Communications to the Editor

Incorporation of a Novel Conformationally Restricted Tyrosine Analog into a Cyclic, δ Opioid Receptor Selective Tetrapeptide (JOM-13) Enhances δ Receptor Binding Affinity and Selectivity

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The incorporation of conformational constraints in analogs of biologically active peptides is a well-established approach for enhancing receptor selectivity, for modulating efficacy, and for minimizing the dynamic averaging that compromises attempts to elucidate the solution and bioactive conformations of the peptide ligand. In the opioid peptide area, conformational restriction via side chain to side chain cyclizations, in particular, has been very effective, yielding highly selective δ opioid receptor agonists[1,2] and highly selective μ receptor agonists[3] and antagonists.[4] Our own efforts have resulted in three key analogs, Tyr-c[D-Pen-Gly-Phe-D-Pen]OH (DPDPE, where Pen, penicillamine, is β,β-dimethylcysteine),[1] its L-Pen[5] diastereomer, DPLPE,[1] and the tetrapeptide, Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13, 1),[2] all of which are cyclized via a disulfide bond formed from side-chain sulfhydryl groups. In all three analogs, conformational flexibility of the backbone is considerably reduced. This is especially true of 1, which was designed as a further constrained analog of DPDPE, lacking the flexible central glycine residue of the pentapeptide. However, in all three analogs mobility of the side chains, especially of the Tyr and Phe residues, is comparatively unhindered. Recently, increasing attention has been directed toward the incorporation of conformationally restricted Phe and Tyr analogs, within a backbone-restricted matrix, in order to better assess the conformational requirements for bioactivity of these residues which are critical for opioid receptor binding. Of major interest are modifications which limit the conformational range of χ^1, the dihedral angle about the C^α–C^β bond. Three such modifications have been employed recently for Tyr and/or Phe substitutions in opioid peptides. These are tetrahydroisoquinoline-3-carboxylic acid (Tic),[5] 2-aminotetralin-2-carboxylic acid (Atc),[6] and 2-aminoindan-2-carboxylic acid (Aic),[6] as phenylalanine replacements, and their appropriate aryl ring hydroxylated counterparts, HO-Tic,[7] Hat,[8,9] and Hai,[8] respectively, as tyrosine replacements (Figure 1). All three types of modification are effective in limiting orientational freedom about the C^α–C^β bond due to the inclusion of this bond in a 5- or 6-membered ring. However, due to their bicyclic structures, these modifications also greatly limit the allowed values of χ^2, the dihedral angle about C^β–C^γ. This latter effect poses a limitation, particularly for the interpretation of reductions in binding affinity, since the respective roles of the χ^1 and χ^2 restrictions cannot be deduced. We report here the use of a novel, conformationally restricted tyrosine analog, *trans*-3-(4′-hydroxyphenyl)proline (*t*-Hpp, Figure 1), which, like the fused bicyclic analogs described above, curtails orientational freedom about χ^1, but which allows unrestricted rotation about χ^2. *t*-Hpp is the tyrosine-like counterpart of *trans*-3-phenylproline, which has been employed as a proline substitution in the opioid peptide morphiceptin,[10] but

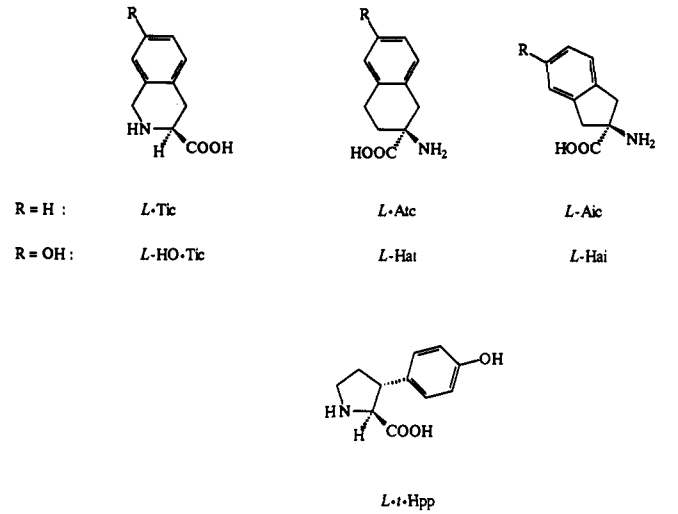

Figure 1. Structures of conformationally restricted tyrosine and phenylalanine analogs.

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Table I. Binding Affinities of Opioid Peptides at μ, δ, and κ Receptors in Guinea Pig Brain Homogenates

peptide	compd	binding K_i (nM) [^{3}H]DAMGO	[^{3}H]DPDPE	[^{3}H]U69,593	$K_i(\mu)/K_i(\delta)$
Tyr-c[D-Cys-Phe-D-Pen]OH (JOM-13)	1	107 ± 1.0	1.79 ± 0.11	>10 000	60
L-*t*-Hpp-c[D-Cys-Phe-D-Pen]OH[a]	2	105 ± 19.1	0.66 ± 0.06	~10 000	159
D-*t*-Hpp-c[D-Cys-Phe-D-Pen]OH[a]	3	~10 000	84.3 ± 6.16	>10 000	~120
Tyr-c[D-Pen-Gly-Phe-D-Pen]OH	DPDPE	810 ± 66	3.98 ± 0.46	>10 000	204

[a] Tentative assignment of *t*-Hpp stereochemistry (see text).

which can also be viewed as a conformationally restricted phenylalanine replacement (Mosberg, et al., in preparation).

As noted above, the tetrapeptide 1 was designed to decrease the residual flexibility found in the backbone of DPDPE, which can be attributed to the conformationally unhindered Gly^3 residue. NMR studies support the notion that the backbone of 1 is less susceptible to dynamic averaging effects than is DPDPE.[11] Despite this increased rigidity, 1 retains high binding affinity for the δ opioid receptor, higher in fact than that of DPDPE, while suffering a small loss in δ selectivity.[2] As in DPDPE, the Tyr and Phe side chains of 1 can be expected to possess a higher degree of flexibility than does the peptide backbone. NMR studies of 1 in aqueous solution, in which $J_{\alpha\beta}$, the coupling constant between the C^α and C^β protons, is interpreted in terms of populations of the three low-energy, staggered rotamers about this bond, indicate that χ^1 for the Tyr residue is dominated by the g^- ($\chi^1 = -60°$) and t ($\chi^1 = 180°$) rotamers, with the calculated g^+ ($\chi^1 = 60°$) rotamer population being less than 10%.[11] Since these side-chain preferences in solution might not be predictive of those required for receptor recognition, analogs in which the motional freedom of the side chain is more limited can provide insights into the receptor requirements. Substitution of Tyr^1 by *t*-Hpp^1 provides a means of evaluating whether the receptor-bound and solution side-chain conformers differ, since χ^1 of *t*-Hpp is limited by the pyrrolidine ring to the range ~−85° to ~−150° with conformations near these limiting values favored. Thus *t*-Hpp^1 substitution in 1 favors χ^1 rotamers near those highly populated for the parent Tyr^1 residue in solution. The synthesis[12] of the 3-(4′-hydroxyphenyl)prolines yielded a mixture of all four stereoisomers. After chromatographic separation from the pair of cis enantiomers, the trans racemic mixture was employed to synthesize the two diastereomeric JOM-13 analogs 2 and 3.[13] Table I summarizes opioid receptor binding data for these *t*-Hpp^1-substituted analogs of 1 and compares them with the corresponding data for 1 itself and for the δ selectivity reference, DPDPE. The data are given as K_i values calculated from binding experiments in which competition with radiolabeled ligands selective for μ ([^{3}H]DAMGO), δ ([^{3}H]DPDPE), and κ ([^{3}H]U69,593) opioid receptors is assessed.[14] As can be seen from Table I, binding affinities at both μ and δ receptors are much higher for 2 than for 3. Although circumstantial, this much higher affinity for 2 suggests that it is the L-*t*-Hpp^1 analog, which is consistent with the very similar affinity difference observed for 1 and its D-Tyr^1 analog.[15] As seen from Table I, analog 2 displays similar μ affinity but ca. 3-fold enhanced δ affinity compared with 1 and is consequently approximately 3-fold more δ selective than is 1. Compared with the δ selectivity standard, DPDPE, 2 exhibits comparable δ selectivity and 6-fold higher δ affinity. In addition to the side-chain restriction, *t*-Hpp differs from Tyr by being an imino acid, which could in principle contribute to the observed high affinity for 2. This appears unlikely, however, since replacement of Tyr^1 in 1 by NMeTyr^1, mimicking the secondary amine in the *t*-Hpp^1 analog, is without significant effect on μ or δ binding.[16] Thus, the high δ affinity observed for 2 indicates that the conformational restriction imposed by the L-*t*-Hpp^1 substitution is conducive to δ receptor binding and that, therefore, the orientation about the C^α–C^β bond of the Tyr^1 residue in the parent peptide 1 also lies between $\chi^1 = \sim -85°$ and $\sim -150°$. This result is in good agreement with the NMR findings for 1 in aqueous solution, suggesting that no major reorientation of the Tyr side chain accompanies δ receptor binding of 1. The results reported here also suggest a more general role for *t*-Hpp (and *c*-Hpp) as a conformationally con-

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(12) Sodium-catalyzed condensation of diethyl acetamidomalonate with *p*-methoxycinnamaldehyde was followed by reduction of the resultant 5-hydroxypyrrolidine with triethylsilane in a manner analogous to that described by Chung et al. for the synthesis of *N*-(*tert*-butyloxycarbonyl)-*trans*-3-phenylproline. (Chung, J. Y. L.; Wasicak, J. T.; Arnold, W. A.; May, C. S.; Nadzan, A. M.; Holladay, M. W. Conformationally Constrained Amino Acids. Synthesis and Optical Resolution of 3-Substituted Proline Derivatives. *J. Org. Chem.* 1990, *55*, 270–275.) The resultant diethyl 1-acetyl-3-(4′-methoxyphenyl)pyrrolidine-2,2-dicarboxylate was subjected to saponification and decarboxylation by the method of Sarges and Tretter (Sarges, R.; Tretter, J. R. Synthesis of Aryl-Substituted 1,3- and 1,4-Diazocine Derivatives. *J. Org. Chem.* 1974, *39*, 1710–1716) to give *cis*- and *trans*-*N*-acetyl-3-(4′-methoxyphenyl)proline. N-Deacetylation by refluxing under the strongly acidic conditions of Chung et al. gave simultaneous deprotection of the methyl ether. Following N-Boc protection, *N*-(*tert*-butyloxycarbonyl)-*trans*-3-(4′-hydroxyphenyl)-proline (*t*-Hpp) was separated from the cis-isomer by reverse-phase high-performance liquid chromatography (RP-HPLC). The two isomers were distinguished on the basis of ^{1}H-NMR spectral analysis and by the straightforward comparison with the NMR spectrum of the corresponding *trans*-3-phenylproline, synthesized by the method of Chung et al.

(13) Solid-phase peptide synthesis was performed as described in ref 2. HF cleavage from the resin gave a pair of linear diastereomeric peptides which were separated by semipreparative RP-HPLC. Oxidation of each of the linear, diastereomeric peptides with $K_3Fe(CN)_6$ was followed by RP-HPLC purification to give the diastereomeric cyclic disulfide peptides 2 and 3. Each peptide was >98% pure as assessed by analytical RP-HPLC (Vydac 218-TP column, 4.6 × 250 mm, using a linear gradient of 0–70% organic component in 70 min at a flow rate of 1 mL/min) monitored at four wavelengths. The solvent system was 0.1% (w/v) trifluoroacetic acid in water and 0.1% (w/v) trifluoroacetic acid in acetonitrile. Retention times were 30.53 min and 30.40 min for 2 and 3, respectively. Each peptide was subjected to fast-atom bombardment mass spectrometry and gave the expected molecular weight of 586.

(14) Binding assays were conducted using membrane preparations from guinea pig brain homogenates, as previously described. (Heyl, D. L.; Mosberg, H. I. Substitution on the Phe^3 Aromatic Ring in Cyclic Delta Receptor-Selective Dermorphin/Deltorphin Tetrapeptide Analogs: Electronic and Lipophilic Requirements for Receptor Affinity. *J. Med. Chem.* 1992, *35*, 1535–1541.) Each reported K_i value and standard error of the mean (SEM) is based upon at least two independent binding experiments, each done in triplicate.

(15) K_i values determined for [D-Tyr^1]JOM-13 were ~10 000 nM at μ and 1200 ± 130 nM at δ receptors.

(16) K_i values determined for [NMeTyr^1]JOM-13 were 176 ± 31 nM at μ and 2.36 ± 0.34 nM at δ receptors.

strained tyrosine analog in other bioactive peptides. As in the example described here, the use of *t*-Hpp in other peptides can provide important insights into side-chain orientation of tyrosine residues in peptide bioactive conformations.

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