FEBS Letters 351 (1994) 308–310

FEBS 14462

Opioid ligands with extraordinarily high μ-selectivity: dermorphin tetrapeptides containing thymine-modified alanine residues

D.A. Kharkevich[a], N.V. Sumbatyan[b], A.N. Topin[b], O.N. Chichenkov[a], S.V. Zaitsev[b], G.A. Korshunova[b],*

[a]*Department of Pharmacology, I.M. Sechenov Moscow Medical Academy, Moscow, Russian Federation*
[b]*A.N. Belozersky Institute of Physico-Chemical Biology and Chemistry Department, Moscow State University, Moscow, 119899, Russian Federation*

Received 15 July 1994

Abstract Four new [D-MetO^2]dermorphin tetrapeptides with substituted N- and C-terminal groups and a thymine-modified alanine residue at position 4 were prepared and tested for their activity. All analogues were found to be μ-opioid receptor ligands. Two of them, H-Tyr-D-MetO-Phe-TalNHR (R=H, Ad) displayed an extremely high μ-opioid receptor selectivity comparable with that of the most μ-selective agonists among opioid peptides.

Key words: Opioid; μ-selective ligand; Dermorphin analogue; 3-(Thyminyl-1)alanine

1. Introduction

A number of opioid peptide analogues showing a selectivity for μ-opioid receptors were obtained by substitution of various natural or artificial amino acids, amino acid deletions and end-group modifications [1,2]. The N-terminal tetrapeptideamide of dermorphin (DM/1–4/) is almost as μ-selective as the parent natural heptapeptide which exhibits a rather high μ selectivity [3]. The tetrapeptide analogues derived from DM/1–4/ and bearing the N-terminal guanidino-group and an adamantylamide moiety at the C-terminal appear to show a high preference for μ receptors over δ ones [4]. Substitution of D-methionine-sulfoxide (D-MetO) for D-Ala^2 results in compounds that are more μ-selective than the parent tetrapeptide [5]. Introduction of aromatic and bulky proteinogenic or other amino acid residues, such as phenylalanine, tryptophan or naphtylalanine ones, into position 4 produces an affinity increase both at the μ and δ receptors and a high preference for μ receptors [6]. In previous papers we have shown that insertion of a 3-(thyminyl-1)alanine residue (Tal) into position 4 of the enkephalin and short dermorphin structures gives rise to analogues with a high μ-opioid selectivity [7,8].

In the search for potent μ-selective ligands we synthesized and tested for opioid activities for dermorphin tetrapeptide [D-MetO^2]analogues bearing Tal at position 4 and N- or C-terminal modifications.

2. Materials and methods

2.1. Chemical synthesis

DL-Tal was prepared by the three-step chemical procedure as described in [9], and its L-isomer was obtained enzymatically [10]. Boc-Pal was synthesized as described previously [11]. Adamantyl- and D-phenylethylamides of Tal were prepared by a method similar to that described by Salvadori et al. [12]. The [D-Met^2]dermorphin analogues were prepared in solution via 3 + 1 coupling, using active esters. Boc-peptide-sulfides were oxidized with hydrogen peroxide in acetic acid to yield sulfoxides as in [5,13]. Guanidino derivatives were obtained by treating the respective peptideamides with 1-amidino-3,5-dimethylpyrazole nitrate as in [14]. The homogeneity of the final peptides and intermediates was demonstrated by TLC, HPLC, electrophoresis and amino acid analysis (Sumbatyan, N.V. and Korshunova, G.A., unpublished).

2.2. Binding assay

The selectivity of the compounds synthesized was characterized by the relative opioid receptor affinity ($IC_{50}\delta/IC_{50}\mu$), as determined by displacement of selective radioligands from the rat brain membrane preparation [15,16]. [^{3}H]DAGO and [^{3}H]DSLET were used as selective μ- and δ-receptor radioligands.

2.3. Biological activity on isolated organs

Isolated guinea pig ileum (GPI) and mouse vas deferens (MVD) were prepared and used as described by Ward et al. [17].

3. Results and discussion

The structures of the newly synthesized compounds can be described by the general formula, X-Tyr-D-MetO-Phe-Tal-NHR, where X stands for H or amidino-group (HN=C(NH$_2$)–) and R represents 1-adamanthyl of D-1-phenylethyl moieties.

The opioid activities of the novel dermorphin analogues synthesized (I–IV) determined by in vitro assays of GPI and MVD and by the binding assays are reported in Table 1 and along with the corresponding activities of the parent tetrapeptide DM/1–4/, DAGO and DSLET.

In the [^{3}H]DAGO and [^{3}H]DSLET binding assays, all new [Tal^4]tetrapeptides I–IV, like DM/1–4/, displayed a greater preference for μ-subtypes over δ-receptors. Compared to the parent DM/1–4/ peptide, analogues I, II and IV were more potent in the [^{3}H]DAGO binding assays. Analogue III showed a somewhat lower affinity for μ-receptors than DM/1–4/. All compounds, except for analogue II, whose activity was almost equal to that of DM/1–4/, were far less potent in the [^{3}H]DSLET binding assays than DM/1–4/. The introduction of bulky hydrophobic residues into the C-terminal abide moiety affected the binding affinity in a different manner. Compared to parent peptide I, adamantly derivative II was more potent both in the

---

*Corresponding author. Fax: (7) (095) 939 3181.

Abbreviations: Ad, 1-adamantyl; DAGO, H-Tyr-D-Ala-Gly-MePhe-Gly-ol; DM/1–4/, dermorphin tetrapeptide, H-Tyr-D-Ala-Phe-Gly-NH$_2$; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; GPI, guinea-pig ileum; MVD, mouse vas deferens; Tal, 3-(thyminyl-1)alanine.

Table 1
The inhibitory effect of dermorphin analogues on the electrically induced contractions of GPI and MVD and the binding of [^{3}H]DAGO (1 nM) and [^{3}H]DSLET (1 nM) in crude rat brain homogenates in IC_{50}[a]

Compound		GPI IC_{50} (nM)	MVD IC_{50} (nM)	[^{3}H]DAGO (μ) IC_{50} (nM)	[^{3}H]DSLET (δ) IC_{50} (nM)	$\frac{IC_{50}(\delta)}{IC_{50}(\mu)}$
H-Tyr-D-MetO-Phe-Tal-NH_2	(I)	31 ± 13	> 10,000	5.0 ± 2.3	7000 ± 2300	1400
H-Tyr-D-MetO-Phe-Tal-NH-Ad	(II)	57.5 ± 23	> 10,000	0.5 ± 0.13	476 ± 67	952
H-Tyr-D-MetO-Phe-Tal-NH-D-CH(Ph)Me	(III)	575 ± 35	> 10,000	45 ± 35.5	7544 ± 181	168
NH=C(NH_2)-Tyr-D-MetO-Phe-Tal-NH_2	(IV)	68 ± 6	950 ± 106	4.55 ± 1.0	1177 ± 300	259
DM(1–4)		58 ± 14	860 ± 80	40 ± 9	890 ± 100	22
DAGO		2.7 ± 0.5	140 ± 31	1.0 ± 0.2	110 ± 16	110
DSLET		66 ± 11	0.12 ± 0.1	95 ± 10	7.0 ± 0.7	0.7

[a] The IC_{50} values are the mean ± S.E.M. for 3–5 observations.

[^{3}H]DAGO and [^{3}H]DSLET binding assays. This confirms the previous data obtained for short dermorphin analogues [18]. By comparison with structurally related peptide I, D-phenylethylamide derivative III exhibited the same affinity for δ-receptors and lower affinity for μ-receptors. The substitution of a guanidino group for the NH_2-terminal group in the parent peptide I left the μ-affinity unaltered and decreased the δ-affinity. The replacement of Gly by Tal^4 in the corresponding structurally related peptides kept constant (I, III, IV) or increased (II) the affinity for the μ-site and lowered (I, III, IV) or maintained (II) the affinity for the δ-site [5,13,19].

As revealed by the binding assays and by the tests on isolated organs (GPI and MVD), all [D-MetO^2,Tal^4]dermorphin tetrapeptides (I–IV) displayed a very high preference for μ-receptors over δ-receptors due to their high affinity for μ-sites and poor affinity for δ-sites. Although it was not possible to derive accurate estimates of the selectivity from GPI/MVD data, one can conclude that peptides I and II are the most potent and, probably, most selective μ-ligands among the synthesized analogues. Similar conclusions can be drawn from the binding assays: the $IC_{50}\delta/IC_{50}\mu$ values for compounds I and II are equal to 1400 and 952, respectively.

Peptides I–IV are 5 to 35 times more selective in comparison to the corresponding [D-MetO^2,Gly^4]dermorphin tetrapeptideamides described previously [5]. The synthesized compounds (I–IV) appear to have a comparable or significantly increased selectivity in comparison with the most selective analogues among the series of [D-MetO^2]dermorphin tetrapeptides, including Gly, Sar and D-Ala at position 4 [5]. Thus, analogues I and II exhibit an extraordinary selectivity comparable to that of morphiceptin, LY164929 (NMe-Tyr-D-Ala-Gly-NEt-CH(CH_2Phe)CH_2NMe_2), and PL017 (H-Tyr-Pro-MePhe-D-Pro-NH_2) and rank below only syndyphalin, DALDA (H-Lys-Tyr-D-Arg-Phe-Lys-NH_2), and Lys-DALDA, the most selective peptide μ-ligands reported to data [1].

The effect of Tal addition on the opioid receptor selectivity of dermorphin tetrapeptides may be rationalized in terms of ligand conformational properties. A conformational restriction on ligand structure is known to be an absolute requirement for its interaction with μ-receptor sites [1,20]. Earlier it has been found that the presence of MetO^2 in the D configuration in the dermorphin tetrapeptide structures is critical for the formation of 'active' conformers [21]. In the case of [D-MetO^2]analogues modified with thymine the biological active conformation may be additionally stabilized due to the presence of the nucleic acid base, which is known to participate in different hydrogen bonds, with peptide groups in particular [22]. Our early CD data have indicated a decrease in the conformational flexibility of nucleoamino acids in solution due to intramolecular hydrogen bonding [23]. Besides, the conformation-function analysis of dermorphin and its pentapeptide analogues indicated that the peptide backbone structure of the [Tal^4]DM/1–5/ analogue is stabilized by hydrogen bonds [24].

In conclusion, the results presented indicate that the substitution of Tal for Gly at position 4 of [D-MetO^2]dermorphin tetrapeptide series gives rise to analogues with a further increased μ-selectivity.

References

[1] Schiller, P.W. (1991) in: Progress in Medicinal Chemistry (G.P. Ellis and G.B. West, Eds.) Vol. 28, pp. 301–340, Elsevier, Amsterdam.

[2] Reisine, T. and Bell, G.I. (1993) Trends Neurosci. 16, 506–510.

[3] Korshunova, G.A. and Sumbatyan, N.V. (1989) Bioorg. Khim. (Russian) 15, 869–963.

[4] Salvadori, S., Sarto, G.P. and Tomatis, R. (1983) Eur. J. Med. Chem. 18, 489–493.

[5] Marastoni, M., Salvadori, S., Balboni, G., Borea, P.A., Marzola, G. and Tomatis, R. (1987) J. Med. Chem. 30, 1538–1542.

[6] Schiller, P.W., Nguyen, T.M.-D. and Lemieux, C. (1989) in: Peptides 1988 (Jung, G. and Bayer, E., Eds.) pp. 613–615, W. de Gruyter, Berlin.

[7] Ryabtseva, O.N., Petrova, M.G., Korshunova, G.A., Chichenkov, O.N. and Shvachkin, Yu.P. (1987) Pharmacol. Toxicol. (Russian) 6, 20–23.

[8] Chichenkov, O.N., Sumbatyan, N.V., Ryabtseva, O.N., Korshunova, G.A., Shorr, V.A. and Shvachkin, Yu.P. (1988) Pharmacol. Toxicol. (Russian) 5, 17–20.

[9] Doel, M.T., Jones, A.S. and Taylor, N. (1969) Tetrahedron Lett. 27, 2285–2288.

[10] Korshunova, G.A., Semiletov, Yu.A., Ryabtseva, O.N. and Shvachkin, Yu.P. (1982) Vestnik MGU, Ser. Khimiya (Russian) 23, 412–413.

[11] Semiletov, Yu.A., Tyaglov, B.V., Permogorov, V.I. and Shvachkin, Yu.P. (1981) J. Obsch. Khim. (Russian) 51, 230–238.

[12] Salvadori, S., Marastoni, M., Tomatis, R. and Sarto, G.P. (1983) Il Farmaco Ed. Sc. 38, 640–646.

[13] Salvadori, S., Marastoni, M., Balboni, G., Sarto, G.P. and Tomatis, R. (1986) J. Med. Chem. 29, 889–894.

[14] Salvadori, S., Sarto, G.P. and Tomatis, R. (1983) Eur. J. Med. Chem. 18, 489–493.

[15] Zaytsev, S.V. and Kurochkin, I.N. (1984) Biokhimiya (Russian) 49, 1127–1133.

[16] James, I.F. and Goldstein, A. (1984) Mol. Pharmacol., 25, 337–342.

[17] Ward, S.J., Lopresti, D. and James, D.W. (1986) J. Pharmacol. Exp. Ther. 238, 625–628.

[18] Tomatis, R., Salvadori, S. and Sarto, G. (1983) in: Peptides 1982. Proc. of 17th Eur. Pept. Symp. (Blaha, K. and Malon, P.B., Eds.) pp. 496–499, W. de Gruyter, New York.

[19] Marastroni, M., Salvadori, S. and Tomatis, R. (1987) Il Farmaco Ed. Sc. 42, 125–131.

[20] Hruby, V.J. and Gehrig, C.A. (1989) Med. Res. Rev. 9, 343–401.

[21] Pastore, A., Temussi, P.A., Tancredi, T., Salvadori, S. and Tomatis, R. (1984) Biopolymers 23, 2349–2360.

[22] Niu, C.-H., Han, K., Yeh, H.J.C. and Black, S. (1987) Biochem. Biophys. res. Commun. 148, 456–462.

[23] Romanov, V.V., Voskova, N.A., Korshunova, G.A. and Shvachkin, Yu.P. (1979) Bioorgan. Khim. (Russian) 5, 536–545.

[24] Shendorovich, M.D., Liepinya, I.T., Nikiforovich, G.V., Sumbatyan, N.V., Korshunova, G.A. and Shvachkin, Yu.P. (1989) Bioorgan. Khim. (Russian) 15, 1161–1172.