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PROPOSALS FOR CONFORMATION OF ENKEPHALINS RELATED TO OPIATE μ-PHARMACOPHORE

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SUMMARY

Conformational similarities between morphine and the enkephalin analogue Tyr-D-Ala-Gly-Phe which interact preferentially with opiate μ-receptors were investigated using a constrained energy minimization procedure. This method takes into account several structural features of morphine-like substances including enkephalin analogues and uses them to search for conformations of peptides exhibiting low energies and good similarity with the μ-opiate pharmacophore. This latter involves as critical components the A-ring, the N-atom of D-ring and the C_6-O_2 bond is morphine which correspond to the N-terminal tyrosine moiety and the Gly^3-CO group in Tyr-D-Ala-Gly-Phe respectively. Several low energy conformers present a good similarity with rigid opiates and are consistent with activity of sterically constrained enkephalins. Conformational changes of peptides from solid or solvated states to μ-receptors bound state involve a transconformational binding process.

INTRODUCTION

The discovery in brain extracts (1) of endogenous pentapeptides, enkephalins, which exhibit morphine-like properties has induced considerable interest about the activity (see 2 and 3 for reviews) and conformational properties (4-7) of these opioid peptides. Thus, a large amount of works has been devoted to the synthesis of peptide analogues in order to investigate the structural components required for opioid activity (2,3). Moreover, as enkephalins and "rigid" opiates seem to compete for the same receptors (8), it is reasonable to suppose the existence of chemical and conformational similarities between these two classes of compounds. Nevertheless, this assumption is complicated by the existence of at least two kinds of binding sites for enkephalins in brain (8-10) as well as in peripheric organs (11). From displacement experiments using radiolabelled opiates and enkephalins and from pharmacological assays it was established that the low affinity binding site (K_D = 5-10 nM)

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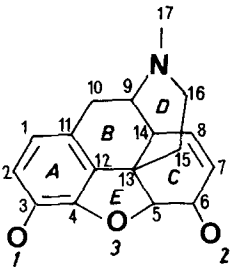

Figure 1. Morphine structure and atom numbering.

for enkephalins corresponds to the binding sites of morphinic substances called μ-receptors (8). The various studies on the topological analogy between enkephalins and morphine-like compounds have been recently discussed by Gorin et al. (12). However none of these studies have taken into account the heterogeneity of enkephalin-receptors. Moreover, it has been pointed out from theoretical work (13) that the conformations of enkephalins which mimic the stereochemistry of morphine are obtained with a loss of conformational stability. Therefore, the hypothesis of a morphine-enkephalins structural relationship must be taken with caution and needs further support from both experimental and theoretical approaches.

As a contribution to this problem, we present an original conformational analysis allowing to find energy minima for peptide conformations which also evidence the main structural features observed in morphine-like opiates. The most important starting hypothesis for these calculations were our findings that the removal of the phenylalanine moiety in enkephalin analogues leads to μ-specific agonists and that the carbonyl of the Gly^3 residue is an absolute requirement for morphine-like activity (14). Results have been obtained using a constrained energy minimization procedure working with the set of (ϕ, ψ, χ) angles in the model sequence Tyr-D-Ala-Gly-Phe. This tetrapeptide is a very potent analog of enkephalins and exhibits a high preference for the μ-opiate receptors (15).

METHOD

Topological requirements for the μ-opiate pharmacophore in enkephalins. Several models of the opiate pharmacophore have been proposed (16-18,12). All these studies suggested that opiate activity essentially requires the correct spatial disposition of the phenolic A-ring and ammonium group (see fig. 1 for definitions). However, from the most recent structure-activity studies, these requirements appear necessary but not sufficient. Additional atoms of the morphine C-ring could thus provide another site for the essential pharmacophore. So, Gorin et al. (12,19) assume that the phenolic side chain

Table 1. Dihedral angles (°) and relative computed energies for the most stable conformations (B) of Tyr-D-Ala-Gly-Phe fitting the μ-opiate pharmacophore.

Forms	TYR 1			D-ALA 2		GLY 3		PHE 4			$\Delta E^{a)}$
	χ_1^1	χ_1^2	ψ_1	ϕ^2	ψ^2	ϕ^3	ψ^3	ϕ^4	χ_4^1	χ_4^2	
1A[b)]	63	70	170	74	11	90	0	-71	168	70	-
1B	166	84	-75	118	74	108	41	-173	-174	75	0
2A[c)]	-43	-89	126	59	25	97	-7	145	-62	90	-
2B	28	-101	64	38	43	-162	52	-91	-51	112	0
3A[d)]	-106	-163	129	160	-87	-118	98	-87	-87	-56	-
3B	-175	-129	151	-176	-68	-85	127	-48	-78	-23	0.9
4A[e)]	30	-20	-150	60	30	160	-70	50	10	-50	-
4B	-162	-140	-176	109	37	133	-99	-33	56	80	3.9

a) Relative energies (in Kcal/mole) between the computed fitted conformations 1B - 4B, with E 1B ~ 2B = -30.5 Kcal/mole. b) Starting values from RX of Tyr-Gly-Gly-Phe (28). c) Starting values from RX of Leu-enkephalin (29). d) Starting values from computed fitted conformation of Tyr-D-Ala-Gly-Phe (12). e) Starting values from NMR and theoretical study of Tyr-D-Ala-Gly-Phe (27).

and nitrogen terminus of the Tyr residue in enkephalins correspond to the tyramine moiety of morphine and that the C-ring of the alkaloid and the aromatic Phe^4 side chain in peptides are in close correspondence in the opiate pharmacophore. More accurately, the C_5-C_6 atoms of the C-ring and the para or meta C-atoms of the Phe^4 ring could interact with the same subsite in the opiate-receptors. In fact, the removal of the phenylalanine ring does not change the potency of enkephalin analogues whereas the only reduction of the CO group of Gly^3 in the highly potent tetrapeptide Tyr-D-Ala-Gly-Phe leads to a complete loss of activity (14). Therefore the C_6O_2 bond in morphine could correspond to the CO bond of Gly^3 in enkephalins. Such a crucial role for an oxygen atom in opiates have already been assumed (20,21). Consequently, the following correspondence between Tyr-D-Ala-Gly-Phe and morphine was searched for during the present conformational analysis.

OH group and aromatic ring of Tyr → A-ring.
N-terminal nitrogen in peptide → N-atom of the D-ring.
CO carbonyl bond of Gly^3 → C_6-O_2 bond of the C-ring.

Theoretical conformational analysis.

The nomenclature and conventions adopted by the IUPAC-IUB Commission (22) were used. The potential functions taken for the conformational energy calculations are those previously used in our Monte-Carlo calculations on Enkephalin (23). A standard geometry is given to aminoacids and atomic coordinates associated to each (ϕ, ψ, χ) set of dihedral angles are calculated. All dihedral angles are taken as variables for the minimization procedure except those around the peptide bonds (ω) which are maintained trans-planar. Charged NH_3^+ and COO^- groups are taken as end-groups, and solvent effects are not included in the present calculations.

The constrained energy minimization is performed using the CONMIN procedure of Haarhoff et al. (24) which has been found to be one of the most efficient for constrained minimization problems in conformational analysis (25). The constraint function is obtained from the "distance", in a least-square

Table 2. Distances (Å) between the crucial components of the μ-opiate pharmacophore in computed conformations of Tyr-D-Ala-Gly-Phe and in morphine (26).

Conformations	$H_3\underline{N}^+...\underline{C}OO^-$	$H_3\underline{N}^+...Tyr^{a)}$	$H_3\underline{N}^+...\underline{O}CGly^3$	$Gly^3\underline{C}\underline{O}...Tyr^{a)}$
1B	6.0	5.1	7.8	4.4
2B	7.8	3.4	5.4	4.6
3B	9.5	5.1	6.9	4.6
4B[b)]	6.4	5.1	6.9	4.7
		$CH_3\underline{N}^+...Phenol^{a)}$	$CH_3\underline{N}^+...\underline{O}_2\text{-}C_6$	$C_6\underline{O}_2...Phenol^{a)}$
morphine		4.7	6.5	4.7

a) Ring center. b) Atomic coordinates of this model are available on request.

sense, between selected atoms in the tetrapeptide and morphine as described above. The atomic coordinates of morphine are taken from Gylbert (26). The atomic coordinates of both peptide and morphine molecules are calculated in the same axes system (origin at the center of the phenolic ring, X and Y axes in the plane of this ring, Z axis perpendicular to this plane).

The conformations used for starting the constrained minimization are taken from litterature (Table I) or from another study (27) on the conformational properties in solution of peptides with sequences Tyr-X-Gly-Phe-Y where X can be L-Ala, D-Ala or Gly and Y is a carboxy, carbomethoxy or H (decarboxylated peptide) substituent.

RESULTS AND DISCUSSION

Results of the calculations are summarized in Table 1, which presents the most stable conformations obtained among all the different fitted conformations. Firstly it appears that several good conformations exist which can equally well fit the spatial structural requirements defined presently as the essential features of the opiate pharmacophore. This is evidenced by the close correspondence between the crucial distances in the different conformers (Table 2). Although these conformations show similarities as concerns the overall shape of the N-terminal Tyr residue which fits the tyramine moiety of "rigid" opiates, the remaining parts of the molecules show different behaviours.

The conformation of lowest energy 1B is obtained starting from the (ϕ, ψ, χ) dihedral angles found for the Tyr-Gly-Gly-Phe peptide 1A in both solution and solid state (30). The computed conformation 1B presents a compact folded structure stabilized by favourable $NH_3^+...COO^-$ ionic interaction (N...C end-to-end distance of about 6 Å) and a weak 4 → 1 β-turn tendency. The Tyr^1 and Phe^4 aromatic side chains are very near with a distance of about 6 Å between the ring centers. This model results from a large modification of the ψ_1 angle in

conformation 1A leading to a completely different orientation of the tyrosine side chain but keeping the remaining backbone almost unmodified.

Another minimum of similar energy 2B is obtained starting from the (ϕ, ψ, χ) dihedral angles taken from an X-ray diffraction study on Leu-enkephalin (29). It must be noted that two different conformers have been found in the crystal structure, and that low-energy minimum is reached from only one of these conformers. The conformation resulting from the constrained energy minimization procedure is more extended that the previous minimum (N...C end-to-end distance of about 8 Å), but the relative position of the aromatic side-chains remain the same, with a rings centers distance of 6 Å. As compared to the conformation 1B the model 2B corresponds to changes in the ψ_1 and ϕ_3, ψ_3 dihedral angles.

The third most favourable conformation is obtained starting from the (ϕ, ψ,χ) values proposed by Gorin et al. (12) for the receptor-bound conformation of enkephalins. This conformer presents a more extended conformation for the peptide backbone than the two previous ones (N...C end-to-end distance of about 10 Å). This is mainly due to the conformation of the D-Ala^2-Gly^3 residues for which the dihedral angles (ϕ, ψ) must appear in the β-region of the Ramachandran ernergy-map. As expected, this third model 3B is closely related to the computed active conformation 3A of Gorin et al. (12) but with a change around the Gly^3 residue. This feature results from the change in the imposed fit between our model (Gly^{3}CO corresponding to C_6O_2 of morphine) as compared to that of Gorin.

Another favourable conformation can be obtained from the dihedral angles determined for Tyr-D-Ala-Gly-Phe in solution by ^{1}H NMR spectroscopy (27). The calculated conformation 4B is obtained essentially by a change of the backbone around the Gly^3 residue. It can be observed that this structure is also highly folded but does not exhibit the favourable orientation of the NH_3^+ and COO^- groups required for electrostatic interactions.

All the proposed conformers (1B-4B) correspond only to secondary minima of the conformational energy surface which are obtained when constraints are imposed during the minimization procedure. Indeed, deeper minima can be found when the search for low energy conformation is performed without any constraint (27) but none of these computed conformers mimic the morphine structure. The energy difference between the best non-constrained conformation obtained (27) and the best constrained one (conformation 1B in table 1) is of 4.0 kcal/mole.

The biological significance of these computed conformations must be discussed taking into account the following very important structure activity relationships in enkephalin series : i) the presence of a free carboxyl group is not required for μ-agonist potency (14-15), ii) the replacement in Met-enke-

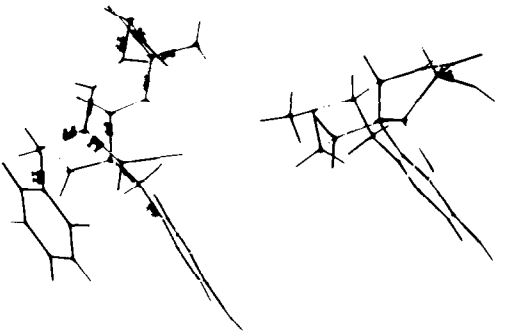

Figure 2. Right, dreiding model of morphine.
Left, dreiding model of Tyr-D-Ala-Gly-Phe (conformation 4B).

phalin of Gly^2 by an α-aminoisobutyric residue leads to a peptide with a severe steric strain around the ϕ_2, ψ_2 angles. Nevertheless this compound is a potent morphine-like peptide (31). Such a high opioid activity was also found for the Z-isomer of D-Ala^2-Δ^Z-Phe^4-Methionine enkephalinamide (32). In this compound the phenyl ring is necessarily oriented towards the N-terminal part of the peptide which takes a T-shape structure, iii) the Tyr^1-Gly^2 and Gly^2-Gly^3 amide groups seem to be not directly involved in μ-receptor subsites recognition since their reduction (33) or the replacement of Tyr^1-Gly^2 amide bond by an ethylenic one (34) does not reduce significantly opiate activity. On the contrary the flexibility of the tyrosine residue seems to be a necessary requirement for receptor interaction as evidenced by the loss of potency resulting from the incorporation of the Tyr ring in a strained cyclic moiety (35,36).

The four computed conformations 1B-4B fit the opiate pharmacophore and therefore could equally well interact with the μ-receptors. However only the structure 1B and 4B adopt the characteristic T-shape of morphine. Moreover, in the conformation 4B : i) the dihedral angles ϕ_2-ψ_2 roughly satisfy the requirement of the Ramachandran energy map for the introduction of an α-aminoisobutyric residue in place of Gly^2, ii) the ionic favourable head to tail interaction does not appear. Such an intramolecular interaction is not required for the receptor-binding since the decarboxylated derivative of Tyr-D-Ala-Gly-Phe present a higher μ-potency than its parent compound (15), iii) only weak backbone perturbations occur during the changes from the solvated 4A to the computed conformation 4B. For instance, the flexibility of the tyrosine moiety is not affected by such changes. So, despite its higher energy, the conformer 4B seems to be the more adapted model to fit the defined μ-pharmacophore (Fig. 2).

In all cases, the fitted conformations are slightly different of the solid or solvated states which are unable to fit the pharmacophore. The conformer of lowest energy calculated for an isolated molecule is stabilized by 7-8 kcal/mole as compared to the computed receptor-bound conformation. Obviously, as already reported (12), there is no reason that the receptor-bound conformation must be in a state of minimum energy.

Therefore, the loss of energy which could reach 8 kcal/mole in the extreme situation must be counterbalanced by the large free energy changes through hydrophobic, hydrogen-bonded and electrostatic interactions within the probably lipophibic binding-site. Moreover, the required flexibility of tyrosine and the changes between experimental and computed active conformations seem to indicate that the peptide bind to the receptor by a stepwise mechanism (37,38) which is more favourable from a thermodynamical point of view.

Finally this work shows that several conformations of a same ligand, i.e. Tyr-D-Ala-Gly-Phe are able to interact with the μ-opiate receptor. This finding could explain that compounds exhibiting apparent large structural differences in the serie of both peptides and opiates (meperidine, methadone, fentanyl...) could bind an identical receptor provided that the critical interactions in the binding site must be satisfied. Obviously, only a fully interacting conformation, for instance 4B, would active the effector component. In the case of μ-receptors, it seems that the specificity of a ligand for this kind of site depends on its ability to take a highly folded conformation. This is in accordance with CD experiments (39) and NMR studies (40) on the highly μ-specific pentapeptides with a proline in the fifth position.

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