Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 879–881

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Dermorphin and Deltorphin Heptapeptide Analogues: Replacement of Phe Residue by Dmp Greatly Improves Opioid Receptor Affinity and Selectivity

Akihiro Ambo, Harumi Murase, Hideko Niizuma, Hidekazu Ouchi, Yutaka Yamamoto and Yusuke Sasaki*

Tohoku Pharmaceutical University, 4-1 Komatsushima 4-chome, Aoba-ku, Sendai 981-8558, Japan

Received 15 November 2001; accepted 7 January 2002

Abstract—The usefulness of 2,6-dimethylphenylalanine (Dmp) as a Phe surrogate in two opioid peptides, dermorphin (DM) and deltorphin II (DT), was investigated. Compared to DM, [L-Dmp^3]DM (**1**) showed a 170-fold increase in μ affinity and only a 4-fold increase in δ affinity, resulting in a 40-fold improvement in μ receptor selectivity. Compared to DT, [L-Dmp^3]DT (**3**) showed a 22-fold increase in δ affinity and somewhat of a loss in μ affinity, and consequently a marked (75-fold) improvement in δ receptor selectivity. The D-Dmp replacement, however, resulted in a great loss in receptor selectivity in each of the peptides. The specific receptor interactions of **1** and **3** were confirmed by in vitro bioassays. Analogues **1** and **3** seem to be useful as pharmacological tools for the study of opioid systems.

In opioid peptides, two aromatic amino acids Tyr^1 and either Phe^3 or Phe^4 are important structural elements that interact with the opioid receptors.[1] Recent structure–activity studies of opioid peptides have demonstrated that the introduction of 2,6-dimethyltyrosine (Dmt) in place of Tyr^1 produces vastly improved opioid receptor affinity.[2–16] Very recently, we have shown that the use of 2, 6-dimethylphenylalanine (Dmp) in place of Phe^4 in combination with Dmt^1 in Leu-enkephalin produced an antagonist that is active primarily toward the μ receptor.[17] That study suggested that the Dmp residue would be a useful Phe surrogate in the design of opioid mimetics with unique opioid activities. Nevertheless, to date there is no other example of the Dmp residue being incorporated into other opioid peptides. To investigate further the usefulness of Dmp as a Phe surrogate in opioid peptides, we applied this unnatural amino acid to two peptides, dermorphin (DM: Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) and deltorphin II (DT: Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2); these ligands are highly selective toward μ and δ opioid receptors, respectively.[18,19] The present study deals with hexapeptide analogues produced by the single residue (Dmp) replacement, and demonstrates these analogues' extraordinarily high affinity and selectivity toward either μ or δ opioid receptors.

Chemistry

All peptides were synthesized (Tables 1 and 2) by a solid-phase method using Fmoc chemistry, as previously described.[16,20] For the syntheses of analogues, protected peptides **1** and **2**, or **3** and **4**, were constructed on solid support using Fmoc-D/L-Dmps. After the peptides were cleaved from the resin and deprotected, diastereomeric peptides were separated by a medium-pressured HPLC as previously reported.[16,20] The L- or D-configuration of Dmp in the peptides was determined by comparison with authentic ones on a chiral TLC plate[17] after acid hydrolysis of the peptides.[21]

Biological Results and Discussion

Table 1 shows the binding affinity of each analogue to its opioid receptor in comparison with the respective parent peptide. These comparisons were made using rat brain synaptosomes as previously reported.[16] DM and DT showed high affinity and selectivity toward μ and δ

*Corresponding author. Tel.: +81-22-234-4181; fax: +81-22-275-2013; e-mail: ysasaki@tohuku-pharm.ac.jp

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00035-5

Table 1. Opioid receptor binding assay of synthetic analogues

Peptides	Receptor binding affiniy $K_i \pm$SE (nM)		Selectivity	
	μ[a]	δ[b]	δ/μ	μ/δ
Dermorphin (DM)	0.092±0.024	192±51	2087	—
[L-Dmp^3]DM (**1**)	0.00054±0.00021	45.7±11.8	84,630	—
[D-Dmp^3]DM (**2**)	4.43±1.85	3300±702	745	—
Deltorphin II (DT)	314±53	0.0226±0.0077	—	13,894
[L-Dmp^3]DT (**3**)	1098±111	0.00105±0.00043	—	1,045,714
[D-Dmp^3]DT (**4**)	1956±177	111±17	—	18

[a]Versus [^{3}H]DAMGO.
[b]Versus [^{3}H]DT.

Table 2. In vitro biological assay of Dmp replacing DM and DT analogues

Pepetides	$IC_{50} \pm$SE (nM)[a]		Ratio	
	GPI (μ)	MVD (δ)	MVD/GPI	GPI/MVD
Dermorphin (DM)	3.74±0.57	34.4±4.8	9.2	—
[L-Dmp^3]DM (**1**)	1.21±0.23	4.62±0.82	3.8	—
[D-Dmp^3]DM (**2**)	44.4±6.1	358±45	8.1	—
Deltorphin II (DT)	5437±812	0.582±0.029	—	9342
[L-Dmp^3]DT (**3**)	6705±992	0.022±0.003	—	304,772
[D-Dmp^3]DT (**4**)	8214±872	145±15	—	56

[a]Values are the mean of 4–8 experiments±SE.

receptors, respectively. Interestingly, the replacement of Phe by L-Dmp in μ-specific ligand DM (**1**) induced a great increase (170-fold) in μ affinity and only a modest (4-fold) increase in δ affinity. As a consequence, the μ receptor selectivity of **1** was markedly improved (δ/μ ratio = 84,630). The D-Dmp replacement (**2**), however, decreased affinity by 48-fold and 17-fold at the μ and δ receptors, respectively, indicating that the L-chirality at this position is crucial to both receptor interactions. This result is in accord with the results of other peptide analogues that are structurally related to DM[22] and enkephalin.[16] On the other hand, the replacement of L-Dmp in δ-specific DT produced **3** with a 22-fold increase in δ affinity and a 3-fold decrease in μ affinity, resulting in a 75-fold improvement in δ receptor selectivity with an unprecedented δ receptor preference (μ/δ = 1,045,714). The configurational inversion of Dmp in DT (**4**) was detrimental to δ affinity and selectivity, a finding similar to the case of μ ligand DM as described above.

In vitro biological activity of synthetic analogues was evaluated by electrically induced smooth muscle contractions of guinea pig ileum (GPI) and mouse vas deferens (MVD) tissue preparations, as previously reported.[23] The GPI tissue contains predominantly μ receptors, while MVD includes mainly δ receptors. As shown in Table 2, **1** showed 3 and 7 times more potency than the parent peptides in the GPI and MVD assays, respectively. However, the degree of increase in GPI potency (only 3-fold) was not well related with that of the receptor binding affinity (170-fold). This may be due to the differences in μ receptor subtypes in the brain and peripheral tissues. As expected from the receptor binding data, **2** showed low potencies in both assays. Consistent with the receptor binding data, **3** showed markedly increased MVD potency without significant changes of GPI potency, resulting in a very high GPI/MVD ratio: 304,772. As expected, D-Dmp^3 analogue **4** possessed a very low MVD potency.

Recent structure–receptor selectivity studies of DM/DT peptides have revealed that the hydrophobicity, but not the aromaticity, of the third residue is an important factor for both μ[24,25] and δ[26] receptor affinities. Thus, the high receptor affinity of analogues **1** and **3** would be attributed mainly to an elevated hydrophobic character of Dmp residue, which is apparently reflected by these analogues' long RP-HPLC elution times.[27] Although the Dmp residues in DM and DT sequences could possibly influence the conformation of the peptides by reducing the rotation of the aromatic ring, the potential alteration in both peptides may also be advantageous to each receptor interaction (**1** for μ and **3** for δ) since both **1** and **3** had markedly improved receptor selectivities.

In summary, the present study demonstrated that the replacement of Phe^3 of DM or DT with L-Dmp produced an analogue (**1** or **3**) with greatly improved receptor affinity and selectivity. The modification with Dmp may serve in the design and development of new opioid mimetics with high affinity and selectivity for opioid receptors. Analogues **1** and **3** would be among the most potent and selective ligands for μ and δ opioid receptors, respectively, identified to date, and would therefore seem useful as pharmacological tools for the study of opioid systems.

References and Notes

1. Hruby, V. J.; Gehrig, C. A. *Med. Res. Rev.* **1989**, *9*, 343.
2. Hansen, D. W., Jr.; Mazur, R. H.; Clare, M. In *Peptides: Structure and Function*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds.; Pierce Chemical Co.: IL, 1985; p 491.
3. Chandrakumar, N. S.; Yonan, P. K.; Stapelfeld, A.; Savage, M.; Rorbacher, E.; Contreras, P. C.; Hammond, D. *J. Med. Chem.* **1992**, *35*, 223.
4. Hansen, D. W., Jr.; Stapelfeld, A.; Savage, M. A.; Reichman, M.; Hammond, D. L.; Haaseth, R. C.; Mosberg, H. I. *J. Med. Chem.* **1992**, *35*, 684.
5. Pitzele, B. S.; Hamilton, R. W.; Kudla, K. D.; Tsymbalov, S.; Stapelfeld, A.; Savage, M. A.; Clare, M.; Hammond, D. L.; Hansen, D. W., Jr. *J. Med. Chem.* **1994**, *37*, 888.
6. Schiller, P. W.; Weltrowska, G.; Schmidt, R.; Nguyen, T. M.-D.; Berezowska, I.; Lemieux, C.; Chung, N. N.; Carpenter, K. A.; Wilks, B. C. *Analgesia* **1995**, *1*, 703.
7. Salvadori, S.; Attila, M.; Balboni, G.; Bianchi, C.; Bryant, S. D.; Crescenzi, O.; Guerrini, R.; Picone, D.; Tancredi, T.; Temussi, P. A.; Lazarus, L. H. *Mol. Med.* **1995**, *1*, 678.
8. Guerrini, R.; Capasso, A.; Sorrentino, L.; Anacardio, R.; Bryant, S. D.; Lazarus, L. H.; Attila, M.; Salvadori, S. *Eur. J. Pharmacol.* **1996**, *302*, 37.
9. Salvadori, S.; Balboni, G.; Guerrini, R.; Tomatis, R.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *J. Med. Chem.* **1997**, *40*, 3100.
10. Schiller, P. W.; Schmidt, R.; Weltrowska, G.; Berezowska, I.; Nguyen, T. M.-G.; Dupuis, S.; Chung, N. N.; Lemieux, C.; Wilkes, B. C.; Carpenter, K. A. *Lett. Pep. Sci.* **1998**, *5*, 209.
11. Wang, C.; McFadyen, I. J.; Traynor, J. R.; Mosberg, H. I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2685.

12. Schiller, P. W.; Fundytus, M. E.; Merovitz, L.; Weltrowska, G.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N.; Coderre, T. J. *J. Med. Chem.* **1999**, *42*, 3520.
13. Capasso, A.; Guerrini, R.; Balboni, G.; Sorrentino, L.; Temussi, P.; Lazarus, L. H.; Bryant, S. D.; Salvadori, S. *Life Sci.* **1996**, *59*, PL93.
14. Bryant, S. D.; Salvadori, S.; Cooper, P. S.; Lazarus, L. H. *Trends Pharmacol. Sci.* **1998**, *19*, 42.
15. Salvadori, S.; Guerrini, R.; Balboni, G.; Bianchi, C.; Bryant, S. D.; Cooper, P. S.; Lazarus, L. H. *J. Med. Chem.* **1999**, *42*, 5010.
16. Sasaki, Y.; Suto, S.; Ambo, A.; Ouchi, H.; Yamamoto, Y. *Chem. Pharm. Bull.* **1999**, *47*, 1506.
17. Sasaki, Y.; Hirabuki, M.; Ambo, A.; Ouchi, H.; Yamamoto, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 327.
18. Montecucchi, P. C.; de Castiglione, R.; Piani, S.; Gozzini, L.; Erspamer, V. *Int. J. Pep. Prot. Res.* **1981**, *17*, 275.
19. Erspamer, V.; Melchiorri, P.; Falconieri-Elspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Kreil, G. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5188.
20. Sasaki, Y.; Ambo, A.; Suzuki, K. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 822.
21. All analogues reported here gave satisfactory FAB-MS and amino acid analytical data.
22. Schiller, P. W.; Nguyen, T. M.-D.; Maziak, L. A.; Wilkes, B. C.; Lemieux, C. *J. Med. Chem.* **1987**, *30*, 2094.
23. Sasaki, Y.; Ambo, A.; Midorikawa, K.; Suzuki, K. *Chem. Pharm. Bull.* **1993**, *41*, 1391.
24. Heyl, C. L.; Mosberg, H. I. *Int. J. Pep. Prot. Res.* **1992**, *39*, 450.
25. Calderan, A.; Ruzza, P.; Ancona, B.; Cima, L.; Giusti, P.; Borin, G. *Lett. Pep. Sci.* **1998**, *5*, 71.
26. Lazarus, L. H.; Bryant, S. D.; Cooper, P. S.; Salvadori, S. *Prog. Neurobiol.* **1999**, *57*, 377.
27. The retention times of **1** and **3** were 30.72 and 34.53 min, respectively, while those of DM and DT were 26.58 and 29.57 min, respectively, on analytical HPLC. The HPLC was performed on a Wakopak column (4.6×150 mm) using a solvent system of 0.1% TFA (A)/80% CH_3CN containing 0.1% TFA (B) with a linear gradient elution of 10–50% B over 40 min.