Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2681–2684

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A HIGH AFFINITY, MU-OPIOID RECEPTOR-SELECTIVE ENKEPHALIN ANALOGUE LACKING AN N-TERMINAL TYROSINE

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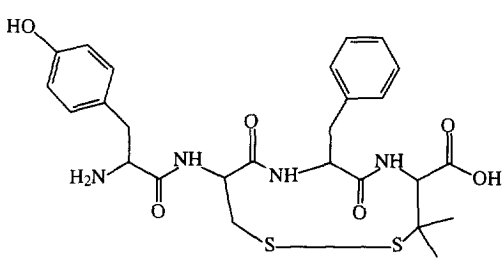
Received 8 June 1998; accepted 7 August 1998

Abstract: We report a high affinity, μ opioid receptor selective enkephalin analogue in which the N-terminal tyrosine residue thought to be required for such high affinity is replaced by phenylalanine. The high affinity can be traced to a shift of the ligand's N-terminal residue within the μ receptor binding pocket, which diminishes the importance of the usual hydrogen bond between the tyrosine phenolic moiety and the receptor.

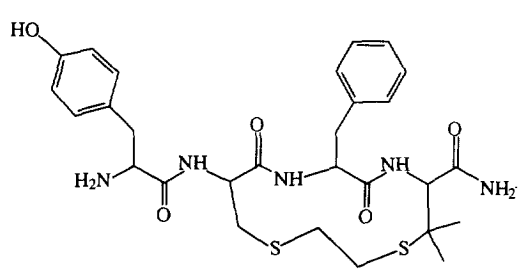
It has long been accepted that the key opioid pharmacophore elements of enkephalin-like peptides are an amino terminal tyrosine residue, in which the free amine and phenolic side chain are both required, and a second aromatic side chain, typically arising from residue 3 or 4 in the peptide sequence.[1] Of these elements, the tyrosine residue, which corresponds directly to the essential tyramine moiety of morphine and related morphinans and epoxy-morphinans, is particularly well entrenched as a presumed requirement. Although these tenets are based upon observations in many enkephalin analogues, it is clear that ligand receptor interactions of structurally similar ligands interacting with the same binding site may vary. Thus, for example, a missing pharmacophore element might be overcome by compensatory interactions available with a specific ligand, and/or structural features of a particular ligand might necessitate a slight shift within the binding pocket that reduces the importance of a usually key ligand-receptor interaction. The latter effects might especially be expected for larger, relatively rigid ligands, which are less able to conformationally adapt to the binding pocket.

JOM-13 and JOM-6, two structurally related, conformationally constrained opioid peptides,[2] are shown in Figure 1. Although these peptides differ only in the nature of the C-terminal moiety (carboxylic acid in

Figure 1: Structures of Cyclic Opioid Tetrapeptides



JOM-13



JOM-6

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PII: S0960-894X(98)00476-4

JOM-13, carboxamide in JOM-6) and in the size and type of the tripeptide cycle (11-membered disulfide in JOM-13, 13-membered ethylene dithioether in JOM-6), they differ significantly in their interactions with opioid receptors: JOM-13 binds with high affinity and moderate selectivity to δ (vs. μ) opioid receptors, while JOM-6 exhibits high affinity and moderate selectivity for μ (vs. δ) receptors.[2] We have examined the conformational preferences of these two peptides and related analogues with further conformational constraints and have deduced the conformational features required for interaction of these compounds with the δ and μ opioid receptors.[3-8] A key difference in these pharmacophore models is the orientation of the Phe^3 side chain, which requires a *gauche* orientation (χ^1 ~ -60°) for optimal binding to the δ receptor, but assumes a *trans* conformation (χ^1 ~ 180°) for binding to the μ receptor.

We have recently described a novel approach for the calculation of high resolution structural models of the transmembrane domain of G protein-coupled receptors[9] and have applied this approach to μ and δ opioid receptors.[10] Docking of the pharmacophore conformations of JOM-13 and JOM-6 (and related peptides) to the δ and μ receptor models, respectively, revealed that the differing requirement for the side chain conformation of Phe^3 arises from a Trp for Leu substitution in transmembrane helix VII of the μ (vs. δ) receptor.[10] Steric interaction between this Trp side chain and the tripeptide cycle of peptides in the JOM-6 and JOM-13 series causes a slight shift of the peptide ligand within the binding pocket, which, in turn, results in the required reorientation of the Phe^3 side chain. A secondary consequence of this shift is a slight difference in orientation of the ligand N-terminal Tyr residue within the corresponding region of the binding site, which is common to both receptors. As a result, the distance between the phenolic oxygen of Tyr^1 of JOM-6 and the imidazole nitrogen of its presumed hydrogen bonding partner, His^{297} of the μ receptor, is ~0.7 Å greater than the corresponding distance for JOM-13 in the δ binding site. This increased distance raises the possibility that this hydrogen bond may be of lesser importance for ligand-receptor recognition in the former case and, by extension, that the Tyr phenolic substituent may play a subordinate role in the binding of JOM-6 to the μ receptor.

To examine the possibility raised above, Phe^1 substituted analogues of JOM-13 and JOM-6 (KSK-58 and JH-54, respectively) were synthesized[11] and their affinities for μ, δ, and κ opioid receptors were examined.[12] The results for μ and δ receptor binding are shown in Table 1 (κ receptor binding affinities of all compounds were much weaker than μ or δ affinities, ranging from 5 – 100-fold lower than the weaker of the μ and δ affinities). As can be seen from Table 1, Phe^1 substitution in either cyclic series leads to considerable reduction in δ receptor binding affinity (~40-fold for JH-54 vs. JOM-6; ~55-fold for KSK-58 vs. JOM-13), while binding affinity to the μ receptor is less affected (~4.5-fold and 12-fold, respectively). As a result of the disparate effect of the Phe^1 substitution on μ and δ binding affinity, JH-54 displays considerably improved μ binding selectivity: $K_i(\delta)/K_i(\mu) = 750$. This high selectivity is comparable to that displayed by DAMGO ($K_i(\delta)/K_i(\mu) = 570$), the prototypical μ receptor-selective opioid ligand[13] (Table 1). The relatively small effect

Table 1: Receptor binding affinities of cyclic opioid tetrapeptides

Compound	Sequence	Cyclization	K_i μ (nM)	K_i δ (nM)
JOM-13	Tyr-c[D-Cys-Phe-D-Pen]OH	S-S	51.5	0.74
KSK-58	Phe-c[D-Cys-Phe-D-Pen]OH	S-S	610	42.4
JOM-6	Tyr-c[D-Cys-Phe-D-Pen]NH_2	S-Et-S	0.29	24.8
JH-54	Phe-c[D-Cys-Phe-D-Pen]NH_2	S-Et-S	1.36	1020
DAMGO	Tyr-D-Ala-Gly-NMePhe-Gly-ol	-----	0.99	567

of Phe^1 substitution on μ receptor binding affinity in the peptides described here is entirely consistent with the suggestion that a hydrogen bond involving the phenolic Tyr hydroxyl of JOM-6 (and its related analogues) is only a minor contributor to the overall binding energy of this peptide to the μ receptor. This suggestion, itself, follows from comparison of the ligand-receptor complex models derived for JOM-13 and JOM-6 with δ and μ receptors;[11] hence the results described here serve as further support for these models.

It is important to emphasize that the reduced importance of the phenolic hydroxyl for μ receptor binding in the the cyclic tetrapeptides described here results from the shift of the tyramine moiety in the complementary region of the binding site of the μ receptor. This shift, in turn, is due to a steric interaction of the bulky Leu^{300} side chain of the μ receptor with the tripeptide cycle of the peptide. The absence of a similar effect in other native and synthetic opioid peptide reflects the absence of a corresponding 'shift-inducing', steric ligand-receptor interaction. It should also be mentioned that another series of opioid peptides, derived from somatostatin, lacking an N-terminal tyrosine has been reported;[14,15] however these peptides are structurally remote from endogenous opioid peptides and the nature of the pharmacophore in this series is unclear. Further, the somatostatin-based analogues are all μ selective opioid antagonists, suggesting that they may interact quite differently with the μ receptor than do the native agonists. By contrast, JH-54 is a potent (IC_{50} = 9.1 nM) full agonist in the in vitro guinea pig ileum opioid bioassay (F. Porreca, personal communication).

Acknowledgments This work was supported by grants DA03910 and DA00118 from the National Institutes of Health. We are grateful to Carol Mousigian for performing the opioid receptor binding assays.

References and notes

1. Casy, A. F.; Parfitt, R. T. *Opioid Analgesics. Chemistry and Receptors*; Plenum: New York, 1986; pp 342-353.

2. Mosberg, H. I.; Omnaas, J. R.; Smith, C. B.; Medzihradsky, F. *Life Sci.* **1988**, *43*, 1013.

3. Lomize, A. L.; Flippen-Anderson, J. L.; George, C.; Mosberg, H. I. *J. Amer. Chem. Soc.* **1994**, *116*, 429.

4. Mosberg, H. I.; Lomize, A. L., Wang, C.; Kroona, H.; Heyl, D. L.; Ma, W.; Sobczyk-Kojiro, K.; Mousigian, C.; Porreca, F. *J. Med. Chem.* **1994**, *37*, 4371.

5. Mosberg, H. I.; Omnaas, J. R.; Lomize, A.; Heyl, D. L.; Nordan, I.; Mousigian, C.; Davis, P.; Porreca, F. *J. Med. Chem.* **1994**, *37*, 4384.

6. Lomize, A. L.; Pogozheva, I. D.; Mosberg, H. I. *Biopolymers* **1996**, *38*, 221.

7. Mosberg, H. I.; Dua, R. K.; Pogozheva, I. D.;. Lomize, A. L. *Biopolymers* **1996**, *39*, 287.

8. Ho, J. C. *Development of a Model for the μ Opioid Receptor Pharmacophore*, Ph.D. Thesis, University of Michigan, Ann Arbor, 1997.

9. Pogozheva, I. D.; Lomize, A. L.; Mosberg, H. I. *Biophys. J.* **1997**, *70*, 1963.

10. Pogozheva, I. D.; Lomize, A. L.; Mosberg, H. I. *Biophys. J.* **1998**, *75*, 612.

11. All peptides were synthesized on a Vega 250C solid phase peptide synthesizer using techniques described in references 2, 4, 5, 7, and 8.

12. Binding to μ, δ, and κ opioid receptors in guinea pig brain membranes was determined as described in Heyl, D. L.; Mosberg, H. I. *J. Med. Chem.* **1992**, *35*, 1535. K_i values reported for each analogue in Table 1 represent the mean of 2-4 independent determinations, each performed in triplicate. Standard errors of the mean (s.e.m.) were <10%.

13. Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. *Eur. J. Pharmacol.* **1981**, *70*, 531.

14. Pelton, J.; Kazmierski, W.; Gulya, K.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1986**, *29*, 2370.

15. Kazmierski, W.; Wire, W. S.; Lui, G. K.; Knapp, R. J.; Shook, J. E.; Burks, T. F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1988**, *31*, 2170.