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Structure-Activity Studies of Morphiceptin Analogs: Receptor Binding and Molecular Determinants of μ-Affinity and Selectivity

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SUMMARY

In this study we report the systematic investigation of conformational profiles and electronic properties of a series of analogs of the μ-selective opioid peptide, morphiceptin, together with receptor-binding studies of some of these analogs. In particular, we have investigated the effect of: substitution in the second position, substitution of D-Pro for L-Pro in the second and fourth positions, the addition of an *N*-methyl group at the third position, and variations in the carboxyl end group. The binding studies confirm the preference of these analogs for μ- versus δ-receptor-binding sites and also indicate differences in μ-receptor affinity among them. The theoretical analyses allow identification of a preferred conformation leading to high μ-receptor affinity and two reliable indicators of relative μ-receptor affinities. These properties are the energy required to obtain the candidate μ-binding conformer and the extent to which each compound overlaps with the highest affinity compound in this conformation. In addition, electronic interactions deleterious to high affinity μ-binding are identified.

With the discovery of multiple opioid receptors, a great deal of attention has been focused on the molecular requirements for high affinity binding to each specific receptor subtype. The two receptor-binding sites which have been best characterized for enkephalin-type opioid peptides are the opioid receptor subtypes known as "μ" and "δ". The μ-site is also the high affinity binding site for classical fused ring opiates, such as morphine, which bind with lower affinity to the δ-site. A number of theoretical studies on the conformational profiles of enkephalin-type peptides (1–3) have identified active conformers of these peptides and similarities to fused ring opiates which might lead to high affinity at the μ-receptor site.

In a continuing effort to investigate molecular requirements for high μ-receptor-binding affinity of opioid peptides, we report here the structure-activity study of a series of analogs of morphiceptin, Tyr-Pro-Phe-Pro-NH_2, an amidated fragment of the natural bovine milk protein β-casomorphin (4). This peptide is of particular interest because it was found to have morphine-like physiological activity, to bind with fairly high affinity, and to be extremely selective for the "μ"-receptor (5). Moreover, a series of morphiceptin analogs has been reported which vary in μ-receptor affinity and retain μ-receptor selectivity (6).

In the work reported here, we have selected 11 analogs (Table 1) for study. These analogs were chosen for theoretical studies in order to investigate the effect of adding a methyl group to the amide nitrogen of the phenylalanine residue, changing L-Pro to D-Pro in the second and fourth positions, making chemical modifications to the L-Pro^2 residue, and altering the carboxyl end group. In addition, detailed receptor-binding studies of four of the analogs (1, 4, 5, and 11) were done in order to further verify the μ-selectivity suggested by the data reported in Table 1.

A central hypothesis in the theoretical studies is the assumption that a conformer common to each analog is required to produce the intermolecular interactions necessary for binding and activity at the μ-receptor. As a corollary, it is assumed that analogs which cannot attain the proper conformation will have low affinity. By comparing receptor affinities and possible low energy conformations for various morphiceptin analogs, we were able to identify a likely μ-binding conformer. The relative energies required to attain the common μ-interacting conformation, and the extent of overlap of the conformer with that of a high affinity analog, appear to be predictors of relative μ-receptor affinity.

In addition to optimum accommodation at the receptor, crucial local interactions of side chain and terminal groups with receptor subsites can also be important contributions to affinity and activity. Propitious hydrophobic, electrostatic, H-bonding, and dispersion interactions can occur by an appropriate match between functional groups of the peptide and amino acid resi-

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ABBREVIATIONS: MEP, molecular electrostatic potential; ECEPP, empirical conformational energy program for peptides; rms, root mean square; MNDO, modified neglect of differential overlap; DADL, (D-Ala^2-D-Leu^5)enkephalin; EKC, ethylketocyclazocine; DSLET, (D-Ser^2-Leu^5-Thr^6)enkephalin; DHM, dihydromorphine.

TABLE 1
Morphiceptin analogs studied

	Binding[a] (IC_{50})		In vitro activity[a] (ED_{50})	
	FK33824	DADL	GPI	MVD
	nM		nM	
1. Tyr-Pro-Phe-Pro-NH_2	63	30,000 (100,000)	318	4,800
2. Tyr-D-Pro-Phe-Pro-NH_2	10,000		NT[b]	NT
3. Tyr-Pro-*N*(Me)Phe-Pro-NH_2	37	10,000	225	2,300
4. Tyr-Pro-Phe-DPro-NH_2	4.3	20,000	81	194
5. Tyr-Pro-*N*(Me)Phe-DPro-NH_2	5.5	10,000	34	240
6. Tyr-Pro-*N*(Me)Phe-DPro-OH	186	20,000	255	2,700
7. Tyr-Pro-*N*(Me)Phe-DPro-ol	5	20,000	NT	NT
8. Tyr-Pro-*N*(Me)Phe-DPro-Gly-NH_2	8.7	20,000	31	303
9. Tyr-4OH Pro[c]	800	40,000	NT	NT
10. Tyr-ΔPro[c]	21	30,000	NT	NT
11. Tyr-Pip[c]	16	30,000	NT	NT

[a] Data from Refs. 5 and 6.
[b] NT, not tested.
[c] Analogs **9**, **10**, and **11** are variations of peptide **5** with the 2-position substituted as indicated.

dues in the binding site of the receptor. Conversely, affinity can be greatly reduced by modifications in the peptide which destroy this complimentarity. Thus, in addition to conformational properties, electronic properties which could modulate relative receptor affinities were investigated in two ways. In one type of comparison, crucial functional group overlaps were compared. For example the *p*-hydroxy phenethylamine (tyramine) moiety of the peptide and morphine were compared, as were the c-terminal groups of different peptide analogs. In the other comparison, MEP equi-energy contours have been calculated to characterize electronic properties of the modified proline (9–11) residues in the second position. These properties also appear to modulate relative receptor affinities.

Methods

Theoretical. Energy-optimized conformations were obtained using a modified version of ECEPP (8, 9), a program developed in the laboratory of Dr. Harold Scheraga. The empirical energy expression used in ECEPP consists of five terms: electrostatic, repulsion, Van der Waals (dispersion), hydrogen-bonding, and torsion-angle energy components. Parameters required for this energy expression have been reported elsewhere in the literature (9). This program has been modified in our laboratory to include atom types for nonstandard amino acids and nucleic acid components, and to characterize intermolecular interactions.

Initial conformations to be used as input were constructed using efficient, interactive, structure-generating programs coupled to graphic displays, contained in a package called MOLECULE (10) developed in collaboration with NASA-AMES colleagues.

The extent of overlap between any two conformers was determined by using a program called MOBLS. This program determines the minimum rms of the distance between user-selected, matched atoms in two molecules, without allowing conformational change of either molecule. In this study, when comparing two conformers, all atoms in the two molecules were included in the overlap calculation.

To determine a set of conformations available to each analog for binding at the receptors, a two-part search strategy was used.

The first part employed an "aufbau" procedure for analogs 1 and 3. The procedure entails: 1) optimizing the conformers of each single amino acid (11); 2) combining them to form dipeptide conformers which are then energy optimized; 3) using the resulting low energy dipeptide conformers to form initial tetrapeptides; and 4) energy optimization to attain final possible tetrapeptide conformations for each analog.

In this manner, 49 unique, energy-ordered conformers were obtained for morphiceptin, **1**, and 54 conformers for *N*MePhe-morphiceptin, **3**. The lowest energy conformer of each analog was assumed to be the global minimum, and the remaining conformers spanned a relative energy range, ΔE, of about 70 kcal/mol.

In the second part of our search strategy, a set of common conformers for analogs **1** and **3** was identified with $\Delta E \leq 7.5$ kcal/mol, which was used as a reasonable estimate of energy available for conformational changes at the receptor site. This set of conformers was used to construct initial conformations for all of the remaining analogs studied. Initial conformers of each analog were optimized and energies and conformations compared to those of analog **5**, one of the highest affinity analogs and, thus, presumably one with an excellent fit at the receptor site.

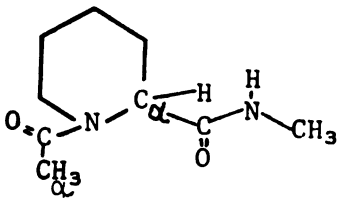
The fused ring and peptide opiates have in common a *p*-hydroxy phenethylamine moiety. In the fused ring systems the relative position of the *p*-OH phenyl and the ethylamine moiety are fixed. The terminal amine and tyrosine side chains which mimic this unit in the peptide are more flexible. Total overlap of this portion of the peptide with the fused ring opioids can be achieved if the side chain angles of the tyrosine are set at $\chi_1 = 267°$, $\chi_2 = 193°$.

The energy required to attain this overlap of the tyramine moiety of the tetrapeptides with the corresponding region of fused ring opiates was determined by setting the side chain dihedral angles (χ_1, χ_2) of the tyrosine residue of conformers of analog **5** to the corresponding values found in morphine ($\chi_1 = 267°$, $\chi_2 = 193°$). The conformers were then optimized with and without allowing these side chain angles to vary.

For analogs **9–11**, involving nonstandard proline residues, optimized geometries for input into ECEPP were obtained using the semi-empirical quantum mechanical method called Modified Neglect of Differential Overlap (MNDO) (12) as developed in the laboratory of Michael Dewar. The optimized structures and electron distributions obtained from the MNDO program were also used in a program (MOLGRAF) developed in our laboratory to calculate the MEP in specified planes around the side chain groups of this amino acid.

The MEP values, expressed as equi-energy contours, are the energy of interaction of the residue with a point positive charge placed anywhere along each contour. Also obtained was the position of maximum interaction, V_{max}, with a point positive charge.

MEP calculations were done for derivatives of proline, the two optical isomers of 4-OH proline, 3,4-dehydro proline, and pipecolinic acid. For each amino acid, MNDO-optimized geometries were used with the backbone angles frozen as in the tetrapeptides. The derivative of each residue (in the scheme shown below) was used to mimic the electronic effects of neighboring amino acids in the peptide.



Opiate receptor binding. Opiate receptor-binding assays were performed essentially as described by Pasternak *et al.* (13) Briefly, rat (Sprague-Dawley) whole brain homogenates were prepared, preincubated at 37° for 1 hr, and resuspended in Tris, pH 7.7, at 6.7 mg of tissue per ml. Receptor-binding incubations contained 1.8 ml of tissue suspension, 0.1 ml of labeled ligand, and unlabeled drugs in a total volume of 2.0 ml. The tubes were incubated in triplicate at 25° for 50 min prior to filtration.

In the present studies, self- and cross-competition experiments were conducted using two different concentrations of the five tritiated ligands: tritiated naloxone, DADL, DSLET, and DHM. In addition to the resulting five-by-five "matrix" of competitive inhibition behavior, inhibition of binding of all five labeled ligands with each morphiceptin analog was performed, again at two labeled ligand concentrations.

Data obtained were analyzed by a modified version of the program LIGAND (14) which predicts a set of self-consistent receptor-binding affinities and capacities assuming different receptor site models by using a weighted, nonlinear, least squares regression analysis procedure.

In the procedure used, all self- and cross-competition studies involving the five labeled ligands were analyzed together assuming one-, two-, three-, four-, five-, and six-site models of receptor binding, and results were compared for statistical significance and other indications of reliability. Inhibition data for morphiceptin and its three analogs were then added to the matrix obtained for the labeled and unlabeled ligands and the data were reanalyzed simultaneously for self-consistent receptor-binding affinities and capacities. Three-, four-, and five-site models for receptor binding were systematically explored.

Materials. [^{3}H]Naloxone, [^{3}H]DADL, [^{3}H]EKC, and [^{3}H]DSLET were from New England Nuclear; [^{3}H]DHM was from Amersham. Morphiceptin and analogs **4** and **5** were from Peninsula Laboratories. Analog **11** was the generous gift of Dr. K. J. Chang of Burroughs Wellcome Inc.

Results and Discussion

Receptor binding. A five-by-five matrix of inhibition experiments using labeled and unlabeled naloxone, DADL, EKC, DSLET and DHM yielded a five-receptor site model statistically more significant than one-, two-, three-, or four-site models. The binding data for the morphiceptin analogs were then added to the matrix. This set of 90 experiments was analyzed simultaneously assuming three, four, and five sites, and again in the five-site fit was statistically the best. The results are shown in Table 2.

In the five-site fit, a site appears which has high affinity of all of the labeled ligands. We have called this site "μ_1", in accordance with Pasternak's finding of a site with high affinity for most ligands (15). Recently, a similar site was described using similar computer analysis techniques (16). Sites were also labeled "μ_2", "δ", and "κ," as defined by high affinity for naloxone, DSLET, and EKC, respectively. In repeated analyses, naloxone always shows high affinity at "κ." The "anomalously" high affinity of naloxone at this site may reflect the increased accuracy in generating affinity constants by computer analysis or it may be defining a site different from that normally called "κ." Computer analysis also indicates one other site with high capacity and relatively low affinity for all ligands. This site may be related to nonspecific binding or may be a composite of sites, and is probably unrelated to opioid activity.

The morphiceptin analogs also seem to have highest affinity at μ_1, with next highest affinity at μ_2, followed by κ, with very low affinity at δ. These detailed binding studies provide further evidence that morphiceptin and analogs possess high affinity at μ with an extremely marked selectivity over the δ-site and are suitable templates for the investigation of peptide conformation leading to μ-receptor binding.

Identification of a set of possible active conformers. Using the aufbau method, as shown in Fig. 1, 27 unique conformers of morphiceptin were found within 7.5 kcal/mol of the lowest energy conformer. This result indicates that, even though morphiceptin is constrained by having two proline rings in its backbone structure, this tetrapeptide retains significant flexibility.

By contrast, when the same method was used for *N*MePhe^3-morphiceptin (also shown in Fig. 1), only 10 unique conformers with relative energies within 7.5 kcal/mol of its lowest energy conformer were found. Although the aufbau procedure converged upon 12 conformers within that energy range, 2 conformers (**2** and **11**) had dihedral angles identical to 2 others (**3** and **10**) and were therefore not further explored. The unique conformers could be classified into two basic types, one resembling a β_{II}-type turn and the other resembling a repeating C_7 structure. Comparing these results to those for morphiceptin, it appears that the steric hindrance of the additional methyl group significantly restricts the possible low energy conformers of morphiceptin analogs.

When the conformers for the two analogs obtained by the aufbau procedure were compared in detail, no significant overlap was found between the stable conformers of morphiceptin and NMePhe^3-morphiceptin. None of the morphiceptin conformations determined from low energy single amino acids and dipeptides by the aufbau procedure could accommodate an *N*-methyl group on phenylalanine. The energy cost of maintaining any low energy conformer of morphiceptin with the additional constraint of the methyl group at Phe^3 was of the order of hundreds of kcal/mol. By contrast, when the methyl group was removed from the 10 low energy NMePhe^3-morphiceptin conformers, 7 of the 10 resulting morphiceptin conformers were within about 14 kcal/mol of the lowest energy morphiceptin conformer found by the aufbau procedure. The remaining three were also within this range after minimization which did not result in major conformational change as indicated by rms values no greater than 1.5. Since morphiceptin could accommodate the 10 low energy NMePhe^3-morphiceptin conformers either directly or with minor conformational change, these 10 conformers were selected as the set of potential conformers used in further investigations of the remaining analogs.

Selection of a candidate conformer. The relative energies obtained when D-Pro^4 was substituted for L-Pro^4 into *N*MePhe^3-morphiceptin and the tetrapeptide reoptimized are given in Table 3a. This substitution produced 10 conformers spanning the same energy range, $\Delta E \leq 7.5$ kcal/mol, as found for the L-Pro^4 analog (Fig. 1). The rms values, a measure of similarity of the two analogs (NMePhe^3-morphiceptin with L- or D-Pro^4), show several relatively low energy conformers with reasonable overlap (i.e., rms $\leq$ 1.2). As shown in Fig. 2, conformer $3\beta_{II}$ is essentially the same for the two analogs, with the rms value of 1.1 primarily reflecting the change in spatial

TABLE 2

Receptor affinities and maximum binding capacities for a five-receptor site model

	K_D (nM)				
	Site 1 μ1	Site 2 μ2	Site 3 δ	Site 4 κ	Site 5
Naloxone[a]	0.50 ± 0.10	4.35 ± 0.60	28.7 ± 2.32	0.44 ± 0.07	92.6 ± 21.7
DADL[a]	1.16 ± 0.25	23.5 ± 3.35	2.65 ± 0.26	410 ± 114	10800 ± 4290
EKC[a]	0.67 ± 0.15	5.71 ± 0.72	20.5 ± 1.71	0.035 ± 0.012	00.0[b]
DSLET[a]	3.88 ± 0.88	79.4 ± 12.3	1.33 ± 0.13	1618 ± 342	183 ± 40
DHM[a]	0.18 ± 0.033	11.1 ± 1.87	167 ± 13.4	690 ± 218	49.3 ± 12.0
5	6.85 ± 1.12	10.3 ± 2.60	58480 ± 6582	310 ± 81.1	11420 ± 5335
11	5.65 ± 1.44	31.0 ± 6.79	40490 ± 3880	439 ± 107	190 ± 83.0
4	4.27 ± 1.09	51.3 ± 10.1	19690 ± 1913	2110 ± 547	193 ± 88.3
1	32.1 ± 9.97	94.3 ± 25.0	122900 ± 20000	1000 ± 236	833 ± 444
B_{max} (pmol/g)	3.78 ± 0.55	26.34 ± 1.88	10.48 ± 0.95	2.93 ± 0.20	96.68 ± 18.34

[a] ^{3}H-ligand used for binding experiments.
[b] Value held equal to zero.

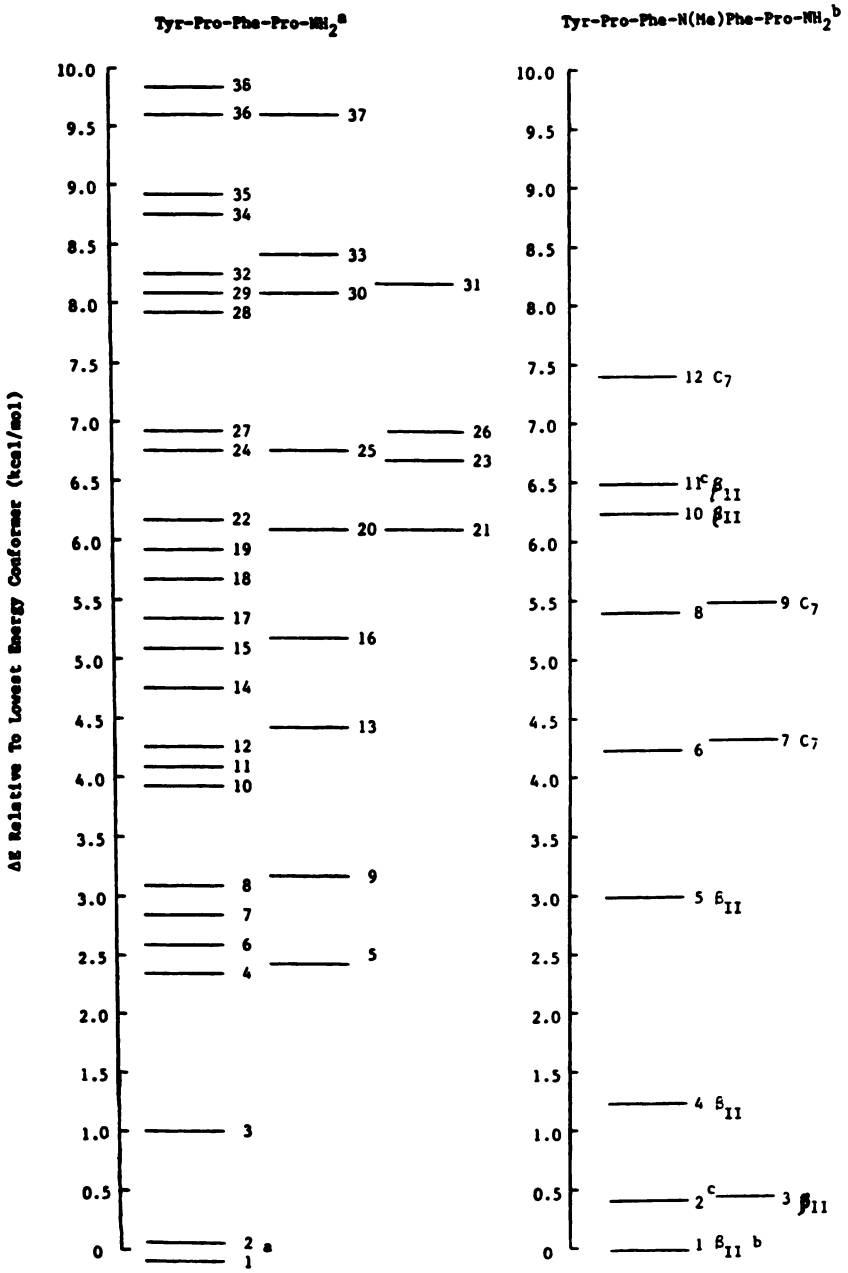


Fig. 1. Relative energies of optimized conformers of two morphiceptin tetrapeptides obtained using empirical energy method (ECEPP) and "aufbau" search strategy. a. Lowest energy conformer with $E = -23.2$ kcal/mol. b. Lowest energy conformer with $E = -8.1$ kcal/mol. c. Conformers 2 and 11 optimized to the same conformations as 3 and 10, respectively, and hence were not further considered.

TABLE 3

Relative energies and extent of overlap of morphiceptin analogs with the highest μ-affinity compound

a. Tyr-Pro-*N*(Me)Phe-DPro-NH_2 (*5*)										
Conf	$1\beta_{II}$	$4\beta_{II}$	$3\beta_{II}$	$6C_7$	$5\beta_{II}$	$7C_7$	$8C_7$	$9C_7$	$10\beta_{II}$	$12C_7$
ΔE	0.0	0.3	1.6	1.9	2.6	3.3	4.1	4.5	5.2	7.0
rms[a]	1.2	1.5	1.1	1.1	1.1	1.3	1.1	1.3	1.7	1.2
b. Tyr-Pip-*N*(Me)Phe-DPro-NH_2 (*11*)										
ΔE	13.3	13.8	0.0	1.8	1.2	14.6	3.0	3.4	12.6	12.6
rms[b]	0.8	0.8	1.2	2.3	1.2	0.8	2.8	1.3	0.9	1.8
c. Tyr-ΔPro-*N*(Me)Phe-DPro-NH_2 (*10*)										
ΔE	0.0	0.6	3.1	3.8	3.8	5.1	5.0	5.5	4.4	3.8
rms[b]	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.7	1.7
d. Tyr-4-OHaPro-*N*(Me)Phe-DPro-NH_2 (*9a*)										
ΔE	0.0	0.4	1.3	1.8	2.2	3.5	3.7	4.5	5.0	6.9
rms[b]	0.04	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.02
e. Tyr-4-OHbPro-*N*(Me)Phe-DPro-NH_2 (*9b*)										
ΔE	0.0	0.4	1.8	2.0	2.8	3.5	4.3	4.9	5.2	7.0
rms[b]	0.04	0.03	0.01	0.02	0.01	0.03	0.01	0.03	0.03	0.02
f. Tyr-Pro-Phe-DPro-NH_2 (*4*)										
ΔE	2.6	2.6	3.7	0.3	5.4	0.0	3.7	5.0	8.4	1.2
rms[b]	0.2	0.2	0.2	2.2	0.4	3.1	0.9	0.8	0.6	1.2
g. Tyr-Pro-Phe-Pro-NH_2 (*1*)										
ΔE	4.4	4.4	5.5	0.00	7.1	0.4	3.5	3.9	12.1	1.6
rms[b]	1.1	1.1	0.8	2.0	1.0	1.9	0.7	0.7	1.0	1.5
h. Tyr-Pro-*N*(Me)Phe-DPro (*6*)										
ΔE	1.1	1.4	2.9	0.0	3.7	1.7	3.2	3.6	6.3	7.1
rms[b]	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2
i. Tyr-Pro-*N*(Me)Phe-DPro-ol (*7*)										
ΔE	0.0	0.0	0.5	1.1	1.5	1.1	3.0	3.0	5.6	5.6
rms[b]	0.2	0.1	0.2	0.1	0.4	0.2	0.1	0.1	0.2	0.1
j. Tyr-Pro-*N*(Me)Phe-DPro-Gly-NH2 (*8*)										
ΔE	3.2	3.2	0.0	2.8	1.2	2.8	4.7	4.7	5.3	6.4
rms[b]	0.4	0.4	1.0	0.8	1.1	0.8	0.8	0.8	0.7	0.2
k. Tyr-D-Pro-Phe-Pro-NH_2[c] (*2*)										
ΔE	12.6		7.8*	12.1[c]	6.71*		6.5			
rms[b]	1.3		1.4	1.5	2.7		2.1			

[a] rms = root mean square overlap of each conformer of Tyr-Pro-*N*(Me)Phe-DPro-NH_2 with corresponding conformer of Typr-Pro-*N*(Me)-Phe-LPro-NH_2 from which it was generated.

[b] For all other analogs the rms = root mean square of the overlap of each optimized conformation with its initial conformation generated from that of analog **5**.

[c] Five optimized energies for this analog obtained from substituting D-Pro for L-Pro in the second position of morphiceptin and adjusting the initial conformer to achieve maximum overlap with the L-Pro analog. The lowest energy conformer was optimized from the lowest energy morphiceptin conformer with no attempt to overlap the two analogs.

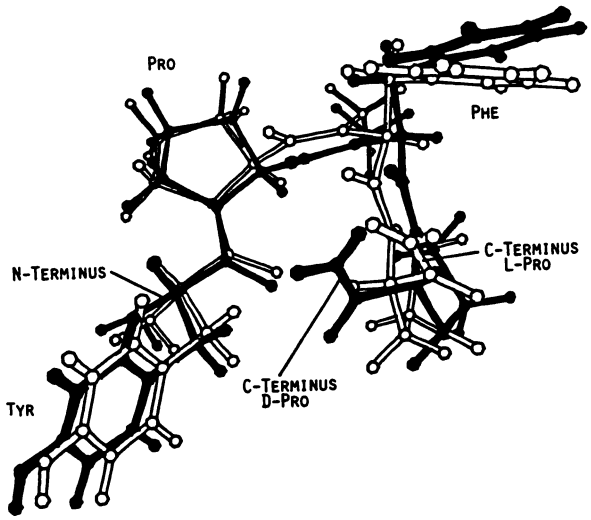


Fig. 2. Maximum overlap of Tyr-Pro-*N*(Me)Phe-Pro-NH_2 (*white*) with Tyr-Pro-*N*(Me)Phe-D-Pro-NH_2 (*black*), both in candidate conformer $3(\beta II)$.

position of the C-terminal amide group caused by replacing L-Pro[4] with D-Pro[4].

The 10 conformers of the high affinity analog, Tyr-Pro-*N*(Me)Phe-DPro-NH_2, shown in Table 3a, were then used as templates for the remaining analogs. Initial geometries for each analog were generated using the corresponding conformation of analog **5** and then reoptimized. A lowest energy conformer was found for each new tetrapeptide analyzed, and the energies of the remaining conformers were compared to its energy. The rms value was determined for the overlap of each optimized conformer of the new peptide with its initial conformer, the one corresponding to the same conformer of analog **5**. All atoms were included in the comparison. The results are given in Table 3, b–k.

Of all the high affinity analogs analyzed, only **11** (Table 3b) produces a clear differentiation, in terms of relative energy, among the 10 conformers. Substitution of the six-membered pipecolinic acid ring for the five-membered proline ring, forming analog **11**, is only accommodated by 5 of the 10 conformers with $\Delta E \leq 7.5$ kcal/mol. Of these five, only three ($3\beta_{II}$, $5\beta_{II}$, $9C_7$) have a reasonable rms value (i.e., rmps ≤ 1.2) indicating

that this substitution does not produce significant distortion from their initial conformations. Thus, these three conformers are the only common low energy conformers found for analogs **5** and **11**. Since both **5** and **11** bind with a reasonably high

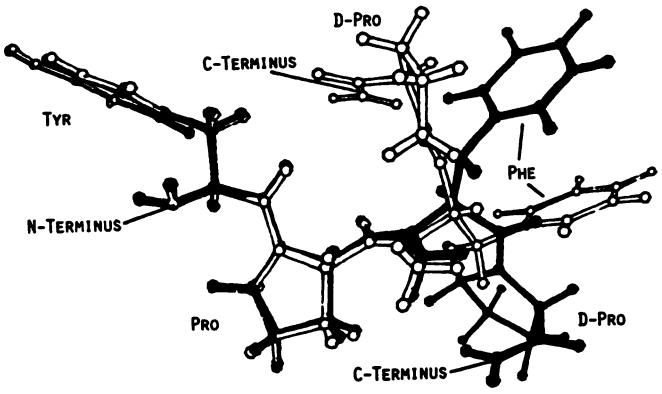


Fig. 3. Comparison of β_{II}-type (*white*) and C_7-type (*black*) candidate conformers for Tyr-Pro-*N*MePhe-D-Pro-NH_2.

TABLE 4

Energies required for tyramine overlap of Tyr-Pro-*N*MePhe-D-Pro-NH_2

Conformer	E_{opt}[a]	$\Delta E_{tyramine}$[b]	$\Delta E_{tyramine\text{-}opt}$[c]	Tyrosine side chain angles[d]		Tyramine overlap
				χ_1	χ_2	
$1\beta_{II}$	0	7.25	0.0	290	277	No
$4\beta_{II}$	0.3	7.28	0.0	290	279	No
$3\beta_{II}$	1.6	10.74	6.77	255	225	Yes
$6C_7$	1.9	12.13	1.74	297	286	No
$5\beta_{II}$	2.6	10.91	7.27	263	214	Yes
$7C_7$	3.3	12.26	1.86	297	286	No
$8C_7$	4.1	10.15	0.0	296	285	No
$9C_7$	4.5	10.13	0.0	296	285	No
$10\beta_{II}$	5.2	3.03	−2.71	250	230	Yes
$12C_7$	7.0	13.18	2.82	297	288	No

[a] Optimized relative energies corresponding to Table 4a.
[b] Additional energy required for induced tyramine overlap for each conformer. $\Delta E_{tyramine} = E_{constrained} - E_{total}$. Constrained optimization means that the tyrosine side chain angles were held fixed at values of $\chi_1 = 267°$, $\chi_2 = 193°$ for total tyramine overlap and all other torsion angles optimized.
[c] The additional energy required to reach the local minimum conformer closest to tyramine overlap:
$\Delta E_{tyramine\text{-}opt} = E_{total}$ (optimized with initial values of $\chi_1 = 262°$, $\chi_2 = 193°$) $- E_{total}$ (optimized original conformer).
[d] Values of χ_1 for local minimum conformer closest to tyramine overlap; values significantly different from $\chi_1 = 262°$, $\chi_2 = 193°$ mean there is no morphine-like local minimum for this conformer.

TABLE 5

Correlation of μ-receptor affinities with calculated relative energies and overlap with candidate active conformer ($3\beta_{II}$)

	IC_{50}	ΔE[a]	rms[b]
	μ	kcal/mol	
Tyr-Pro-*N*(Me)Phe-D-Pro-NH_2	5.3	1.6	0
Tyr-Pro-*N*(Me)Phe-D-Pro-ol	5	0.5	0.19
Tyr-Pro-Phe-D-Pro-NH_2	4.3	3.7	0.23
Tyr-Pro-*N*(Me)Phe-D-Pro-Gly-NH_2	8.7	0.0	0.21
Tyr-Pro-*N*(Me)Phe-Pro-NH_2	37	0.4	1.13
Tyr-Pro-Phe-Pro-NH_2	63	5.5	1.34
Tyr-Pro-*N*(Me)Phe-D-Pro	186	2.9	0.14
Tyr-D-Pro-Phe-Pro-NH_2	≥10,000	7.8	1.92

[a] ΔE values were calculated with respect to lowest energy conformer of each analog.
[b] Residual value of root mean square atom-atom displacement of each analog conformer with respect to the template conformer (rms = 0) after rigid overlap optimization.

TABLE 6

μ-Receptor affinities for analogs with χ^2 substitution with calculated correlation of relative energies and overlap with candidate active conformer ($3\beta_{II}$)

χ^2	Tyr-X^2-(NMe)Phe-D-Pro-NH_2		
	IC_{50}	ΔE[a]	rms[b]
	nM	kcal/mol	
Pro	5.3	1.6	0
Pip	16	0.0	1.28
ΔPro	21	3.1	0.17
4-OHa Pro	800	1.3	0.01
4-OHb Pro		1.8	0.01

[a] ΔE values were calculated with respect to lowest energy conformer of each analog.
[b] Residual value of root mean square atom-atom displacement of each analog conformer with respect to the template conformer (rms = 0) after rigid overlap optimization.

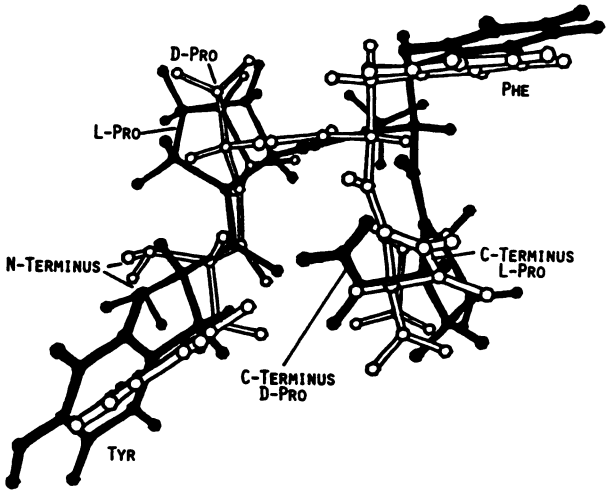


Fig. 4. Maximum overlap of Tyr-D-Pro-Phe-Pro-NH_2 (*white*) with Tyr-Pro-*N*(Me)Phe-D-Pro-NH_2 (*black*), both in candidate conformer $3(\beta II)$.

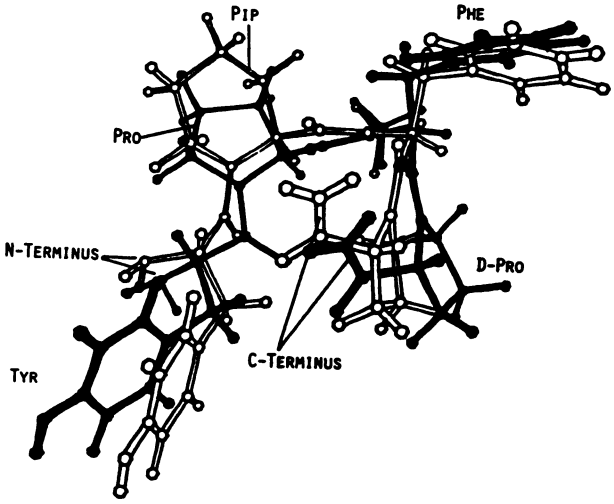


Fig. 5. Maximum overlap of Tyr-Pip-Phe-Pro-NH_2 (*white*) with Tyr-Pro-*N*(Me)Phe-D-Pro-NH_2 (*black*), both in candidate conformer $3(\beta II)$.

affinity at the μ-receptor, these three conformers were considered as possible candidate conformers for binding at the μ-receptor. The remaining high affinity analogs, shown in Table 3, c–j, all have $\Delta E < 7.5$ kcal/mol and low rms values for the three possible candidate conformers. Conformers $3\beta_{II}$, $5\beta_{II}$ are

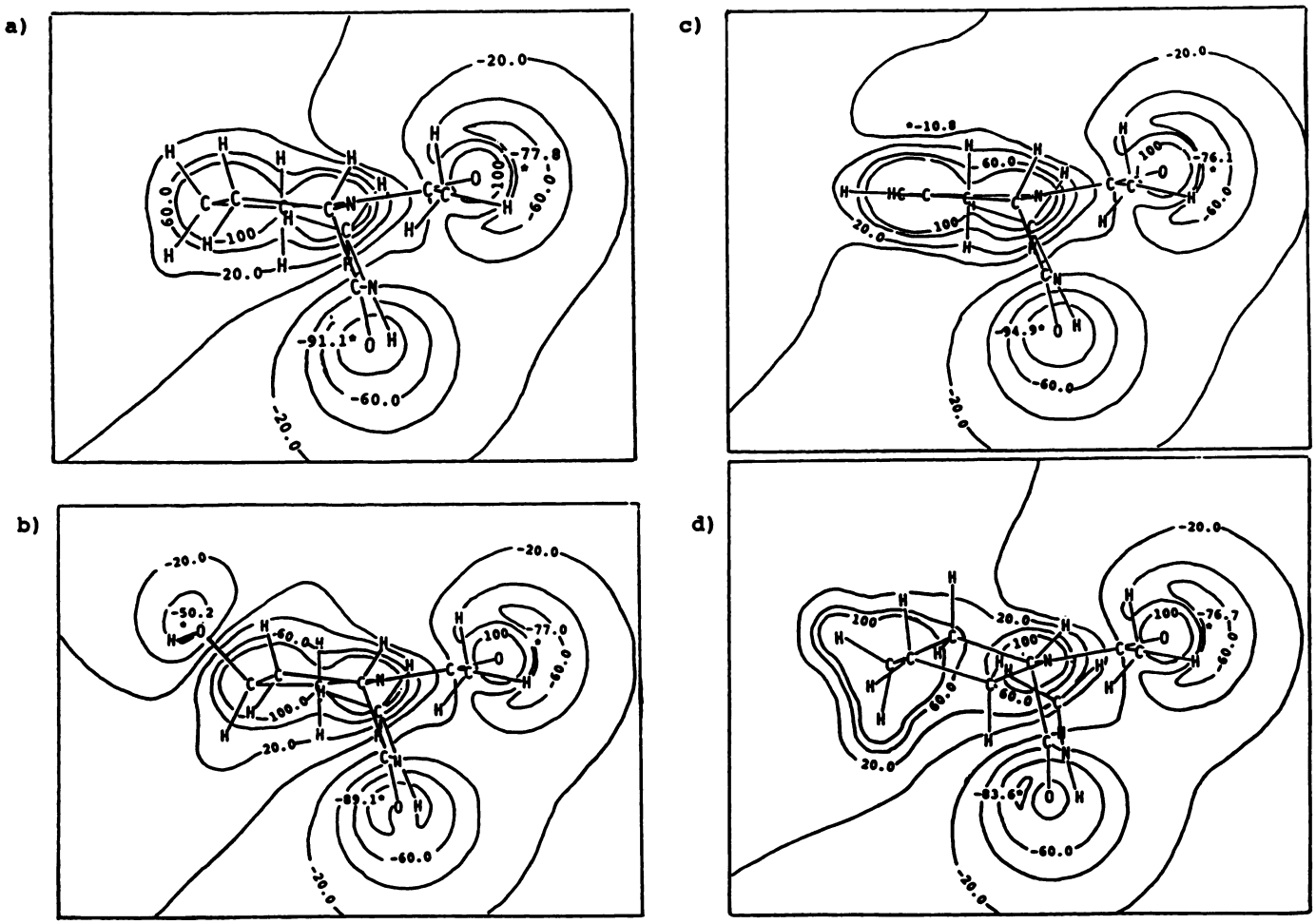


Fig. 6. Molecular electrostatic potential maps, calculated in the plane perpendicular to view with potentials of the ring. a, Proline; b, Δproline; c, 4(OH)proline; d, pipecolinic acid.

identical except for the orientation of the phenylalanine side chain. By contrast as shown in Fig. 3, conformer $9C_7$ differs significantly from the other β_{II} candidates in the conformation of the third and fourth residues. Thus, it would represent a qualitatively different μ-selective conformer.

In addition to being a common feature among the tetrapeptides, a *p*-hydroxy phenethylamine (tyramine) moiety is a structural feature shared by all fused ring opiates and the *N*-terminal tyrosine of opioid peptides. The presence of this tyramine moiety is widely accepted as a requirement for high μ-affinity and analgesia for these classes of opioids. Thus, the tyramine moiety of high affinity peptides should overlap with that of morphine. The ability of the 10 low energy conformers of analog **5** to accept a "tyramine" overlap is shown in Table 4. The energy required for each conformer to retain this overlap in an induced conformation ranges from 3 to 13 kcal/mol. When the side chain dihedral angles were allowed to vary to local minima values, only three conformers (**3**, **5**, and **10**) retained side chain angles close to those required for tyramine overlap. Two of these conformers are the β_{II}-like possible candidate conformers chosen by the criteria discussed above. Conformer $9C_7$ does not accept a tyramine overlap and, therefore, does not appear to be a viable candidate for the μ-binding conformation. Thus, the requirement of tyramine overlap with morphine combined with the requirements for low ΔE and rms for high affinity analogs has identified two candidate conformers, $3\beta_{II}$ and $5\beta_{II}$, of similar backbone and tyramine conformations.

The extent to which the energy required to attain the candidate conformer (ΔE) for each analog and its similarity to that for analog **5** (rms) are reliable indicators of their relative μ-receptor affinities as shown in Tables 5 and 6.

As shown in Table 5, for modifications in the second, third, and fourth positions, when the C-terminal is not charged at neutral pH, both relative energy (ΔE) and rms are reliable indicators of relative affinities. Values of these properties tend to increase as the μ-affinity decreases. Analog **2**, D-Pro^2-morphiceptin, a very low affinity compound has unacceptably high ΔE and rms values for our candidate conformers (Fig. 4).

The similarity of the lowest energy possible candidate conformers for analogs **5** and **11** is shown in Fig. 5. Similar close overlaps were also found between each remaining high affinity analog and analog **5**.

Specific ligand-receptor interactions. Also shown in Table 5 are the effects of alteration of the carboxyl end group. There is no significant change in any of the torsion angles when the C-terminal amide of pro-NH_2 is changed to an alcohol or a free acid or is extended with insertion of a Gly-NH_2. The small changes in ΔE and rms are consistent with the high affinity found for the alcohol and glycyl-amide analogs. In contrast, the free acid has a 10-fold decrease in affinity, indi-

cating that the charged ionic form of the end group, rather than conformational changes, prevents an essential receptor-ligand interaction at the C-terminal end. The implied importance of the C-terminal region for specific receptor interactions is consistent with another observation: that changing L-Pro[4] to D-Pro[4] significantly increases affinity without changing conformation. This change affects the orientation of the C-terminal amide with respect to the rest of the peptide. Thus, the enhanced affinity of the D-Pro[4] analog appears to be due to the localized electronic interactions of the C-terminal end group. Taken together, these two effects indicate that hydrogen bonding, but not electrostatic interactions, occurs between the C-terminal region and specific μ-receptor subunits.

In addition to identifying the favorable type of receptor interactions with the C-terminal region, our results also allow deductions to be made for favorable interaction with the second residue. In particular, as seen in Table 6, the addition of a 4-OH group on the proline has little effect on the ΔE and rms values but produces a marked lowering in μ-affinity (100-fold increase in IC_{50}).

The low affinity of the 4-OH proline analog can be understood by examining the MEP contours generated by the four proline analogs corresponding to the variations in the second position of the morphiceptin analogs **1**, **9**, **10**, and **11**. Shown in Fig. 6, a–d, are the MEP equi-energy contours and position of minimum energy in a plane essentially perpendicular to the proline ring and peptide backbone and passing through the C4 position of proline. We see from this figure that the three analogs with similar receptor affinity (K_D = 9–21 nM) have similar MEPS. The side chains of these residues are essentially hydrophobic, surrounded by small positive potentials representing steric repulsion of these moieties. By contrast, the presence of the 4-OH group generates a large negative potential which would be efficacious for interaction with polar receptor sites. The observation that such a potential diminishes activity allows the inference that hydrophobic receptor residues surround the binding site of the proline.

Conclusions. The results of our studies of this series of morphiceptin analogs demonstrate that relative μ-receptor affinity is altered by both conformational and electronic properties. Criteria used for selecting candidate conformers are low energy and significant overlap for all high affinity compounds, and possible overlap of the tyramine moiety with that of morphine. The ΔE required to maximize overlap with the candidate conformer and the extent of overlap as measured by the rms value proved to be two reliable conformational indicators of relative μ-receptor affinity.

A candidate conformer for high affinity opioid peptide binding at the μ-receptor has been identified as one with a β_{II}-like turn. This conformer is similar to a conformer involving a β_{II}-like turn that was found as a candidate conformer in our previous studies of μ-selective enkephalin analogs (18). These results taken together may indicate a "universal" type of μ-selective opioid peptide conformer. Additional evidence for such a conformer is provided by a recent X-ray crystal structure reporting an enkephalin-like opioid peptide with a β-turn (19). This type of conformer was found for each of several peptide molecules in the unit cell when crystalized from both polar and non-polar solvents.

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