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The Role of Crystallography in Drug Design

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ABSTRACT

Structure and function are intimately related. Nowhere is this more important than the area of bioactive molecules. It has been shown that the enantioselectivity of an enzyme is directly related to its chirality. X-ray crystallography is the only method for determining the "absolute" configuration of a molecule and is the most comprehensive technique available to determine the structure of any molecule at atomic resolution. Results from crystallographic studies provide unambiguous, accurate, and reliable 3-dimensional structural parameters, which are prerequisites for rational drug design and structure-based functional studies.

KEYWORDS: structure, absolute configuration, opioid, pharmacophore, X-ray diffraction

INTRODUCTION

Structure and function are intimately related. X-ray crystallography is the most comprehensive technique available to determine the structure of any molecule at atomic resolution. "X-ray crystallography has become the sine qua non for elucidating the 3-dimensional structures of biologically interesting large and small molecules, providing the proverbial 'picture that is worth a thousand words'."[1] Accurate knowledge of molecular structures is a prerequisite for rational drug design and structure-based functional studies. Results from X-ray crystallographic studies provide unambiguous, accurate, and reliable 3-dimensional structural parameters at times even before complete chemical characterization is available. In addition, crystallography is the only method for determining the "absolute" configuration of a molecule. Absolute configuration is a critical property in biological systems as changes in this may alter the response of the biologic system.

CONFORMATION AND BIOLOGIC ACTIVITY

The endogenous opioid peptides Leu- and Met-enkephalin (Try-Gly-Gly-Phe-Leu [Met]) were isolated from pig brain as a mixture.[2] These endogenous peptides are not receptor subtype specific and show binding affinity for both the μ and δ opioid receptors. Conformational studies indicate that small linear peptides, such as the enkephalins, can have many different conformations.[3] The lack of specificity may be related to the large number of conformations available to the peptides. However, in the crystalline state Leu-enkephalin has been shown to exist in only 3 conformations (Figure 1), extended,[4] single β-bend,[5] and double β-bend,[6] while Met-enkephalin[7] has only been seen in an extended conformation. Thus the lack of specificity may be related to differential binding of these conformers at the various opioid receptors. Other investigators have speculated that the single-bend conformation, with its 2 intramolecular hydrogen bonds, provides the "rigid frame to which side chains are attached in a specific spatial relationship" required for activity.[5] Thus, systematic approaches for the design of potent and selective analogs of enkephalin have involved the application of both conformational and topographical constraints. Attempts to constrain the backbone to the single-bend conformation fall into 3 general categories: incorporation of residues that constrain the backbone conformation, cyclization of the peptide, or incorporation of constrained residues.

It is generally accepted that the most important pharmacophoric parameters in these opioids include the distance from the protonated amine to the tyrosine aromatic ring, the distance from the protonated amine to a second hydrophobic center (generally a second aromatic ring), and the distance between the tyrosine ring and hydrophobic center.[8] The pharmacophoric parameters for opioid peptides for which the X-ray crystallographic studies have been completed are summarized in Tables 1 and 2. Through examination of these parameters and the biologic activity we may begin to understand the relationship between structure and activity in this class of compounds.

Even though the cyclized peptide backbone is much more conformationally restricted than the linear enkephalin analogs, it still possesses significant residual flexibility due in part to the unsubstituted Gly residue at position 3. Attempts to reduce this flexibility have included replacing Gly^3 with bulkier residues (eg, l- and d-Ala^{20}), replacing one of the bridging residues with a more conformationally restricted moiety such as mercaptoproline,[21] or removing the Gly^3 residue altogether to form a more rigid cyclic tetrapeptide.[22]

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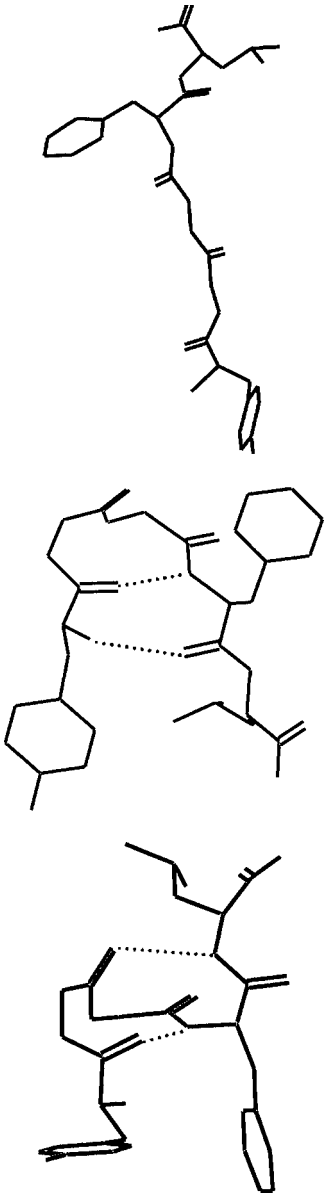

Figure 1. Solid-state conformations of Leu-enkephalin: top—extended, middle—single-bend, bottom—double-bend.

The rationally designed linear peptides in Tables 1 and 2 exhibit only folded conformations in the solid state with the single-bend peptides being agonists and a double-bend peptide acting as an antagonist. The tighter winding in the double bend brings the 2 aromatic rings to within 5 Å of one another, much closer than what is observed for this approach in any of the other phenylalanine (Phe)-containing peptides. For the tetra-hydroisoquinoline carboxylic acid (Tic) peptides, which show activity as μ agonists and δ antagonists, the distances fall in the range observed for the folded peptides. Despite difficulties in predicting which modifications increase selectivity or potency, the constraints applied to the peptide ligands have produced compounds with higher selectivity and potency.

NONPEPTIDE LIGANDS

Petsko[28] noted, "Chirality is fundamental in biology. The building blocks of proteins, the naturally occurring amino acids, are chiral." Milton and coworkers[29] showed that inverting the chirality of an entire enzyme also inverts its enantioselectivity. Thus absolute configuration is critical to proper function in biological systems. Nonbiological systems can also have a handedness. Inequivalence observed in crystallographic data of zinc sulfide was related to the absolute configuration of the crystal.[30] This effect was later shown to be general and applied to a variety of chiral structures to determine their absolute configuration.[31] In general determination of absolute configuration requires a heavy atom in the structure. Alternatively, inclusion of a salt of known chirality can be used to set the hand of the complex. With the advent of area detectors, crystallographers are now collecting more "redundant" data. Small differences in certain data pairs can be exploited to determine the absolute configuration.[32]

The naturally occurring opioid peptides are chiral, as are all proteins. A change in the configuration at a single chiral center can alter the pharmacological properties of a molecule.[33,34] Nonpeptide ligands bind to the same receptors as the endogenous opioid peptides and must mimic the arrangement of binding groups present in the opioid peptides. It is therefore no surprise that the nonpeptide ligands are chiral and that crystallography is used to track chiral syntheses and confirm, or determine, the absolute configuration of products.

The opioid alkaloid, morphine, has been around for almost 200 years,[35] and it is still widely used as an analgesic despite its undesirable side effects that include respiratory depression, reduced heart rate, nausea, vomiting, dizziness, sluggishness, sweating, and with repeated use addiction. Thus, the search for a better analgesic has been the search for a substance with morphine's beneficial properties without its undesirable side effects. There is some evidence of topologic similarity between morphine and the endogenous opioid peptides.[36] Building on this, many nonpeptide opioids are based on the morphine skeleton (Figure 2). Structural elements are modified or eliminated in efforts to circumvent unwanted effects. This often results in simplification that serves another function as it is not economic to synthesize a complex structure, such as morphine, on a large scale.[38,39] Extensive studies on morphine have shown that it requires the 3-hydroxyl group of the phenol ring for maximum activity, and that the hydroxyl group on C-6 be omitted or modified without losing activity.[34]

Further studies showed that the opioid pharmacophore could be simplified by eliminating the tetrahydrofuran ring and one cyclohexyl ring (ie, the bridging O, C6, C7, and C8) from morphine to produce the benzomorphans (Figure 3). Other simplifications of the opioid pharmacophore lead to the development of the phenylmorphans. Like benzomorphans,

Table 1. Selected Pharmacophoric Parameters in Linear Opioid Peptides*

Compound	N -Tyr	N - Hydr	Ring-Ring	Angle	N - O	Tyr χ^1	Phe χ^1	CCDC	Ref
Extended									
LE-1	5.18	10.57	9.37	9.4	7.89	177	−63	BIXNIF10	4
	4.31	11.56	13.27	73.9	6.81	70	−55		
	4.10	11.58	13.90	62.4	6.60	53	−71		
	5.16	10.24	8.89	14.4	7.84	169	−68		
LE-2	5.13	14.26	13.21	67.3	7.83	175	−169	FABJEX	10
	4.10	10.52	11.80	42.1	6.55	62	−69		
ME-1	4.08	13.46	13.60	4.7	6.39	58	53	FABJIB	7
	4.00	13.13	13.63	6.3	6.42	68	65		
Metkephamide	5.10	10.05	12.76	36.6	7.88	176	−63	IDIHEI	11
	5.15	10.63	12.95	38.4	7.84	173	−55		
Range	3.8-5.2	7.5-13.4	8.9-13.9						
Single-bend									
LE-3	4.14	7.86	11.26	48.3	6.64	−80	−59	LENKPH11	5
	3.89	7.76	10.76	21.9	6.26	−53	−61		
	4.25	7.84	11.36	59.3	6.79	−87	−60		
	3.95	7.66	10.73	12.9	6.40	−52	−62		
LE-Br			11.34	54.5		−84	−69	NA	12
LE-Nle	5.19	7.71	9.58	26.0	7.89	−167	−73	CITXEI10	13
	5.13	5.00	8.15	41.3	7.81	−177	−74		
DTLET	5.16	8.84	10.83	70.3	7.88	169	-73	HICJUY	14
DADLE	5.13	7.18	9.34	41.7	7.80	−166	−71	HIHYAY	15
TGGP	3.03	6.14	9.07	46.0	6.34	−63	−168	TGGPDH10	16
Biphalin (1:4)†	5.20	7.20	8.57	84.6	7.90	176	−56	NA	17
Range	4.1-5.2	5.0-8.9	8.1-11.4						
Double-bend									
LE-4	5.18	6.70	4.99	79.1	7.89	177	−67	GEWWAG	6
RTI02	5.15	7.45	4.87	83.4	7.84	−150	−61	SUPBOU	18
Biphalin (8:5)†	5.10	6.70	5.95	67.4	7.80	175	−58	NA	17
Range	5.1-5.2	6.7-7.5	4.8-5.0						

*CCDC indicates Cambridge Crystallographic Data Center; Ref, reference; and NA, not applicable. In all cases except the Tic peptides the second aromatic ring is part of a Phe residue and there are 2 amino acids between the Try and Phe residues (except for JOM-13, which has only 1). For the Tic peptides the distances quoted for N-Hydrophobic and Ring-Ring refer to the relationship between Tyr[1] and Tic[2] (1-3) or Phe[3] (1-3), where possible values were determined from the X-ray coordinates using SHELXTL[9]; all distances are reported in Å.
†Biphalin is a mixed agonist, which binds to both μ and δ opioid receptors. Because of these "mixed" properties, the pharmacophoric parameters have been split into the single- and double-bend sections based on the similarity of the 1 to 4 and 5 to 8 regions to other entries.

phenylmorphans are formed by eliminating the tetrahydrofuran ring and one cyclohexyl ring, but only C10 and the bridging O are removed (Figure 4). This progression has continued with newer ligands being either more potent, more selective, or both. Thomas et al showed that potent antagonists could be produced from phenylmorphans,[41] while Hashimoto and his coworkers found that (-)-(1R,5S,1'R)-3-[2-(1'-methyl-2'-phenylethyl)-2-azabicyclo[3,3,1]non-5-yl]-phenol is a moderately potent opioid antagonist.[42]

Other modifications can convert an agonist to an antagonist. The structurally related family of etorphine, buprenorphine, and diprenorphine (Figure 5) are produced by altering the substituent on N17. Etorphine, with only a methyl substituent on the nitrogen, is an agonist, while buprenorphine and diprenorphine, which both have a methylcyclopropyl substituent on the nitrogen, are partial or complete antagonists. The differences in overall activity of buprenorphine and diprenorphine are attributable to the substitution of a methyl group for a t-butyl group (off C7 of the morphine skeleton) converting a mixed agonist-antagonist (buprenorphine) to a pure antagonist (diprenorphine).

DISCUSSION

Despite overlaps in the range of distances separating the pharmacophores in the opioid peptides, some useful insights

Table 2. Selected Pharmacophoric Parameters in Cyclic and Tic Containing Opioid Peptides*

Compound	N -Tyr	N - Hydr	Ring-Ring	Angle	N - O	Tyr χ^1	Phe χ^1	CCDC	Ref
Cyclic									
DPDPE	4.04	13.35	14.95	47.5	6.49	−68	−67	HESFUG	19
	4.14	12.28	15.91	59.6	6.55	−70	−67		
	2.99	12.82	13.18	41.3	6.36	−61	−69		
[D-Ala]-DPDPE	5.13	12.69	13.98	62.9	7.76	174	−64	WIPYEZ	20
[L-Ala]-DPDPE	5.16	8.00	12.06	20.0	7.82	−174	−66	WIPYAY	20
	5.13	7.54	11.56	35.0	7.83	179	−62		
	3.80	7.74	10.23	53.1	7.83	−174	−56		
	3.83	7.93	10.58	32.9	7.81	−179	−46		
DPMPT	5.17	12.10	13.69	60.2	7.88	171	63	WIPXUD	21
[Nle,Gly]-DPLPE	5.12	12.59	12.93	73.0	7.77	−165	−76	NA	unpub†
	5.23	13.05	14.67	59.0	7.95	174	−86		
[Ser^3]-DPDPE	4.01	11.24	14.20	31.0	6.43	57	177	NA	unpub†
	3.98	9.08	12.45	88.6	6.38	57	−69		
Range	3.0-5.3	7.5-13.35	10.2-15.9						
JOM-13	4.34	8.03	10.95	60.9	6.84	71	−83	YECDUF	22
	5.17	4.57	9.57	78.4	7.86	−171	−70		
Range	4.3-5.2	4.6-8.0	9.6-11.0						
Tic									
TIPP (1-2)	5.16	7.37	5.93	51.5	7.86	168	57	SUPBUA	18
TIPP (1-3)	5.16	8.30	9.21	43.1	7.86	168	−62		
D-TIPP (1-2)	4.18	6.78	6.74	50.0	6.61	−74	47	CALFEB	23
	5.17	6.53	6.27	76.0	7.86	−174	−48		
D-TIPP (1-3)	4.18	9.97	12.67	89.8	6.61	−74	−67		23
	5.17	9.43	9.55	55.0	7.86	−174	−59		
cyclo-[Tyr-Tic]	3.95	6.00	5.35	21.1	6.37	−60	54		24
boc-Tyr-Tic	3.92	6.84	8.45	13.9	6.28	−62	49	QAMWEG	25
Tyr-D-Tic	5.15	6.77	3.90	4.0	7.83	−172	−47	ROHFEZ	26
	5.15	6.90	3.93	1.3	7.84	−178	−55		
Tyr-D-Tic-NH_2	5.15	6.93	4.12	5.8	7.81	−169	−49	ROHFID	26
DmDmT*	5.24	7.30	5.07	13.3	7.94	−170	43	TUSMOJ	27
DmTA*	5.15	7.28	6.27	64.8	7.86	−178	64	TUSMUP	27
DmDmTA*	5.22	7.19	6.54	57.4	7.91	−168	62	TUSNAW	27
Range	3.9-5.2	6.0-10.0	3.9-12.7						

*CCDC indicates Cambridge Crystallographic Data Center; ref, reference; NA, not applicable; DmDmT, N,N-dimethyl-(2,6-dimethyl-Try)-Tic; DmTA, dimethyl-Tyr-Tic-NH-adamantain; and DmDmTA, N,N-dimethyl-(2,6-dimethyl-Try)-Tic-NH-adamantain.
†Deschamps JR, George C, Flippen-Anderson JL, Hruby V. Unpublished data. March 1998.

can be gained from a plot of the separation between the aromatic rings and the distance from the protonated amine to the second hydrophobic center (ie, aromatic ring) (Figure 6). For the purpose of comparison only, structures of potent highly selective ligands were initially used (filled symbols). Examining only these entries, it would appear that the δ-agonists (black squares) cluster along a diagonal, while the μ-agonists (black circles) and δ-antagonists (black triangles) form tight nonoverlapping clusters. Addition of some weak δ-agonists (open symbols) continues to show the same trend as was seen for the strong δ-agonists. The range of separations observed for weak μ-agonists (open circles) overlaps with that of the δ-antagonists in this simplistic 2-dimensional plot.

A large number of poorly selective agonists (denoted by the + symbols in Figure 6) were added in an attempt to classify these compounds. The majority of these "mixed agonists" are enkephalins, thus it is not surprising that they plot in the same general area as the δ-agonists. Enkephalin is considered a δ-agonist, although it has rather poor selectivity.[44] Only one of these mixed agonists plotted in the region of the

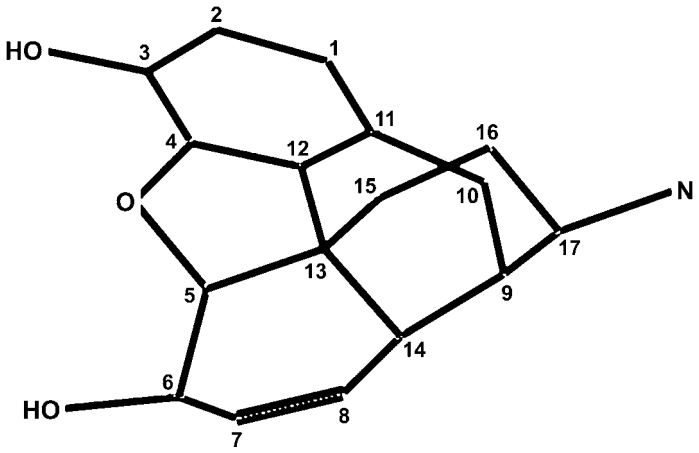

Figure 2. Structure of morphine[37] showing the numbering of the heterocyclic atoms.

Figure 3. Benzomorphans lack the fourth 6-membered ring and the bridging ether linkage, which forms the 5-membered ring. Shown is 4-methylhomobenzomorphan.[40]

μ-agonists. It lies between the strong and weak μ-agonists. This is the double-bend conformation of Leu-enkephalin. The presence of the δ-antagonists in this same region confuses the picture, but it is interesting to note that a protected derivative of the δ-antagonist N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH[18] shares this double-bend conformation.

Part of the difficulty in quantifying the optimal separation of the pharmacophores may be due to conformational flexibility remaining even in the conformationally constrained peptides (Figure 7). This problem was previously noted by Lomize and his colleagues.[22] Although nonpeptide ligands eliminate many of these problems, small structural changes may result in large changes in activity. Our ability to design highly potent and highly specific ligands is limited by our poor understanding of the molecular recognition necessary for proper receptor binding and activation. The final answers may not be

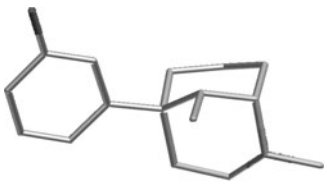

Figure 4. Phenylmorphans are formed by eliminating the tetrahydrofuran ring and one cyclohexyl ring by removing C10 and the bridging O in morphine. Shown is 2,9beta-gimethyl-5-(3-hydroxyphenyl)-2-azabicyclo(3.3.1)nonan-2-ium.[41]

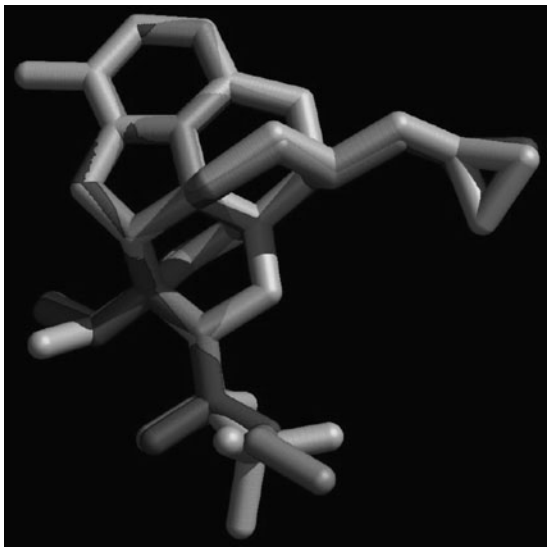

Figure 5. Superimposition of etorphine (green), buprenorphine (yellow), and diprenorphine (red) shows the structural similarity of these compounds despite their divergent activities (ie, agonist, mixed agonist-antagonist, and antagonist, respectively).[43]

available until structural information is obtained for the receptors themselves, both with and without bound ligands.

Highly potent and selective peptide agonists and antagonists have been synthesized. The utility of these compounds as drugs is limited mainly by their nature—they are peptides.

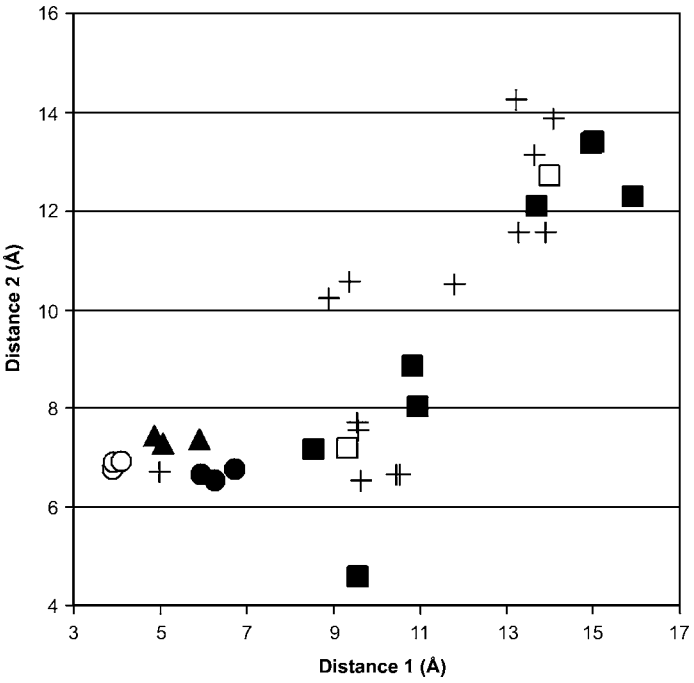

Figure 6. Separation of pharmacophoric elements in opioid peptides. Distance 1 is the distance between the 2 hydrophobic regions (ie, rings); distance 2 is the separation between the amine (ie, N-terminal amino group) and the second hydrophobic region (ie, Phe[4]). Included are strong (■) and weak (□) δ-agonists, strong (●) and weak (○) μ-agonists, δ-antagonists (▲), and poorly selective compounds (+).

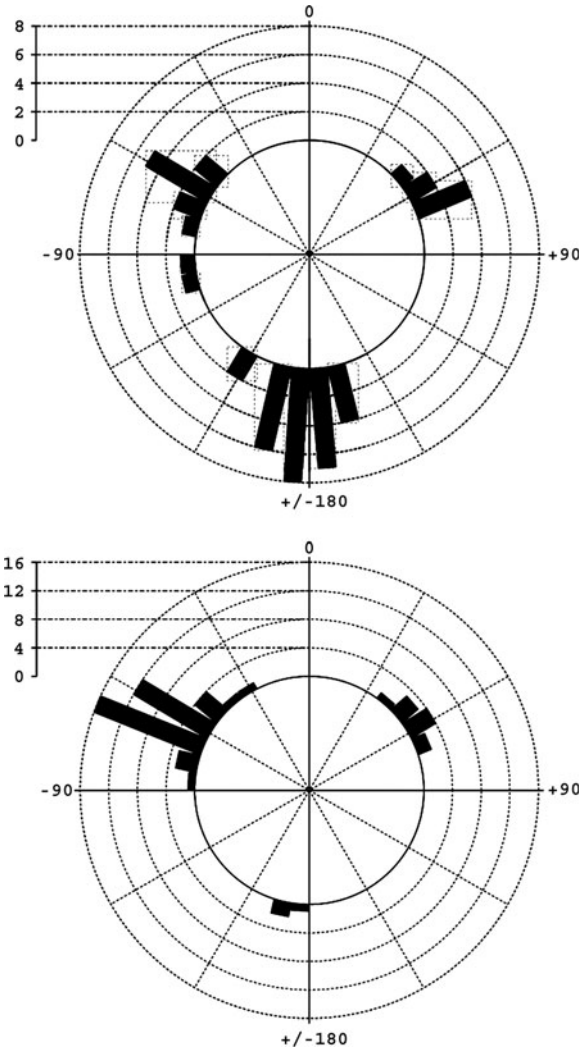

Figure 7. Side chain conformers in the opioid peptides: top—tyrosine (residue 1) χ^1 torsion angle, bottom—phenylalanine (residue 4) χ^1 torsion angle.

In general, the peptide drugs are not given orally but are administered by the parenteral route. This requirement complicates their use and thus limits their utility. The nonpeptide ligands can be administered orally and thus have found their way into widespread use. Nonpeptide ligands also eliminate the problems associated with flexibility found even in highly constrained peptide ligands. The endogenous opioid peptides are somewhat effective in relieving pain and do not exhibit the undesirable side effects associated with the nonpeptide ligands. If problems with drug delivery could be overcome, peptide-based drugs may offer the best of both worlds, effective analgesia and little or no side effects.

CONCLUSIONS

Crystallographic studies play a vital role on drug design. The results from X-ray crystallographic studies provide accurate and reliable 3-dimensional structural parameters. In addition, crystallography is the only method for determining the "absolute" configuration of a molecule, a critical property in biological systems as changes in this may alter the response of the biologic system. Medicinal chemists have made considerable progress in producing more potent and selective opioid peptides by constraining the peptide conformation. Further progress requires translating the linear modifications made to the peptide ligands into the 3-dimensional framework of the receptor. The results of crystallographic studies allow pharmacophoric parameters to be calculated from the 3-dimensional coordinates. These can then be used along with data on biologic activity to guide future development. The use of 3-dimensional data allows comparison of the relative position of groups thought to be important in binding to the receptor even in structurally dissimilar compounds.

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