Vol. 178, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1991 Pages 110-115

OPIOID RECEPTOR SELECTIVITY REVERSAL IN DELTORPHIN TETRAPEPTIDE ANALOGUES

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Received May 17, 1991

Summary: Deltorphin N-terminal tetrapeptides [DEL A: H-Tyr-D-Met-Phe-His-R, where R = -NH_2, -NH-NH_2, -OCH_3, -OH, -NH-NH-CO-R' (R' = -CH_3 or adamantane); DEL C: H-Tyr-D-Ala-Asp-R (R = -OH, -$NHCH_3$)], were used in a receptor binding assay with [^{3}H]DADLE and [^{3}H]DPDPE for δ sites, and [^{3}H]DAGO for μ sites; tetrapeptide $K_i\delta$ values were similar with either [^{3}H]-δ ligand. DEL A tetrapeptides C-terminally substituted with -NH_2, -NH-NH_2, -OCH_3, and -OH had 10 to >1,000-fold *decreased* $K_i\delta$ values, while $K_i\mu$ *increased* 5 to 100-fold to yield μ selectivity. C-Terminal substitution with -NH-NH_2 and -OCH_3 conferred highest μ selectivities; adamantyl and acetyl hydrazide derivatives were non-selective. DEL-(1-4)-OH peptides had decreased δ and μ affinities: DEL A-[Asp^4]-(1-4)-OH and DEL C-(1-4)-OH had low affinities (> 1 μM), however, the $K_i\delta$ of the former was 5-fold greater than the latter, and the $K_i\mu$ was less by 15-fold. The data suggest that the "message" domain of DEL exhibits receptor selectivity different from that of the heptapeptide. © 1991 Academic Press, Inc.

Deltorphin (DEL) A (H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2) and DEL-C (H-Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2) are two of three opioid peptides cloned (1,2) and isolated (2-5). The exceptionally high δ affinity (K_i) and selectivity ($K_i\mu/K_i\delta$) for opioid receptors (3,4,6,7) of these peptides far exceed those observed with the enkephalin-derived peptides (8). The N-terminal tripeptide region, which contains the common "message" domain, H-Tyr-D-Xaa-Phe (where Xaa = D-Met or D-Ala) assumes a 1 — 4 β-turn in solution (9,10). The "message" region affects signal transduction, biological responsiveness (12), and facilitates receptor recognition (9), and is similar to that found with the μ selective dermorphins (10,11). On the other hand, the C-terminal sequences contain the "address"

0006-291X/91 $1.50
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domain responsible for δ selectivity (7,9); this region includes a single anionic residue, Glu^4 or Asp^4, or Asp^7 (3-5,7) which is thought to bind to a positively charged receptor site (13).

In order to expand our understanding of the capabilities of the "message" sequence in DEL in influencing receptor affinity and selectivity, we prepared a series of N-terminal tetrapeptide analogues. These peptides were derivatized at their C-termini in order to determine the effect of ionic and hydrophobic groups on K_i values and selectivity, since hydrophobicity is suggested to influence binding to μ receptors (14,15).

MATERIALS AND METHODS

Peptide Synthesis. DEL A (peptide **1**) and DEL C (peptide **9**) were prepared by solid phase methods described elsewhere (7). The tetrapeptide analogues were synthesized by conventional solution condensation of N-Boc-amino acids to the C-terminal amino acid methyl ester using dicyclohexylcarbodiimide as the coupling agent in the presence of 1-hydroxybenzotriazole (16); His and Tyr were incorporated without side chain protection, while Asp was the *tert*-butylester. Peptides **2-4, 10** and **11** were obtained by treatment of the protected tetrapeptide methyl ester with NaOH, NH_3, NH_2CH_3, and hydrazine; this last hydrazide peptide was subsequently acylated with 1-adamantanecarbonyl chloride (14) to give analogue **6** or with acetyl chloride to give analogue **7** (Table 1). Crude deprotected peptides (TFA-CH_2Cl_2) were purified by a combination of Sephadex gel filtration, partition chromatography, and preparative HPLC (7). The synthetic peptides were homogeneous as assessed by analytical HPLC and TLC; amino acid analyses and NMR properties were consistent with estimates that purities were >99%.

Receptor Assays. Rat brain synaptosomal preparations were prepared in such manner as to remove endogenous opioid peptides (6,7,15,17). Competitive binding assays employed 1-2 hr incubations at 22 °C in the presence of 1.6 mg synaptosome protein in 50 mM HEPES, pH 7.5, 1 μM bestatin, 4 μg bacitracin, 32 μg soybean trypsin inhibitor, 8% glycerol, and 1 mg/ml BSA: δ binding with 0.63 nM [^{3}H]DPDPE was performed in the presence of 5 mM $MgCl_2$ and 100 μM PMSF, while that with 0.68 nM [^{3}H]DADLE used 1 mM $MgCl_2$ and 2.6 μM [N-Me-Phe^3,D-Pro^4]morphiceptin, a selective μ agonist to suppress binding to μ receptors (18); μ binding was conducted using 1.28 nM [^{3}H]DAGO with 1 mM $MgCl_2$ (15). Duplicate samples were filtered through wetted Whatman GF/C glass fibre filters, washed with 3 X 2 ml buffered BSA, dried and counted in CytoScint (ICN). Peptides were tested at 4-8 concentrations using 3-5 synaptosomal preparations, with n = 3-6 binding assays, to ensure statistical reliability. K_i values were calculated according to the equation of Cheng and Prusoff (19). [^{3}H]DPDPE had a K_d = 1.58 nM with these membrane preparations.

RESULTS AND DISCUSSION

Estimates for K_iδ values for DEL A and DEL C were similar with each of the labeled δ-ligands (Table 1), i.e., [^{3}H]DADLE, in the *presence* of the μ agonist (*N*-Me-Phe^3,D-Pro^4)morphiceptin, and [^{3}H]DPDPE. As previously noted, δ selectivities of DEL

Table 1. K_i Values (nM) for Deltorphins and Tetrapeptide Analogues

No.	Peptide	[^{3}H]DADLE	[^{3}H]DPDPE	[^{3}H]DAGO
1.	Deltorphin A	0.18 ± 0.06	0.41 ± 0.02	315.6 ± 22.6
2.	A-(1-4)-OH	1,070 ± 133	1,249 ± 201	403.1 ± 17.8
3.	A-(1-4)-NH_2	689.1 ± 60.1	388.0 ± 23.4	61.5 ± 3.1
4.	A-(1-4)-NH-NH_2	296.8 ± 4.7	125.7 ± 17.9	2.7 ± 0.3
5.	A-(1-4)-OCH_3	784.9 ± 97.2	340.7 ± 6.1	6.0 ± 4.8
6.	A-(1-4)-NH-NH-CO-Ad	29.5 ± 6.8	10.5 ± 1.6	29.2 ± 2.2
7.	A-(1-4)-NH-NH-CO-CH_3	74.4 ± 11.7	190.1 ± 14.1	268.1 ± 48.0
8.	A-[Asp^4]-(1-4)-OH	1,427 ± 88.7	1,268 ± 108	9,010 ± 1,902
9.	Deltorphin C	0.21 ± 0.03	0.25 ± 0.04	398.6 ± 30.1
10.	C-(1-4)-OH	7,441 ± 509	5,094 ± 423	606.6 ± 40.0
11.	C-(1-4)-$NHCH_3$	275.2 ± 18.3	749.1 ± 69.0	318.1 ± 52.6

exceeds that of DPDPE (4,6), one of the most δ selective of the enkephalin-derived peptides (8), by factors of 3 to 10 (3,4,6). Quantitative differences in $K_i\delta$ values using [^{3}H]DADLE and [^{3}H]DPDPE were similar to those observed using [^{3}H]DSLET and [^{3}H]DPDPE (20).

Our results support the argument that the N-terminal tetrapeptide region of DEL A and DEL C are capable of functioning as "message" domains (12,13) of the intact heptapeptides. In agreement with prior observations (9), receptor selectivity for these tetrapeptides is variable even though most exhibited preferential affinity for μ receptors. However, DEL A-1-adamantanecarbonyl hydrazide (Ad) derivative (peptide **6**) and DEL A acetyl hydrazide (peptide **7**) were essentially non-selective; DEL A-[Asp^4]-(1-4)-OH (peptide **8**), exhibited δ and μ affinities in the μM range (Table 1) and was only weakly δ selective (Table 2). Non-selectivity of DEL C-1-Ad was similarly noted (9) based on earlier studies in which Ad enhanced μ binding properties of dermorphin tetrapeptides (14). Alterations in opioid receptor selectivity was previously observed for a deltorphin-dermorphin hybrid (21), as well as with the analogues of the κ selective dynorphin A-(1-

Table 2. Selectivity of Deltorphins and Tetrapeptide Analogues

No.	Peptide	$K_i\mu/K_i\delta$ [^{3}H]DADLE	$K_i\mu/K_i\delta$ [^{3}H]DPDPE
1.	Deltorphin	1,753	745
2.	A-(1-4)-OH	0.38	0.32
3.	A-(1-4)-NH_2	0.09	0.16
4.	A-(1-4)-NH-NH_2	0.009	0.02
5.	A-(1-4)-OCH_3	0.008	0.02
6.	A-(1-4)-NH-NH-CO-Ad	0.99	2.8
7.	A-(1-4)-NH-NH-CO-CH_3	3.6	1.4
8.	A-[Asp^4]-(1-4)-OH	6.3	7.1
9.	Deltorphin C	1,898	1,594
10.	C-(1-4)-OH	0.08	0.12
11.	C-(1-4)-$NHCH_3$	1.16	0.42

17). In the latter peptide, the N-terminal dynorphin A-(1-7) exhibited μ/δ selectivities, while the crossover to κ selectivity occurred with dynorphin A-(1-11) (22).

DEL A-Ad exhibited an intermediate value for $K_i\mu$, which was similar to that for $K_i\delta$; replacement of Ad by Me (peptide 7) resulted in diminution of both δ and μ affinities (Table 1). The bulky hydrophobic Ad moiety may be sterically constrained so that it is capable of facilitating interactions in either receptor type; alternatively, it may directly reposition the hydrazine moiety to enhance stronger binding within the receptor. The replacement of Ad by Me (peptide 7) led to loses in both δ and μ affinities; however, this analogue retained a higher δ affinity than peptides **2-5.** These data suggest that Ad either directly promotes binding to μ receptor sites, or is consistent with the recovery of the hydrophobic residues existant in the C-terminal region of deltorphins.

The most effective DEL A tetrapeptides derivatives to enhance μ selectivity were those C-terminally substituted with hydrazide and methyl ester derivatives (peptides 4 and

5). The K_μ of peptide **4** exceeded those of DEL A (peptide **1**) and DEL A-(1-4)-NH_2 (peptide **3**) by greater than 100 to 20-fold, respectively. The δ and μ affinities and μ selectivity of DEL A-(1-4)-NH_2 differed considerably from that reported (23).

A C-terminal free carboxyl group in the tetrapeptides (peptides **2,8,10**) increased both K_δ and K_μ (μM range) (Table 1). Increased negativity in DEL A heptapeptide analogues, through deamidation or replacement of His^4 or Leu^5 with Asp (7), elicited similar effects. Although peptides **8** and **10** differ by only the amino acid at position 2 ([D-Met^2] and [D-Ala^2], respectively), marked differences occurred in their δ and μ affinities (Table 1) and, as a consequence, their selectivities varied by factors of 60 to 80-fold (Table 2).

In summary, specific tetrapeptide analogues of DEL A and DEL C exhibit preferential μ selectivities, while others display non-selective receptor binding properties. Thus, the concept of "sychnologic organization" (24) would appear to represent one which has limited predictive significance with a heptapeptide; i.e., while a particular peptide hormone is capable of containing proximal domains classified as "message" and "address" (12,24), such domains are not completely exclusive in relatively small peptides. In spite of the relatively restricted molecular dimensions of deltorphin, the peptide nonetheless has apparent structurally and functionally recognizable regions, in which hydrophobic (14,15) and anionic interactions (7,9) appear to be instrumental in modulating affinity and selectivity.

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