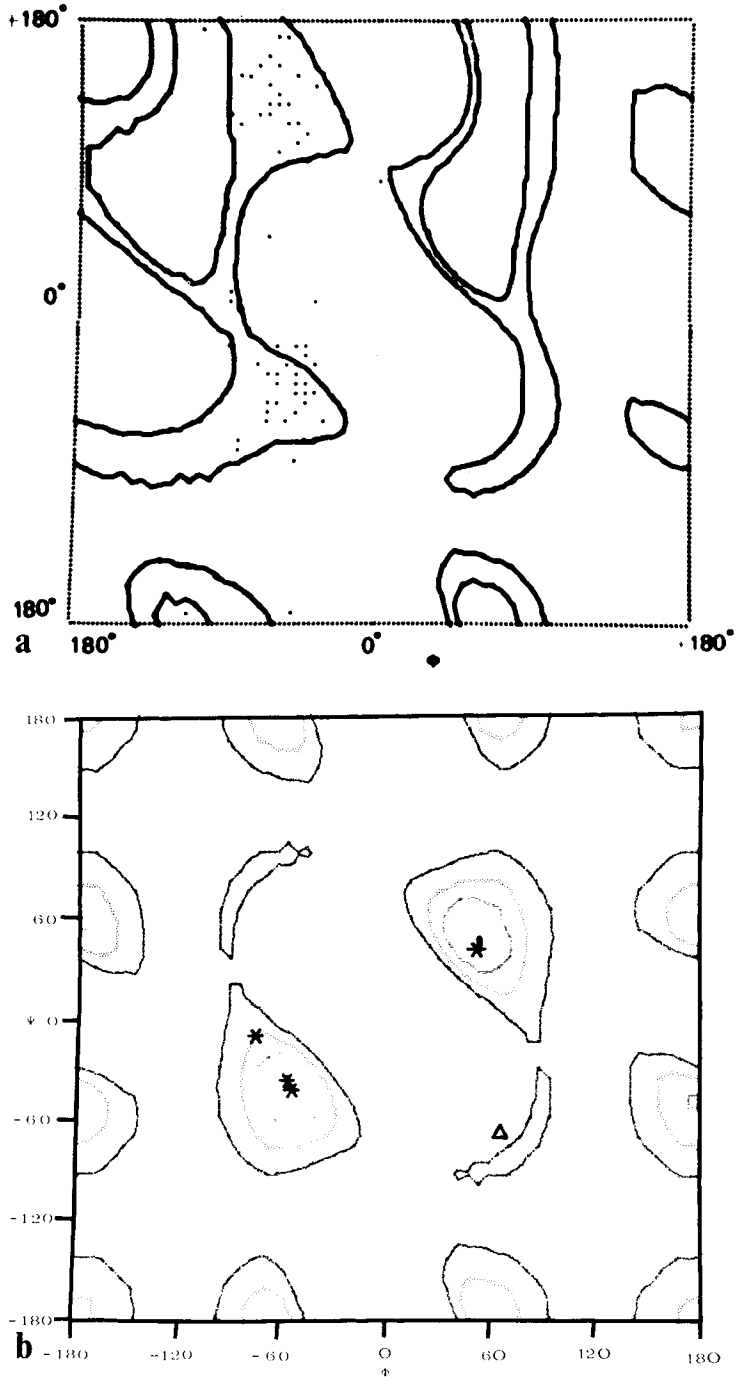


Fig. 1. a) Kitaigorodsky potential plot for acetyl-N-methyl-L-alanine methylamide with dots representing reported crystallographic determinations of ϕ and ψ [4]. b) Potential plot for acetyl-aminoisobutyric acid methylamide with four reported crystallographic determinations (*) from linear peptides [56, 57] and one determination (△) from the cyclic tetrapeptide, dehydroclamydocin [5]. (See text for further discussion.)

(ϕ, ψ = 71.8, −63.7) with the predicted minima regions for aminoisobutyric acid (Aib) is reported for the cyclic tetrapeptide dihydroclamydocin where all the amide linkages in the peptide deviate from planarity by 15–20° [5], implying severe steric strain. Structures predicted by conformational studies to be those of receptor-bound enkephalin should have peptide backbone conformations which can sterically accommodate the more conformationally restricted and yet biologically active analogs containing these methylated amino acids. That is, a proposed conformation for receptor-bound enkephalin should have ϕ_i, ψ_i values which are compatible with the allowed ϕ,ψ torsional space of a methylated amino acid in the i-th residue if such an amino acid substitution results in biologically active analog.

CRYSTAL STRUCTURES

X-ray crystallographic structures have been reported for LENK [6] and the enkephalin fragments tyrosyl-glycyl-glycyl-phenylalanine (TGGP) [7], glycyl-glycyl-phenylalanyl-leucine (GGPL) [7], and tyrosyl-glycyl-glycine (TGG) [8]. It is noteworthy that the hydrogen bonding scheme and β-bend seen in the crystal structure of LENK is different from any proposed by energy calculations or solution NMR. Several points argue against the relevance of the crystal structure of enkephalin to biological activity. The backbone conformation in the crystal is stabilized by an intramolecular hydrogen bond involving the amide proton of Phe^4 and the carbonyl oxygen of Tyr^1 which would be incompatible with the exceptional biological activity of the N-methyl amino acid analogs, [D-Ala^2, $MePhe^4$, Leu-OMe^5] [9] and [D-Ala^2, $MePhe^4$, Met(O)ol^5] enkephalin [10]. Furthermore, ϕ_4, ψ_4 backbone torsional angles are incompatible with the activity of [D-Ala^2, Phe(αMe)^4]-leucine enkephalin (Fig. 2 and [22]). In addition, the tyrosine residue in the crystal is disordered, occurring in two conformers, neither of which correspond to the tyramine conformation seen in the rigid opiates such as morphine (Fig. 3). Structure-activity data of the enkephalins are cited later in this article which support the proposal of several investigators that the amino terminus and phenolic side chain of the tyrosine residue correspond to the hydroxyphenylethylamine moiety seen with the morphine, morphinan, oripavine, and benzomorphan classes of opiates [11–14].

The crystal structure of the fragment TGGP, the minimal structure unit of enkephalin demonstrating biological activity, has hydrogen bonding similar to that of the pentapeptide, but the orientation of the backbone angles and the side-chain angles of Tyr^1 and Phe^4 are quite different (Fig. 3). The other fragments of enkephalin have no intramolecular hydrogen bonding. For small, linear molecules with low internal barriers to rotation, intermolecular interactions can dominate intramolecular forces, making correlation between solution or receptor conformation with solid state structures extremely tenuous [15]. Similar problems have been noted when comparing the numerous crystal structures of acetylcholine with structures predicted by solution studies and by theoretical energy calculations [16].

THEORETICAL ENERGY CALCULATIONS

Although the enkephalins are small peptides, they have a minimum of seventeen variables, ie the rotatable bonds, if the amide unit is assumed to be planar and bond angles and bond lengths remain invariant. For 20° increments of each torsional angle, this would require analysis of approximately 10^{22} conformers. Consequently, all reported semiempirical energy calculations on enkephalin utilized a limited, nonsystematic exploration of conformational space. Isogai et al [17], using neutrally charged methionine-enkephalin, found

the most favorable conformer to be a 1–4 βII′-bend stabilized by a hydrogen bond between the phenolic hydroxyl of Tyr[1] and the carboxyl oxygen of Phe[4] (Fig. 4). Their energetically most favored conformers are incompatible with the observation that [D-Ala[2]]-methionine enkephalin retains essential activity [18], while a large decrease in binding and biological activity accompanies the [L-Ala[2]] analog [19]. The authors conclude that their theoretically

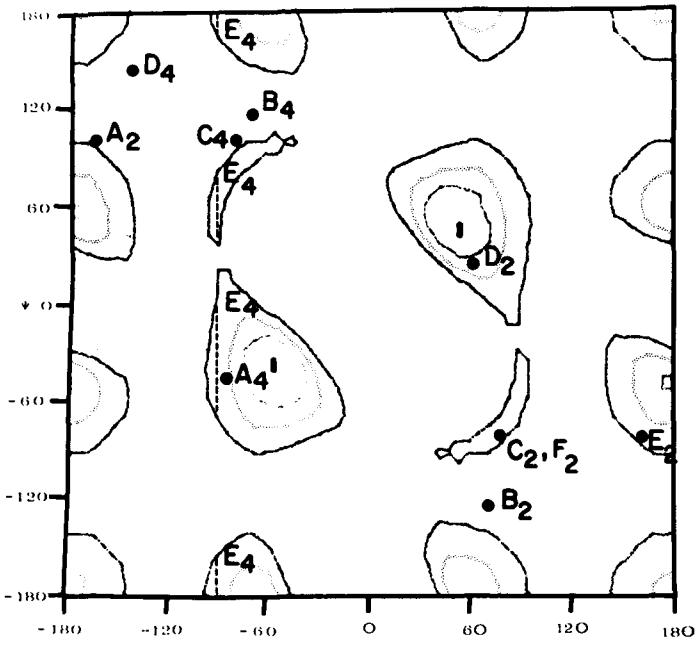


Fig. 2. ϕ, ψ plot of backbone torsional angles for the second (R_2) and fourth (R_4) peptide residues compatible with [Aib[2]]-enkephalin and [Phe(αMe)[4]]-enkephalin. R = (A) Isogai et al [17], minimum energy conformer; (B) Momany [20], V1C conformer; (C) De Coen et al [23], biologically active conformer; (D) Smith et al [6], crystal structure of LENK; (E) Gorin and Marshall [48], topographical comparison of Tyr-D-Ala-Gly-Phe with rigid opiates; (F) Balodis et al [27], "biologically active" conformer. (See text for further discussion.)

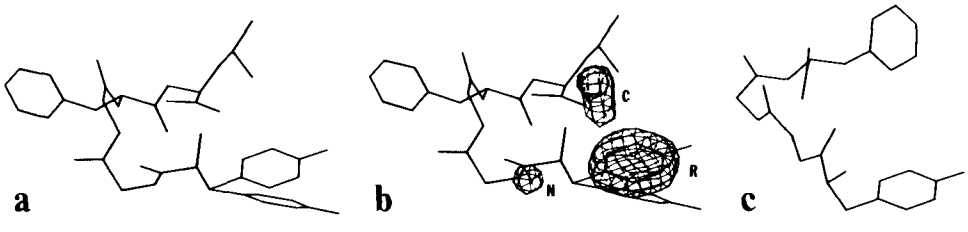


Fig. 3. a) Computer-graphics display [58] of the configuration of LENK reported for its crystallographic determination and demonstrating the two side-chain conformers of Tyr[1] coexisting in the crystal. b) Superposition of a space-filling representation of the phenolic ring of morphine (R) with one of the Tyr[1] conformers of Figure 3a. There is no alignment of the nitrogen terminus of LENK with the nitrogen of morphine (N), and no substituents of the peptide align with the C-ring atoms, C5 and C6 (C) of morphine. A similar lack of correspondence is seen with superposition of (R) using the other Tyr[1] conformer. c) The configuration of Tyr-Gly-Gly Phe reported for its crystallographic determination.

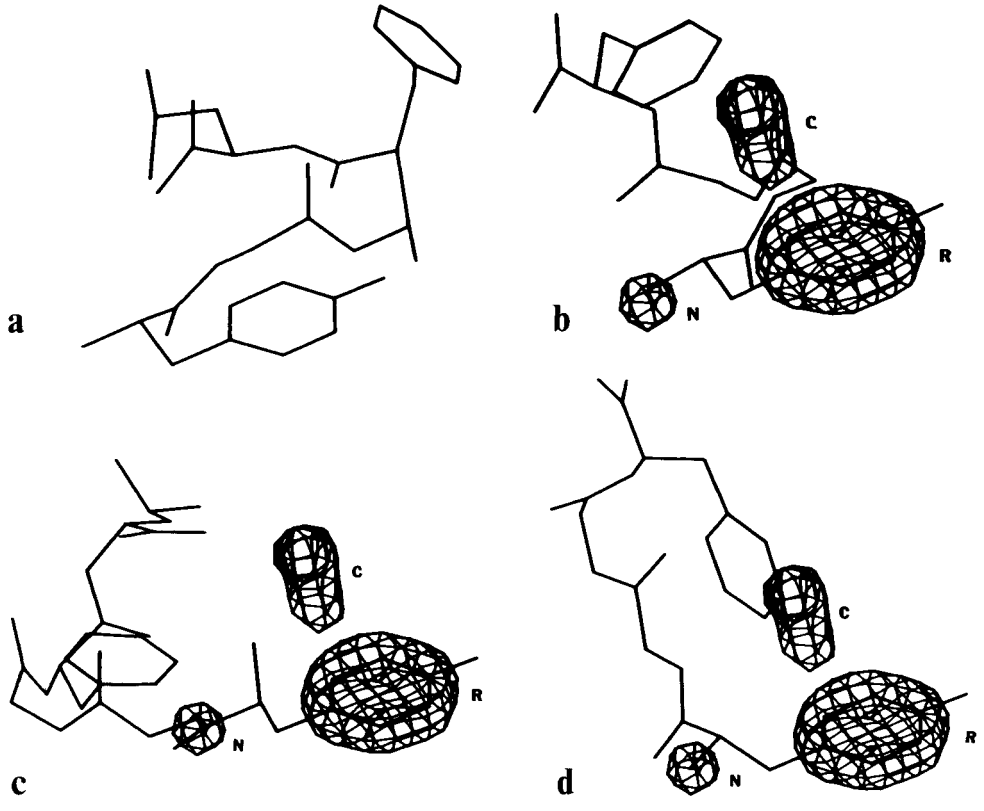


Fig. 4. a) The configuration of MENK reported as the minimum energy conformer by Isogai et al [17]. b) The configuration of LENK reported as the "biologically active" conformer by De Coen et al [23] with opiate topography superimposed as in Figure 3b. Alignment of the phenolic rings (R) results in lack of correspondence of the nitrogen terminus of LENK with the nitrogen of morphine (N). c) The configuration of MENK reported as the "biologically active" conformer by Momany [20]. Alignment as in 4b results in lack of correspondence between the nitrogen moieties of MENK and morphine (N). d) The configuration of Tyr-Gly-Gly-Phe found to fit the opiate pharmacophore of Gorin and Marshall [48]. Alignment of the phenolic rings produces correspondence between the nitrogen moieties (N) and the meta position of the aromatic ring of Phe^4 with C6 of morphine (C).

predicted structures have little in common with the receptor-bound conformation. Momany [20] has attempted to resolve this discrepancy while using the same potential functions as Isogai et al by examining the lowest-energy conformers of the neutrally charged, zwitterionic, and C-terminal amide forms of [D-Ala^2] and [L-Ala^2] analogs of methionine-enkephalin. The conformation selected as being compatible with the active opiate conformer accommodates a D-alanine but not an L-alanine substitution in the second residue. This conformer is in conflict however, with the observations that [Aib^2, Leu-OMe^5] enkephalin [9], [Aib^2, Met-NH_2^5] enkephalin [21], and [Phe(αMe)^4]-leucine enkephalin [22] are active (Fig. 2). The proposed structure is further incapable of explaining the intolerance of substitution of glycine-3 by either a D-alanine or L-alanine substitution [2]. Humblet and De Coen investigated the zwitterionic forms of MENK, LENK, and three analogs: [D-Ala^2],

[D-Ala^3], and [D-Phe^4]-methionine enkephalin [23]. Minimum energy starting conformers were generated by examining the local interactions of each isolated residue with a backbone torsional increment of 20° and with a side-chain torsional increment of 30°. Unlike Momany, who compared relative energies of each analog normalized to its respective minimum energy conformer, De Coen et al advances a probabilistic argument by calculating both the energies and number of side-chain arrangements available for each of their 400 starting conformers [24]. This latter approach is certainly preferable when attempting to correlate conformer population with drug activity [25]. There is the shortcoming however, that certain of the calculated minima for isolated residues, most notably, those representing helical structures, restrict side-chain mobility because of the rather precise geometric constraints imposed by the backbone conformation. Hence, bias is introduced by using starting conformers based solely on the local interactions of the isolated residues. De Coen obtains, by analog comparison, a backbone conformation, which is not an energy minimum for MENK. It is the minimum energy conformer for the biologically active [D-Ala^2]-methionine analog and is not obtained for the inactive [D-Ala^3] or [D-Phe^4] analogs [26]. Strictly speaking, this conformer would contradict structure-activity data by predicting the [Aib^2] analogs of enkephalin and [Phe(αMe)^4]-leucine enkephalin to be inactive. However, because of the coarseness of the torsional scan a ± 20° shift of predicted backbone angles could accommodate known structure-activity data (Fig. 2). Balodis et al [27] use a different approach to generate starting minimum energy conformers but also compare [D-Ala^2] and [D-Ala^3] analogs with MENK and obtain a backbone configuration similar to that of De Coen (Table I). The proposal of Balodis et al is incompatible however, with the activity of [Phe(αMe)^4]-leucine enkephalin (Fig. 2). Neither the proposals of De Coen et al or of Balodis et al position the side chain of Tyr^1 to correspond with the tyramine moiety of the rigid opiates (Fig. 4).

Several groups have incorporated analog information into their energy studies in an attempt to deduce the receptor-bound conformation of enkephalin. The approach has the advantage that some of the tacit assumptions made by the calculations, such as parametrization and neglect of solvent, are internally consistent with such comparisons. The criterion used in all studies for biological relevance, however, is the commonality of minimum energy conformers shared by the biologically active ligands and precluded by inactive ligands. There is no assurance that the receptor-bound conformers need occupy minimum energy states calculated for isolated ligands. Comparisons of the NMR spectrum of acetylcholine and the conformation of semirigid analogs which have muscarinic properties indicate that the preferred solution conformer is not the "active" conformer [28]. Richards et al [29, 30] have suggested that dopamine and serotonin, as well as histamine and the catecholamines, use their flexibility to adapt to the receptor binding site. Lowe and Burt [31] reinvestigated the minimum energy conformers found by Isogai et al [17] using PCILO quantum methods as well as semiempirical energy calculations. Both types of energy calculations demonstrated that significant increases in energy were required for the minimum energy conformers of Isogai et al to adopt a tyramine conformation like that of morphine and that considerable increases in energies were needed for MENK to position the side chain of Phe^4 to correspond to the 19-phenethyl substitution of the potent opiate 7-(1-phenyl-3-hydroxybutyl-3-) endoethenotetrahydrothebaine (PET). It would not be surprising that a conformeric ensemble representing receptor-bound enkephalin is not highly probable in a state isolated from the opiate receptor. One can also question the appropriateness of these calculations in mimicking the solution state of enkephalin prior to receptor interaction; ie solvent interactions may significantly change the potential surface.

NMR STUDIES

Numerous proton studies of MENK [32–37] as well as carbon-13 studies [37–39] have been reported; limited studies of LENK indicate only minor differences [37, 38, 40]. A number of discrepancies in the early studies have been resolved, since it appears that significant conformational differences exist between the zwitterionic and cationic forms of MENK. The zwitterionic form is compatible with a 2–5 βI-turn in DMSO with an intramolecular hydrogen bond between the amide proton of methionine-5 and the carbonyl oxygen of glycine-2 [33, 34]. The cationic form does not appear to be stabilized by intramolecular hydrogen bonds but appears to have a well-ordered solution conformation [39]. This is suggested by the nonequivalence of the Cα protons of Gly^2, Gly^3, and Cβ protons of Phe^4 in D_2O and DMSO [40] and by ^{13}C relaxation studies in a DMSO/D_2O mixture which propose that the side chain of tyrosine is held in a restricted configuration relative to the overall tumbling of the module [39].

These observations are complicated by the report of intermolecular association of enkephalin in a variety of solvents. The aforementioned studies used concentrations in the 10–100 mM range. Khaled et al [37], using PMR and CMR for the zwitterionic form of MENK and LENK in DMSO, demonstrated that there is a concentration-dependent shift of amide protons and carbonyl carbons assignments in this range. Significantly, in DMSO the temperature-dependent shift coefficient (dδ/dt) of the amide proton of methionine-5 is much lower (–1.8) at high concentrations (0.1 M) than at low concentrations (–4.1 at 0.001 M), suggesting that proposed hydrogen bonding to the fifth residue of the 2–5 βI-bend might be artifactual. In another study, the NT_1 values of the α-carbons of the zwitterionic LENK [38] are very similar and compatible with the antiparallel β dimer proposed for the aggregated state by Khaled et al. Khaled et al also suggest that the data in aqueous solution can fit a monomeric, 2–5 βI-hydrogen-bonded structure analogous to the proposals of Jones et al [34] and Rogues et al [33], but with additional hydrogen bonding between Gly^3(NH)-Tyr^1(CO) and Tyr^1(OH)-Gly^3(CO).

OTHER SOLUTION STUDIES

Schiller et al [41–43] have used fluorescence energy transfer with the biologically active [Trp^4]-methionine enkephalin analog to examine the solution conformation. Using this tryptophan analog at significantly lower concentrations (2×10^{-5} M) than in the NMR studies, Schiller finds no conformational changes occurring in the pH range from 1.5 to 5.5 [42] in contrast to NMR studies using 10^{-1} M solutions of MENK [32]. Furthermore, the fluorescent studies in aqueous solvents and in butanol [41] discount the possibility of the predominant solution conformer having a hydrogen-bounded tyrosine, as predicted by the theoretical calculations of Isogai et al [17] and by the NMR studies of Khaled et al [37]. Such hydrogen bonding has been shown to quench phenolic fluorescence completely and quenching is not observed with enkephalin.

Jones et al, in comparing the zwitterionic [D-Ala^2]-methionine enkephalin with the parent compound [36], and Bleich, in comparing the cationic forms of biologically active MENK and [Nle^5] analogs with the inactive [Phe^1] analog of MENK [40], see no significant conformational differences between the analogs in DMSO or D_2O using PMR chemical shift data. Similarly, Schiller and Yan, measuring tyrosine-tryptophan separations of [D-Ala^2, Trp^4]-, [Ala^2, Trp^4]-, and [Trp^4]-methionine enkephalin, report no significant conformational differences [43].

The solution studies suggest that solution conformation of enkephalin and its analogs have some degree of ordered structure, but little difference can be seen between the time-averaged solution conformations of different enkephalin analogs. This is in concert with the recent observation on luliberin (Gn-RH) that a predetermined solution conformation is not required for biological activity [44]. Interpretation of NMR results is hampered by the probability of intermolecular association of enkephalin at the high concentrations used in those studies. However, the fluorescent studies have been conducted at concentrations at least three orders of magnitude more dilute than the NMR studies, and insignificant conformational differences are still observed between biologically active and inactive analogs of enkephalin.

PROPOSED HYDROGEN-BONDING SCHEMES

Structure-activity data discount the necessity of most of the possible intramolecular hydrogen bonding schemes for receptor-bound enkephalin. The proposed 2–5 β-bend schemes are inconsistent with potent [des-Leu^5] and [des-Met^5] enkephalin analogs synthesized by Morgan [45] and by our laboratory (Table II). Various 1–4 β-turns or 2–4γ-turns stabilized by hydrogen bonding to either the amide proton or carbonyl oxygen of Phe^4 can be ruled out as being essential for receptor activation by the potent activity of the N-methyl analog [D-Ala^2, MePhe^4] -leucine enkephalin and by the des-carboxyl, tetrapeptide analogs Tyr-D-Ala-Gly-NMe-$(CH_2)_2$-Ph and Tyr-D-Ala-Gly-NH-$(CH_2)_2$Ph [45]. The hydrogen bonding scheme Tyr^1(OH)-Gly^3 (CO) has been suggested [37], but prevents Tyr^1 of enkephalin from adopting the tyramine conformation seen with morphine and other rigid opiate compounds. Existing analog data do not rule out the remaining hydrogen-bonded schemes, Tyr^1 (CO)-Gly^3(NH) or Tyr^1(NH_3^+)-Gly^3 (CO), as well as possible receptor-peptide hydrogen bonding schemes utilizing some portion of the first three residues of enkephalin.

TABLE II. Relative Activity of Enkephalin Analogs

Compound	$^a EC_{50}$[D-Ala^2, D-Leu^5] / EC_{50} (compound)
Tyr-D-Ala-Gly-Phe-D-Leu	1.00[b]
Morphine·SO_4	0.62
Tyr-D-Ala-Gly-Phe(αMe)-Leu	0.46
Tyr-D-Ala-Gly-Phe(αMe)-Val	0.29
Tyr-D-Ala-Gly-Phe(αMe)	0.16
Tyr-D-Ala-Gly-NH-CH_2-CH_2- 	0.19
Tyr-D-Ala-Gly-NH-CH($CH_2C_6H_{11}$)-CO-D-Leu	0.25
Tyr(αMe)-D-Ala-Gly-Phe-D-Leu	< 0.01
Tyr-D-Ala-Gly	< 0.001
p-tyramine	< 0.0001
Glu(OEt)-D-Ala-Gly-Phe-D-Leu	< 0.001

[a] Effective concentration to inhibit 50% of the amplitude of electrically-stimulated, intact, guinea pig ileum using conditions of I. Creese, S.H. Snyder, J Pharm Exp Ther 14:205 (1975).

[b] All compounds tested in the presence and absence of bacitracin and all active compounds are naloxone-reversible.

Details of peptide syntheses will be published elsewhere.

TOPOGRAPHIC COMPARISON OF THE ENKEPHALIN WITH THE OPIATES

An approach unique to the enkephalins has been to compare the structural similarities between these neuropeptides and the opiates. That such a comparison is reasonable is supported by the following observations: 1) The phenolic group of Tyr^1 is important for activity. Chemical modification of the hydroxyl group by converting it to an O-methyl ether [46] reduces potency analogous to the lowering of activity seen with the O-methylated morphine derivative codeine and the systematically lower activity of O-methylated thebaine derivatives as compared to their phenolic oripavine analogs. Removal of the hydroxyl group giving [Phe^1]-methionine enkephalin almost completely eliminates activity [13]. 2) [des-Amino Tyr^1]-methionine enkephalin is devoid of biological activity [47].

These two observations have prompted comparisons of the tyramine (hydroxyphenylethylamine) portion of morphine and the morphinans with the phenolic side chain of Tyr^1 and its amino terminus. Horn and Rodgers have used crystallographic comparisons of the tyramine moiety in different opiate compounds to quantify the dimensions of this tyramine moiety [11]. However, p-tyramine is inactive as an opiate at concentrations up to 10^{-4}M (Table II), suggesting the tyramine moiety is a necessary but an insufficient condition for opiate recognition and binding. Other sites must play an essential role in opiate recognition.

Furthermore, it has been observed that the tripeptide fragment Tyr-Gly-Gly is inactive, while the tetrapeptide fragment Tyr-Gly-Gly-Phe appears to be the minimal active unit for enkephalin. Although Tyr-Gly-Gly-Phe has 1–3% the activity of MENK [19], proteolytically resistant tetrapeptide analogs such as Tyr-D-Ala-Gly-phenethylamine and Tyr-D-Ala-Gly-Phe(αMe)-OH are potent analogs (Table II). In order to check if proteolytic lability was responsible for the reports of the inactivity of Tyr-Gly-Gly, we synthesized and tested Tyr-D-Ala-Gly (Table II). This peptide was totally inactive at concentrations of 10^{-4}M. Several investigators have related the aromatic side chain of Phe^4 with the 19-phenethyl substituent (F-ring) of PET [12, 14, 41]. We felt that such proposed structural homology did not explain the apparent importance of the fourth residue in enkephalin since many biologically potent morphine, morphinan, and thebaine compounds lack this phenethyl side group. Instead, using 2,9-dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphan (GPA 1657) as a model, we attempted to correspond atoms of the aromatic ring Phe^4 with atoms C5 and C6 of the nonaromatic C-ring of morphine [48]. The considerable activity of [D-Ala^2, L-Cha^4, D-Leu^5] enkephalin (Table II and [49]) and [carboranylalanine^4]-leucine enkephalin [50] supports the assumption that the aromaticity of Phe^4 is not essential.

A minimum of ten rotatable bonds was required to fit the tetrapeptide fragment of enkephalin to an opiate pharmacophore consisting of a phenolic ring, nitrogen atom, and atoms C5 and C6 of the C ring of morphine. The side-chain torsional angles of Tyr^1 were set at $\chi_1 = 197°$ and $\chi_2 = -106°$ to create a tyramine moiety compatible with that of morphine. A systematic search of the remaining eight rotatable bonds using 31° torsional increments was performed, and all conformers were saved which positioned para or meta carbon atoms of the aromatic ring of Phe^4 so as to correspond with atom positions of C5 and C6 of the C ring of morphine. A single conformer was found and a summary of its backbone angles is given in Table I. Based on this conformation, we predicted the activity of N-methyl- and α-methyl-substituted enkephalin analogs [22]. The predictions are consistent with published structure-activity data to date as shown in Table III. It is possible

TABLE III. Comparison of Activities With Predictions Based on Models

Analog	Correct prediction	Analog	Untested prediction
D-Tyr[1]	inactive	L-Phe(αMe)[4]	active
D-Ala[2]	assumed	N-Me-L-Phe[4]	active
L-Ala[2]	active	Aib[3]	inactive
Aib[2]	active	N-Me-Ala[2]	inactive
Pro[2]	inactive	N-Me-D-Ala[2]	active
Sar[2]	active	N-Me-Ala[3]	active
L-Ala[3]	active	N-Me-D-Ala[3]	inactive
D-Ala[3]	inactive	β-Phenyl-Pro[4]	active
Pro[3]	inactive		

that our identification of the opiate pharmacophore and the corresponding chemical groups in enkephalin is incorrect. Furthermore, because of the necessarily coarse incremental scan required for a systematic conformational search, it is also possible that other conformations of enkephalin consistent with our proposed opiate pharmacophore have been overlooked. Conformationally restricted analogs have been synthesized in our laboratory and are currently being evaluated biologically. These analogs should test the assumptions of the model and allow us to refine the precision of our conformational analysis.

Generally, the correspondence between enkephalins and the flexible classes of opiates has been ignored. Some of these flexible opiates do not have a readily identifiable tyramine moiety like that of morphine, and there is suggestive evidence that these compounds either bind in a different fashion [51] or to a different receptor [3] than the rigid opiates. The former possibility has been discussed by Portoghese [51] and more recently by Galt [52]. Clarke et al [53] also have postulated a model by which the different opiate classes and the enkephalins might be accommodated at a single receptor. This group proposed a triangular pharmacophore with a basic nitrogen binding site at the apex with phenyl binding sites on either side. Unique to this proposal is a separate carbonyl binding site to accommodate the nonphenolic meperidine and prodine classes of compounds. Enkephalin is envisioned to adopt a 2–5 βII′ bend at the receptor with the carbonyl of Tyr[1] corresponding to the carbonyl binding site for the meperidines. The first three residues of the pentapeptide are thought to occupy the pharmacophore for morphine, the phenolic benzomorphans, and morphinans; and the Met[5] carboxylate anion is deemed to be important for stabilizing the receptor-bound conformation. Structure-activity data suggest that this latter requirement by Clarke et al is not critical for enkephalin recognition; some C-terminal amide analogs as well as the previously mentioned tetrapeptide analogs have biological activity which exceed that of morphine. Furthermore, the proteolytically resistant tripeptide fragment of enkephalin Tyr-D-Ala-Gly would fit the pharmacophore for morphine proposed by Clarke et al but is totally inactive. On the other hand, Tyr-D-Ala-Gly-(benzyl ester) is weakly active [54], suggesting that the receptor site for enkephalin must recognize some portion of the fourth residue of the peptide. The first position of the potent enkephalin derivative [D-Ala[2], D-Leu[5]]-LENK was substituted by L-Glu(OEt) as a homolog of the meperidines (Table II). It is totally inactive suggesting that substitution of carbonyl π electrons for the phenolic ring [55] is not straightforward (Table II).

CONCLUSIONS

For flexible molecules such as the enkephalins, solid state, solution state, and theoretical energy calculations contribute little to the elucidation of the receptor-bound conformation of these peptides. This is because peptide–receptor interactions are likely to mutually induce conformational changes which are not accounted for by any current conformational technique. Solution conformational studies of enkephalin are of intrinsic interest, but only a subset of the solution conformeric ensemble need be recognized by the receptor. This subset need not be a highly probable state in solution and need not predominate in the time-averaged conformation detected by spectroscopy.

The inability of these conformational studies to propose, a priori, conformations of receptor-bound enkephalin consistent with structure-activity data suggests that an alternative approach might be more useful. The serendipitous existence of classes of conformationally rigid and semirigid opiate alkaloids which act at the same in vitro receptors as the flexible enkephalin peptides can be exploited by using these opiate alkaloids as steric probes to "map" the molecular recognition site of opiate receptor. Structure-activity data of analogs of enkephalin can be used to identify the important chemical moieties in these neuropeptides which correspond to the chemical groups defining the opiate pharmacophore. Finally, one can systematically explore all the possible conformations of conformationally restricted analogs of enkephalin and identify those conformations which are consistent with the spatial topography of the opiate pharmacophore. The ultimate success of such an approach requires that all compounds are evaluated in a biological system where they are known to act competitively on homogeneous class of receptors.

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