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Chemistry

Energy conformation study of Met-enkephalin and its D-Ala^2 analogue and their resemblance to rigid opiates

(opiate-like peptides/tyramine overlap/low-energy conformer/induced fit)

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ABSTRACT **Conformational similarities of Met-enkephalin and its D-Ala^2 analogue to rigid opiates were studied by both empirical and quantum mechanical methods. By both methods, conformers with maximum resemblance to rigid opiates have the highest energies. Conformers with the lowest energy had no resemblance to rigid opiates. However, several low and intermediate energy conformers were identified in which at least the NH_2-terminal tyrosine residue overlaps with the phenethylamine moiety of rigid opiates and which could equally well accommodate either Gly^2 or D-Ala^2. The conformer among these with greatest additional resemblance to other binding sites of rigid opiates is proposed as the most likely candidate for an induced fit at the receptor site.**

Two endogenous pentapeptides with morphine-like activity have been isolated from mammalian brain (1, 2). These pentapeptides have been characterized as methionine-enkephalin and leucine-enkephalin, and have the amino acid sequence Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu, respectively. Both pentapeptides mimic the actions of morphine in the guinea pig ileum (1, 2) and the mouse vas deferens assays (3), exhibit cross tolerance to morphine (4), and inhibit the stereospecific receptor binding of [^{3}H]naloxone in rat brain homogenates (5). In addition, intracerebroventricular injections of enkephalin can produce transient analgesia (6, 7) which can be blocked by the opiate antagonist naloxone (8). These findings, plus the fact that enkephalin appears to be localized in synaptosomal fractions that are rich in nerve terminals (9), provide evidence that the enkephalins may be involved in analgesia or pain suppression.

Since the enkephalins and rigid opiates appear to compete for the same receptors, it would seem that there must be chemical and conformational similarity between these two different classes of opiates. The most striking feature common to both the enkephalins and the rigid opiates is the presence of a *p*-OH phenethylamine (tyramine) moiety formed by the terminal amino group and the tyrosine residue. It has been established by structure-activity studies that for potent opiate activity the tyrosine residue is critical and, in common with rigid opiates, the amine and *p*-OH groups must remain intact (10).

Initial conformational comparisons with rigid opiates were done by model building and comparing overlaps of critical regions of Met-enkephalin and 7-[1-phenyl-3-hydroxybutyl-3-]endothenotetrahydrothebaine (PET) (11). More recently, extensive conformational analyses of Met-enkephalin have been made by means of empirical energy calculations (12, 13). Of the 52 conformations reported (12), the lowest energy structures, i.e., those with $\Delta E \leq 2.5$ kcal/mol, were found to be G-PβII′-type bends with the glycine^3 and phenylalanine^4 taken as the central residues. Many other conformers were in the relative energy range of $5 < \Delta E < 11$ kcal/mol.

The lowest energy structure was found to be stabilized by a hydrogen bond between the tyrosine OH group and the glycine^3 backbone C=O group. This low energy structure is consistent with chemical shifts and coupling constants in reported nuclear magnetic resonance studies of Met-enkephalin (14, 15). Both types of studies indicate a rigidity for the backbone of the last three residues, but considerable flexibility for the tyrosine backbone angles and side chain angles. A G-G β bend, as originally proposed (11), is not consistent with the reported nuclear magnetic resonance data. However, previous work of Ramachandran and his coworkers (16) and others (17, 18) has emphasized the tendency of Gly^2-Gly^3 sequences in particular to form just such bends in proteins.

While valuable in describing numerous low and medium energy conformers of Met-enkephalin, none of the optimized conformers previously reported (12) nor any of the nuclear magnetic resonance structures obtained have significant spatial overlap with any functional groups in morphine-like opiates thought to be crucial to opiate activity (19). These conformers could, however, be altered at the receptor site by an induced fit allowing enhanced resemblance to rigid opiates and hence, presumably, enhanced interaction with the receptor. Energy required for such conformational change could be provided by the increased interaction energy.

In this paper we report energy-conformation studies, using both empirical and quantum mechanical methods, to determine the energy of Met-enkephalin conformers with varying degrees of similarity to rigid opiates that could also accommodate D-Ala^2 but not L-Ala^2 in place of the Gly^2 residue. This second criterion is consistent with the observation of the essential retention of activity when Gly^2 is replaced by D-Ala (20) but a large decrease of receptor affinity upon replacement by L-Ala (21). In fact, as has recently been pointed out (22), this preference for D-Ala^2 could have been predicted from Ramachandran's group's work (16), which shows that D-Ala^2-Gly^3 as well as Gly^2-Gly^3 sequences tend to form β turns.

Starting with the 52 low and medium energy conformers previously obtained (12), successive imposition of these criteria allowed the selection of a small number of low energy conformers as most likely candidates for receptor site interaction. While the empirical and quantum mechanical energy calculations did not give identical results, a number of likely candidates common to both methods were obtained. Both methods also showed a trend towards higher energies for conformers with increasing resemblance to rigid opiates.

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Abbreviations: PET, 7-[1-phenyl-3-hydroxybutyl-3-]endothenotetrahydrothebaine; ECEPP, Empirical Conformation Energy Program for Peptides; PCILO, Perturbative Configuration Interaction using Localized Orbitals.

METHODS

The conformational energy calculations reported here were performed with the Empirical Conformational Energy Program for Peptides (ECEPP) and the Perturbative Configuration Interaction using Localized Orbitals (PCILO) methods of quantum chemistry. In both methods standard residue geometries, supplied with ECEPP, were used. The end groups were considered to be uncharged NH_2 and COOH groups and solvent effects were not included. The nomenclature and conventions used are those adopted by an IUPAC-IUB Commission (23). In the ECEPP method, the empirical potential energy function and energy parameters are those described by Momany *et al.* (24). Energy minimizations were carried out by a quasi-Newton method (25), which uses a gradient search. The convergence criterion was 0.01 kcal/mol. Local minima were also found by parabolic fits to sequentially varied sets of torsion angles.

The PCILO program used was developed by Diner *et al.* (26), and has been successfully applied to a number of structure activity studies of drugs and conformations of dipeptides (27) and nucleotides (28). In this method, local minima were found only by parabolic fits.

Preliminary calculations by both methods were made for the dipeptide NH_2-Tyr-Gly-COOH. Tyrosine side chain angles were varied, pairwise variations of backbone angles ($\phi_i \Psi_i$) were made, and local minima were obtained. Comparisons of the results obtained indicated that empirical ECEPP calculations are orders of magnitude more rapid than the quantum mechanical PCILO method. Local minima with the empirical method are also local minima with the PCILO method, but the reverse is not necessarily true. The calculated minima are much steeper with the ECEPP method and barriers between them are often nearly discontinuous ($\Delta E \geq 10^5$ kcal/mol). PCILO yields more gradually varying minima with smaller and smoother barriers between them.

Based on these comparisons, the following procedure, making optimum use of both methods, was used. The faster ECEPP method was used to obtain totally optimized conformations and energies for each conformer considered. The PCILO method was then used as an alternate method to calculate the energies of the totally optimized conformations obtained from ECEPP calculations after it was determined, for a number of trial conformers, that they are also local minima by the PCILO method. For important features such as the tyramine (*p*-OH phenethylamine) overlap with morphine, local minima were obtained by both the ECEPP and PCILO methods.

The search for candidate conformers of Met-enkephalin that resemble rigid opiates was made in three ways. In each case the goal was the selection of the lowest energy conformers that are local minima with at least tyramine-overlap with rigid opiates and that could accommodate both Gly^2 and D-Ala^2 (but not L-Ala^2) in similar conformations.

The first approach was to calculate the energy of a series of conformers beginning with one that had maximum overlap with PET and relaxing it to one with only tyramine overlap. The conformer with maximum overlap is shown in Fig. 1. It was constructed by starting with the overlap of the terminal amine group and tyrosine side chain with the phenethylamine moiety of PET and then proceeding to overlap the glycine residues with the B and C rings, so that carbonyl carbons and oxygens of the pentapeptide backbone matched corresponding functional groups in the rigid opiate. These overlaps required that ω_1 have the anomalous value of 0° rather than the standard value of 180°.

The other two approaches used the 52 optimized structures

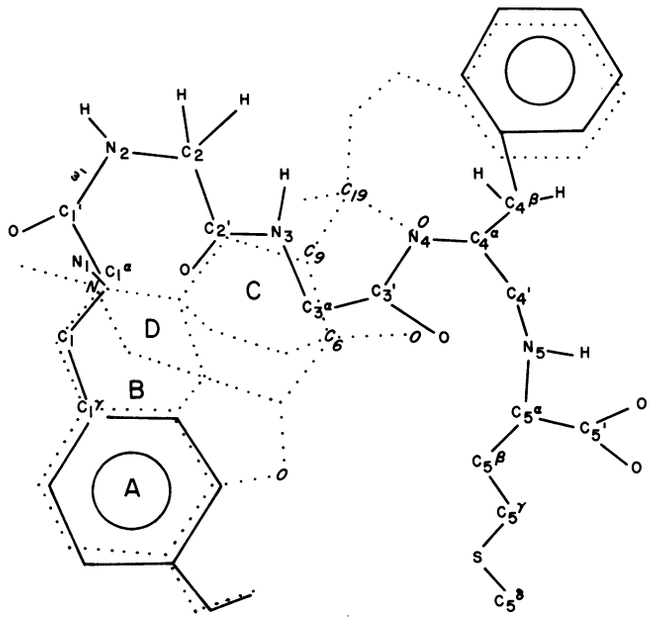

FIG. 1. A conformer of Met-enkephalin constructed by matching overlap of the NH_2-Tyr-Gly-Gly residues with the tyramine, B, C ring positions of PET. In this conformer $\omega_1 = 0$ and the phenylalanine side chain overlaps with the C_{19} substituent of PET, but the methionine residue has no analogue region in PET.

for Met-enkephalin previously obtained by means of empirical energy calculations (12). Values for the 24 torsion angles that define each structure were kindly given to us by H. A. Scheraga.

In one procedure, a morphine overlap with the tyramine moiety ($\chi_1 \approx -90°$, $\chi_2 \approx 180°$) was imposed on the 52 previously optimized Met-enkephalin conformers (12) and their energies recalculated by the ECEPP and PCILO methods. Conformers that had excitation energies less than 30 kcal/mol by the ECEPP method were reoptimized first with the constraint that the tyramine overlap remain fixed, and then allowing the tyrosine side-chain angles to relax to a local minimum. D-Ala and L-Ala were substituted for Gly^2 in the 22 lowest energy conformers with morphine overlap and the energies were recalculated by ECEPP.

In the other approach, D-Ala^2 was substituted for Gly^2 in the optimized Met-enkephalin structures. These analogues were reoptimized with and without the constraint of morphine overlap of the tyramine moiety. To obtain Met-enkephalin conformers similar to these D-Ala^2 analogues, we reinserted the Gly^2 residue and again minimized the conformations with and without morphine overlap. The results of this series of calculations gave four sets of similar Gly^2 and D-Ala^2 conformers with and without morphine overlap. The PCILO method was also used to calculate the relative energies of these four sets of conformers optimized by the ECEPP method.

RESULTS

As shown in Table 1, the conformer with maximum overlap with PET gave extremely high energies by both the ECEPP and PCILO methods. These high energies are due to the crowding of the terminal nitrogen and hydrogen atoms with the Gly^2 carbonyl carbon and oxygen atoms. Relaxation of the Tyr^1 and Gly^2 backbone angles to a local minima, while maintaining $\omega_1 = 0°$, decreased the energy significantly. Step-wise relaxation of another starting structure with ω angles $\sim 180°$ yielded a series of relatively high energy structures by both methods.

Imposition of the morphine overlap on the tyramine moiety

Table 1. Energy of enkephalin conformers with maximum overlap with the rigid opiate PET

Conformer	ΔE[a]	ΔE[b]
Maximum PET overlap ($\omega_1 = 0$)[c]	$\sim 10^7$	613
Relaxed PET overlap ($\omega_1 = 0$)[c]	135	25
Modeled GG II′ ($\omega_1 = 180°$)[c]	628	—[d]
Modified GG II′ (no optimization)[c]	119	—[d]
Optimized GG II′ (Ψ_1, $\chi_1\chi_2$ fixed[e]	25.5	14.4
Optimized GG II′($\chi_1\chi_2$ fixed)[f]	17.3	9.7
Totally optimized GG I′ ($\chi_1\chi_2 = -110, -130$)[g]	13.3	10.7
Totally optimized GG II′ ($\chi_1\chi_2 = -168, -100$)[h]	6.5	5.4

[a] ΔE in kcal/mol was calculated by the ECEPP method.
[b] ΔE in kcal/mol was calculated by the PCILO method.
[c] These conformers were chosen by similarity to PET and were not optimized (Fig. 1).
[d] Energy not calculated.
[e] Optimized GG II′ keeping Ψ_1, χ_1, and χ_2 at values that mimicked PET.
[f] Optimized GG II′ letting Ψ_1 relax.
[g] Totally optimized GG bend that gave closest tyramine overlap.
[h] Lowest energy GG bend. It has no similarity to PET.

of the 52 previously optimized Met-enkephalin conformers (12) resulted in the 22 conformers ([Gly^2]morphine) listed in Table 2 with energies in the range of 9–20 kcal/mol above the lowest energy conformer without the imposed overlap, whose energies are also given for comparison. A number of conformers, among them the lowest energy one, were altered substantially to obtain the best tyramine overlap, and for some the tyramine overlap was not a local minimum.

Related energies calculated by the PCILO method for the same 22 Met-enkephalin conformers both in their original conformations (Gly^2) and for those optimized with morphine overlap ([Gly^2]morphine) are also given in Table 2. The PCILO method gives much smaller energy differences among conformers and hence many more low energy forms of Met-enkephalin. Also, by the PCILO method, much less energy is required for morphine overlap of the tyramine moiety.

Substitution of D-Ala and L-Ala for Gly^2 in the 22 conformers with morphine overlap result in only 12 that accommodate D-Ala^2 better than L-Ala^2 (Table 2). Neither of the surviving lowest energy conformers (35 and 50) have a G-G or G-P bend nor do they overlap with other regions of potent morphine-like opiates.

Optimization of D-Ala^2 analogues of Met-enkephalin by the ECEPP method led to a different energy ordering than for the Gly^2 analogue shown in Table 2. Conformers 7, 15, 16, and 24 changed substantially when reoptimized with D-Ala^2 and are labeled 7′, 15′, 16′, and 24′. For these conformers, Gly^2 was substituted back for D-Ala and the conformations were reoptimized to obtain similar new Gly^2 conformations. Fig. 2 gives

Table 2. Calculated energies of selected Met-enkephalin conformers

	Gly^2		[Gly^2]morphine[b]		
Conformer[a]	ΔE[d]	ΔE[e]	ΔE[f]	ΔE[g]	D-Ala^2[c]
7[h]	0	1.4	8.5 (d,c)	2.5	No
11[h]	3.5	1.6	13.6 (d,c)	8.2	No
15[i]	5.2	0.0	14.9 (d,c)	6.5	No
44[j]	5.8	1.6	14.5 (d,cn)	7.4	No
19[h]	6.5	1.4	11.6 (d,c)	5.3	No
16[k]	7.5	4.2	15.6 (m,c)	4.2	Yes
24[h]	7.7	1.8	11.7 (s,c)	4.2	No
29[k]	7.7	2.5	12.3 (s,nc)	5.5	No
17[k]	7.8	2.8	14.5 (s,c)	3.0	Yes
31[k]	7.8	3.8	15.1 (s,c)	7.2	Yes
32[k]	8.0	4.3	14.3 (s,nc)	7.5	No
50[j]	8.3	0.0	13.7 (s,nc)	1.0	Yes
34[k]	8.3	5.2	14.6 (s,nc)	7.8	Yes
1[l]	8.8	0.9	16.0 (s,nc)	1.5	Yes
42[i]	9.5	1.8	18.5 (d,c)	7.9	Yes
35[k]	9.6	4.1	13.1 (s,c)	7.0	Yes
52[i]	10.7	4.2	14.3 (d,c)	5.6	Yes
38[k]	10.8	4.2	16.0 (s,nc)	5.0	Yes
43[i]	10.8	3.6	19.6 (s,c)	6.7	Yes
2[l]	10.8	5.2	18.1 (s,c)	3.9	No
3[l]	10.9	3.1	17.0 (s,nc)	3.6	No
39[k]	10.9	6.9	16.1 (s,nc)	8.5	Yes

[a] Original conformers that best accommodate tyramine overlap with morphine. Numbered according to Isogai *et al.* (12).
[b] Energy in kcal/mol of reoptimized Met-enkephalin conformers with tyramine overlap relaxed to nearest local minimum.
[c] Energies were calculated for D-Ala^2 and L-Ala^2 analogues of Met-enkephalin without reoptimization. This column indicates whether or not the D-Ala^2 was accommodated better than the L-Ala for each conformer.
[d] Energy of optimized Met-enkephalin conformers in kcal/mol (ref. 12 and unpublished work) relative to lowest energy conformer (no. 7) with $\Delta E = -3.1$ kcal/mol.
[e] Energy of Met-enkephalin conformers in kcal/mol calculated by the PCILO method relative to minimum energy conformer 15.
[f] Energies calculated by ECEPP are relative to lowest energy conformer 7, without overlap. Letters in parentheses indicate whether new conformer is the same (s) or different (d) from the original without tyramine overlap and whether (c) or not (nc) it had a local minimum with this overlap.
[g] Energies calculated by PCILO are with reference to conformer 15 without tyramine overlap.
[h] Conformers with initial G-P bends.
[i] No bend, conformers derived from model building.
[j] No bend, conformers derived from lowest energy single residues.
[k] Conformers with initial G-G bends
[l] Reference conformers: 1 = α helix, 2 = extended chain, and 3 = C_7 equatorial.

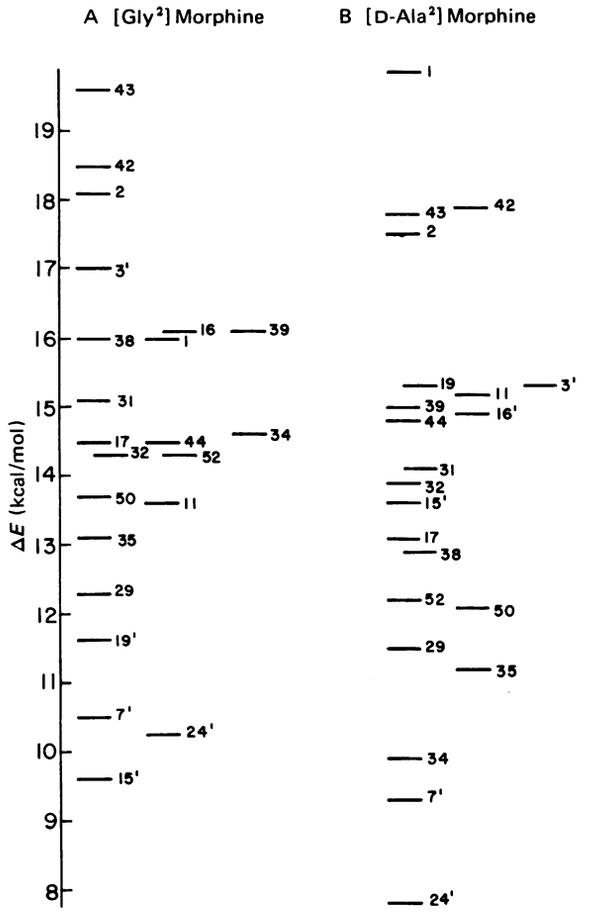

FIG. 2. Calculated empirical energies for optimized Met-enkephalin and similar D-Ala² analogues with the imposed rigid opiate overlap of the side-chain angles in the tyrosine residue. (*A*) Met-enkephalin; ΔE is relative to the lowest energy conformer reported in ref. 12. (*B*) D-Ala² analogues of Met-enkephalin; ΔE is relative to the lowest energy analogue calculated in this study.

the energies of these optimized D-Ala² and Gly² analogues of Met-enkephalin reoptimized with the constraint of having tyramine overlap with rigid opiates. Relative energies from PCILO calculations of these same conformers, i.e, similar D-Ala² and Gly² analogues with tyramine overlap, are given in Fig. 3.

DISCUSSION

While both the ECEPP and PCILO methods give qualitatively similar results in many instances, the comparative studies made reveal a number of obvious differences between them due mainly to the fact that the empirical method is parameterized to yield peptide conformations normally found in proteins. Thus: (*i*) backbone bends rather than extended conformers are favored; (*ii*) side-chain angles of each residue that have been observed in x-ray structures of proteins are favored and; (*iii*) local minima are steep with high energy barriers between them. The PCILO method has no such adjustments built into it and the same criteria are applied to the energetics of all conformers. As a result, for example, regular repeating conformers such as extended chain conformers 2 and 50, α-helix conformer 1, and C_7-bend conformer 3 are more energetically favored by PCILO than ECEPP. Also, changes in energy with side-chain angle variations are more monotonic with less severe energy penalty for conformers that do not mimic proteins such as those favoring tyramine overlap ($\chi^1 \simeq -90°$, $\chi^2 \simeq 180°$) with rigid opiates.

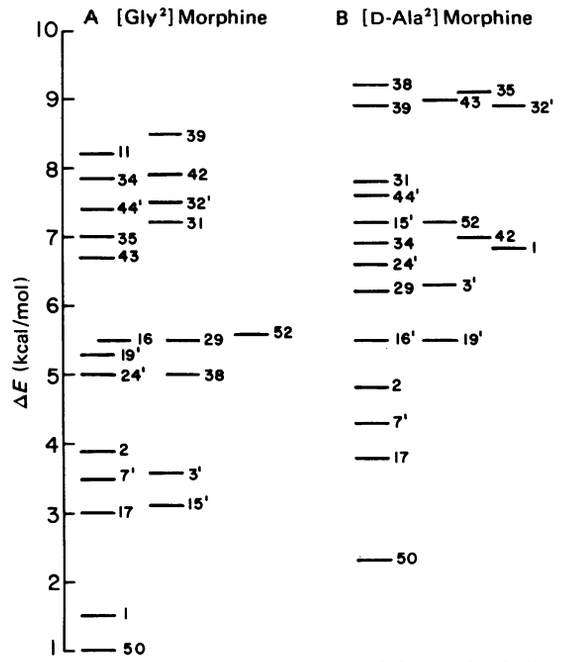

FIG. 3. Relative energies of optimized Met-enkephalin conformers and similar D-Ala² analogues with morphine overlap in the tyramine moiety calculated by PCILO. (*A*) Gly² conformers; ΔE is relative to minimum energy conformer 15′ without the morphine overlap. (*B*) D-Ala² analogues; ΔE is relative to 16′ without the morphine overlap.

Despite these differences, systematic comparisons between the methods allow a common pool of most likely conformers at the receptor site to be chosen.

Selection of likely conformers of Met-enkephalin for interaction at the opiate receptor site depends on the requirements for structural similarities with potent opiates and the energy available for conformational change (Table 3). The tyramine moiety has been shown to be important by numerous structure activity studies, but is not the only necessary feature since the synthetic tripeptide Tyr-Gly-Gly is inactive. The inactivity of the tripeptide implies that secondary binding sites that may not totally overlap with those of rigid opiates are an important feature of Met-enkephalin activity. Thus, conformers of Met-enkephalin that maximally mimic rigid opiates, while of rather high energy by both methods, cannot be totally ruled out by accommodation at the receptor site. The results obtained,

Table 3. Relative energies of Met-enkephalin conformers with increasing overlap with morphine-like opiates

Description[a]	ΔE[b] (ECEPP)	ΔE[c] (PCILO)
No D-Ala², no tyramine	0 (7)[d]	0 (15)
D-Ala² fit, no tyramine	1.5 (15′)	−0.5 (16′)
D-Ala fit, tyramine	9.6 (5′)	2.3 (50)
D-Ala fit, minimal PET overlap	14.5 (17)	3.8 (17)
Increased PET overlap ($\omega_1 = 180°$)	25.5	14.4
Constrained PET overlap ($\omega_1 = 0°$[e])	135.0	25.0

[a] This table is organized to show the energy expenditure required as the lowest energy [Gly²]-Met-enkephalin conformers are progressively changed in order to accommodate D-Ala², the tyramine overlap, and increased mimicking of the rigid opiate PET.
[b] ΔE in kcal/mol relative to the original conformer number 7.
[c] ΔE in kcal/mol relative to the original conformer number 15.
[d] Numbers in parentheses refer to conformers defined in Table 1.
[e] A modeling attempt with $\omega = 0°$ that was made in order to mimic as much as possible of the rigid opiate PET (Fig. 1).

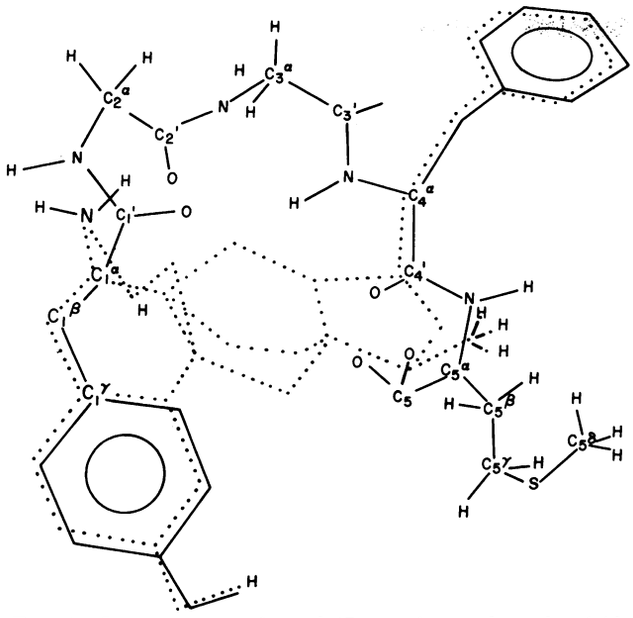

FIG. 4. Low energy conformed 17 superimposed on the rigid opiate PET to show overlap of the various moieties. In particular, these are overlap of the phenethylamine moieties; overlap of the phenylalanine side chain with the phenethyl C_{19} substituent of PET; and close proximity of the methionine backbone C=O and side chain with the C_6 methoxy group of PET.

coupled with structure activity studies, indicate that relatively low energy conformers such as 17 and 16′, which still retain similarity to PET beyond the region of tyramine overlap, are the most likely candidates for an induced fit by interaction at the receptor site. PCILO results particularly favor 17 as a very low energy conformer with ΔE = 3.0 and 3.8 kcal/mol for the Gly^2 and D-Ala^2 analogues, respectively. Fig. 4 shows the overlap of this structure with PET. Not only do the important phenethylamine moieties overlap, but the phenylalanine side chain overlaps with the phenethyl C_{19} substituent of PET and the methionine backbone C=O group and side chain are in the region of the C_6 methoxy group of PET. None of the other relatively low energy conformers, such 7′, 24′, and 50′, obtained by either method have an appreciable overlap with PET beyond the initial overlap with the tyramine moiety. Thus conformer 17, very similar to the one previously proposed by model building (11), is confirmed by the PCILO results as a very likely candidate at the receptor site.

CONCLUSION

The energy-conformation studies reported here for Met-enkephalin and its D-Ala^2 analogue indicate that low energy conformer 17 is accessible at the opiate receptor with a modest energy expenditure of 3.5 kcal/mol. This conformer allows overlap of Met-enkephalin with critical regions of the potent rigid opiate PET (Fig. 4). Other low energy structures do not share this resemblance, while structures with significantly greater overlap (Fig. 1) have much higher energies. Thus, if resemblance to rigid opiates is important and extremely large pertubations of conformation at the receptor site do not occur, conformers like 17 are most likely to be involved in interactions with the receptor. An implication from this study is that Met-enkephalin is binding by an induced fit intiated at the crucial tyramine region, followed by binding of the COOH-terminal residues. This mode is similar to a proposed "zipper" mechanism (29) or the concept of a dynamic pharmacophone (30) rather than a lock and key mode more appropriate for rigid opiates.

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