Elucidation of the conformational features within a series of tetrapeptides which determine the selective recognition of μ versus δ opioid receptors

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We have previously described the cyclic μ opioid receptor selective tetrapeptide Tyr-c[*D*-Cys-Phe-*D*-Pen]NH_2 (S-Et-S) (JOM-6) [1]. In the present study we report the development of a μ receptor pharmacophore model using residue 1 and 3 JOM-6 analogs. The μ opioid pharmacophore groups of JOM-6 (i.e., the phenol and Nα group of Tyr^1 and the phenyl group of Phe^3) lie outside the cyclic portion of the tetrapeptide and are conformationally labile. In contrast to the pharmacophore groups, the tripeptide cycle (a 13-membered ring) experiences only moderate flexibility by virtue of the ethylene dithioether cyclization. To reduce peptide flexibility several residue 1 and 3, and peptide cycle analogs of JOM-6 were prepared. The residue 1 and 3 analogs include: *trans*-3-(4'-hydroxyphenyl)proline (*t*-Hpp) and 2-amino-6-hydroxytetralin-2-carboxylic acid (Hat) in the place of Tyr^1, and Δ*E*Phe in the place of Phe^3. The peptide cycle analogs incorporate disulfide (S-S) or ethyne dithioether (S-*cis*-HC=CH-S) bridges instead of an ethylene dithioether (S-Et-S) bridge. The low energy conformations of each of these analogs were generated using molecular mechanics and then compared to deduce the probable μ receptor bound conformation of JOM-6 and its analogs.

Results and Discussion

In comparison with the *t*-Hpp^1, Hat^1, and ethyne dithioether derivatives of JOM-6, all of which displayed high affinity ($K_i\mu < 4$ nM) to μ receptor sites, the fourth analog employed for this study, Tyr-c[*D*-Cys-Δ*E*Phe-*D*-Pen]NH_2(S-S) (JH-42), displayed slightly reduced μ affinity ($K_i\mu = 8.74$ nM). After identifying all possible low energy conformations for each analog (with ΔE < 4 kcal/mol), the sets of conformations were overlaid to determine the probable μ receptor bound geometry of these tetrapeptides. The receptor bound conformation of JOM-6 requires a χ^1 orientation of *trans* (~ 180°) for the sidechains of both Tyr^1 and Phe^3.

The μ receptor bound conformation of JOM-6 was compared with the previously reported δ receptor bound conformation of Tyr-c[*D*-Cys-Phe-*D*-Pen]OH(S-S) (JOM-13) [2,3] to delineate the conformational features which determine μ versus δ receptor selective binding (Fig. 1). Overlap is observed between the conformations of the Tyr^1

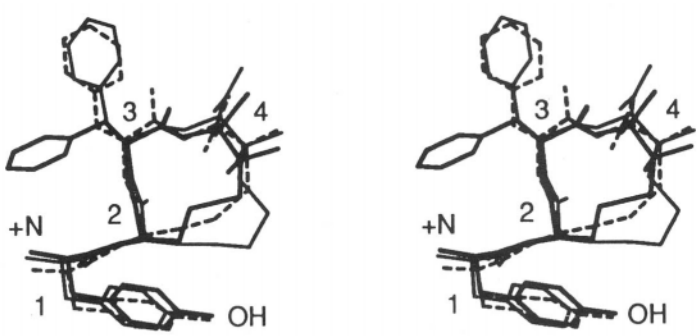

Fig. 1. Superposition (stereoview) of the μ receptor bound conformations of JOM-6 (solid line) and the disulfide-containing tetrapeptide analog JH-42 (dashed line) and the δ receptor bound conformation of JOM-13 (bold solid line). The Cα atom of residue 3 and the functionally important Nα, Oη, Cη, Cε1, and Cε2 atoms of Tyr¹ were used for the superposition.

residue as well as the mainchain atoms within the peptide cycles, including the C-terminal functional groups. The most apparent difference lies in the orientation of the aromatic ring of residue 3. Unlike the *trans* ($\chi^1 \sim 180°$) orientation required for residue 3 of the μ bound geometry, the δ bound geometry requires a *gauche*$^+$ ($\chi^1 \sim -60°$) orientation. By comparing the μ and δ pharmacophore models developed from the structurally similar JOM-6 and JOM-13 tetrapeptides, the conformational feature underlying μ versus δ receptor selectivity in this series appears to be the orientation of the aromatic ring of **Phe**^{3}.

Acknowledgments

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