Cyclic Enkephalin Analogues Containing α-Amino-β-mercapto-β,β-pentamethylenepropionic Acid at Positions 2 or 5[1]

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Analogues of the highly potent and δ-receptor-selective enkephalins **1–4** were prepared with α-amino-β-mercapto-β,β-pentamethylenepropionic acid (Apmp) replacing the β,β-dimethylcysteine (Pen) at positions 2 or 5. The peptides **5–8** were prepared by employing D,L-Apmp and, following oxidative cyclization, the resulting diastereomeric peptides were separated and purified by preparative high performance liquid chromatography. Compounds **7** and **8**, with D- or L-Apmp substituted at position 5 are approximately 5 orders of magnitude more potent in the MVD assay than analogues **5** or **6** with D- or L-Apmp at position 2. While displaying less δ-receptor selectivity than the corresponding Pen-containing compounds, **7** and **8** are an order of magnitude more potent. All the analogues showed diminished δ-receptor selectivity in the rat brain binding assay. Compounds **7** and **8** displayed δ-receptor affinity comparable to the corresponding Pen-containing analogues.

The opioid pentapeptides [Met^5]enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) and [Leu^5]enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH), while exhibiting some δ-receptor selectivity, are capable of interacting with several classes of opioid receptors.[2-4] Determination of the physiological role of these receptor classes requires analogues with a high specificity for a single receptor class. One approach toward such receptor specificity involves the use of conformationally restricted analogues that may possess the correct pharmacophore for recognition at only a single receptor type. Several efforts to gain receptor selectivity through analogues that have been conformationally restricted by way of cyclization have attempted to distinguish the structural requirements for interaction at the μ[5-9] and δ[10-13] opioid receptors. In particular, the penicillamine (β,β-dimethylcysteine) containing enkephalins **1–4** display

H-Tyr-D-X-Gly-Phe-Y-OH
1: X = Pen, Y = Cys
2: X = Pen, Y = D-Cys
3: X = Cys, Y = Pen
4: X = Cys, Y = D-Pen

substantial δ-receptor selectivity and potency.[11,12] Besides the conformational constraints imposed by their cyclic structure, the geminal dimethyl substituents of the penicillamine residue may serve to further restrict the conformational freedom of the disulfide ring in these molecules as has been shown in penicillamine-containing oxytocin analogues.[14-17]

To further investigate the conformational requirements necessary for opioid receptor specificity as well as binding and transduction at the opioid receptors, we prepared the cyclic enkephalins **5–8** that incorporate α-amino-β-

H-Tyr-X-Gly-Phe-Y-OH
5,6: X = L-(or D)-Apmp, Y = Cys
7,8: X = D-Cys, Y = L-(or D)-Apmp

mercapto-β,β-pentamethylenepropionic acid (Apmp) at position 2 or 5. In this amino acid the geminal dimethyls at the β carbon of penicillamine have been replaced with a cyclohexyl ring. The cyclohexyl ring of this residue should increase both the hydrophobicity and steric bulk at the β carbon. We now report the pharmacological effects resulting from this substitution at position 2 or 5 in enkephalin analogues **5–8**.

Chemistry

All peptide analogues were prepared by solid-phase peptide synthesis.[18] Compounds **5** and **6** were synthesized on *N*-(*tert*-butyloxycarbonyl)-*S*-(4-methylbenzyl)cysteine-4-(oxymethyl)phenylacetamidomethyl-resin (Boc-Cys(4-MeBz)-PAM-resin). Compounds **7** and **8** were prepared on hydroxymethyl-resin. The *tert*-butyloxycarbonyl (Boc) group was employed for all α-amine protection. Amino acid side chains were protected with 4-methylbenzyl for sulfhydryl and 4-bromobenzyloxycarbonyl for the phenolic hydroxyl of tyrosine. The *N*-(*tert*-butyloxycarbonyl)-*S*-(4-methylbenzyl)-β,β-penta-

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(2) Chang, K.-J.; Cuatrecasas, P. J. *J. Biol. Chem.* **1979**, *254*, 2610.
(3) Lord, J. A.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. *Nature (London)* **1977**, *267*, 495.
(4) Wolozin, B. L.; Pasternak, G. W. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6181.
(5) Schiller, P. W.; DiMaio, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7162.
(6) Schiller, P. W.; DiMaio, J. *Nature* **1982**, *297*, 74.
(7) Schiller, P. W.; Eggiman, B.; DiMaio, J.; Lemieux, C.; Nguyen, T. M.-D. *J. Biochem. Biophys. Res. Commun.* **1981**, *101*, 337.
(8) Schiller, P. W.; Nguyen, T. M.-D.; Maziak, L. A.; Lemieux, C. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 558.
(9) Schiller, P. W.; Nguyen, T. M.-D.; Maziak, L. A.; Wilkes, B. C.; Lemieux, C. *J. Med. Chem.* **1987**, *30*, 2094.
(10) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Galligan, J. J.; Burks, T. F.; Gee, K.; Yamamura, H. I. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 506.
(11) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Galligan, J. J.; Burks, T. F.; Gee, K.; Yamamura, H. I. *Life Sci.* **1983**, *32*, 2565.
(12) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Akiyama, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. *Life Sci.* **1983**, *33*, 447.
(13) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871.
(14) Meraldi, J. P.; Yamamoto, D.; Hruby, V. J.; Brewster, A. I. R. In *Peptides: Chemistry, Structure and Biology*; Walter, R., Meinenhofer, J., Eds.; Ann Arbor Sci.: Ann Arbor, MI, 1975; p 803.
(15) Meraldi, J. P.; Hruby, V. J.; Brewster, A. I. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1373.
(16) Mosberg, H. I.; Hruby, V. J.; Meraldi, J. P. *Biochemistry* **1981**, *20*, 2822.
(17) Mosberg, H. I.; Hruby, V. J. In *Peptides: Synthesis, Structure, Function*; Rich, D. H., Gross, E., Eds.; Pierce: Rockford, IL, 1983; p 375.
(18) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

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Table I. Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assay of Enkephalin Analogues 5–8

no.	analogue	IC_{50},[a] nM GPI	IC_{50},[a] nM MVD	GPI/MVD
1	[D-Pen^2,Cys^5]enk[b]	213 ± 63	0.32 ± 0.03	666
2	[D-Pen^2,D-Cys^5]enk[b]	1350 ± 340	6.27 ± 1.20	215
3	[D-Cys^2,Pen^5]enk[c]	40 ± 1.5	0.75 ± 0.05	53
4	[D-Cys^2,D-Pen^5]enk[c]	67 ± 1.3	0.13 ± 0.06	515
5	[L-(or D)-Apmp^2,Cys^5]enk	29900 ± 300	>2040	<14.7
6	[L-(or D)-Apmp^2,Cys^5]enk	70900 ± 5700	>4240	<16.7
7	[D-Cys^2,L-(or D)-Apmp^5]enk	13.7 ± 3.5	0.0564 ± 0.0028	242
8	[D-Cys^2,L-(or D)-Apmp^5]enk	13.3 ± 2.5	0.0745 ± 0.0107	178
	[Leu^5]enk	246 ± 39	11.4 ± 1.1	21.6

[a] Mean of three determinations ± SEM. [b] Data obtained from ref 12. [c] Data obtained from ref 11.

Table II. Binding Affinities of Enkephalin Analogues 1–8

no.	analogue	K_i, nM [^{3}H]DAGO[a]	[^{3}H]DSLET[a]	[^{3}H]DPDPE[b]	[^{3}H]naloxone	[^{3}H]DADLE
1	[D-Pen^2,Cys^5]enk[c]				178 ± 15.8	11.7 ± 1.2
2	[D-Pen^2,D-Cys^5]enk[c]				157 ± 73.6	26.0 ± 0.5
3	[D-Cys^2,Pen^5]enk[d]				53 ± 2.3	5.4 ± 0.1
4	[D-Cys^2,D-Pen^5]enk[d]				22 ± 2.8	3.5 ± 0.8
5	[L-(or D)-Apmp^2,Cys^5]enk	1800 ± 540	31.3 ± 1.2	15		
6	[L-(or D)-Apmp^2,Cys^5]enk	4010 ± 1380	683 ± 282	387		
7	[D-Cys^2,L-(or D)-Apmp^5]enk	20.9 ± 1.7	2.30 ± 0.80	0.63		
8	[D-Cys^2,L-(or D)-Apmp^5]enk	14.4 ± 0.5	3.27 ± 0.24	0.94		
	[Leu^5]enk	9.43 ± 2.07	2.53 ± 0.35	1.5		

[a] Mean of three determinations ± SEM. [b] Calculated from a log dose–response curve which was generated from values which were the average of three determinations. [c] Data obtained from ref 12. [d] Data obtained from ref 11.

methylenepropionic acid[19] (Boc-Apmp(4-MeBz)) was prepared by an improved procedure.[20] Couplings were performed with 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt). The couplings of Boc-D,L-Apmp(4-MeBz) were performed with DCC and 1 equiv of (dimethylamino)pyridine (DMAP). Protected peptide resins were cleaved and deprotected with anhydrous, liquid hydrogen fluoride (HF). Following HF cleavage the crude linear peptides were oxidatively cyclized with potassium ferricyanide. Peptides 5–8 were prepared by employing Boc-D,L-Apmp(4-MeBz) and, following oxidative cyclization, the resulting diastereomeric peptides were separated and purified by high performance liquid chromatography (HPLC).

Bioassay Methods

Opioid receptor binding of the analogues was determined in a rat brain membrane preparation by displacement of the μ-selective radioligand [^{3}H]DAGO and the δ-selective radioligands [^{3}H]DSLET and [^{3}H]DPDPE as described elsewhere.[21,22] The radiolabels [^{3}H]DAGO and [^{3}H]-DSLET were used at concentrations of 0.72 and 0.78 nM, respectively, and incubations were performed at 0 °C for 2 h. The radiolabel [^{3}H]DPDPE was used at a concentration of 1.8 nM and incubated at 25 °C for 1.0 h. The binding inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff.[23] Dissociation constants of [^{3}H]DAGO and [^{3}H]DSLET were 1.3 nM and 2.6 nM, respectively. The dissociation constant of [^{3}H]-DPDPE was 1.8 nM.

The analogues were also tested in vitro for their ability to inhibit electrically induced contractions of the guinea pig ileum (GPI) and of the mouse vas deferens (MVD).[21,24] A log dose–response curve was determined with [Leu^5]-enkephalin as standard for each ileum or vas preparation. The IC_{50} values of the analogues tested were normalized as described in the literature.[25]

Results and Discussion

Because the inhibition of smooth muscle contraction in the MVD preparation is mediated primarily by δ-receptors and the μ-receptor in the GPI assay, the ratio of the IC_{50} obtained in the MVD to the IC_{50} in the GPI expresses δ- vs μ-receptor selectivity. As indicated from the GPI/MVD ratio in Table I, analogues 7 and 8 are δ-receptor selective. Compounds 5 and 6 are less potent in both the GPI and MVD than either analogues 7 and 8. Compounds 5 and 6 show both reduced potency and δ-receptor selectivity when compared to the Pen-containing analogues [D-Pen^2,Cys^5]enkephalin 1 and [D-Pen^2,D-Cys^5]enkephalin 2. Analogues 7 and 8 are approximately an order of magnitude more potent in the MVD assay than either [D-Cys^2,Pen^5]enkephalin 3 and [D-Cys^2,D-Pen^5]enkephalin 4 and are more potent than any previously reported δ-selective enkephalin. However, they show only modest δ-receptor selectivity, roughly comparable to that found for 3 and 4. In the GPI assay analogues 7 and 8 showed K_e values for naloxone as antagonist between 1 and 2 nM. These low values are typical for μ-receptor interactions and rule out the possibility that these compounds have significant affinity for κ-receptors, since κ-receptor interactions would be characterized by much higher K_e value.[26,27]

Analogues 5–8 were also evaluated in a rat brain binding assay for their ability to displace the μ-receptor-specific radioligand [^{3}H]DAGO and the δ-receptor-specific radio-

(19) Stanfield, C. F.; Cody, W. L.; Hruby, V. J. *J. Org. Chem.* **1986**, *51*, 5153.
(20) Yim, N. C. F.; Bryan, H.; Huffman, W. F.; Moore, M. L. *J. Org. Chem.* **1988**, *53*, 4605.
(21) Schiller, P. W.; Lipton, A.; Horrobin, D. F.; Bodansky, M. *Biochem. Biophys. Res. Commun.* **1978**, *85*, 1322.
(22) Codd, E. E.; Walker, R. F. *Prog. Opioid Res.* **1986**, *75*, 351.
(23) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(24) DiMaio, J.; Nguyen, T. M.-D.; Lemieux, C.; Schiller, P. W. *J. Med. Chem.* **1982**, *25*, 1432.
(25) Waterfield, A. A.; Leslie, F. M.; Lord, J. A. H.; Ling, N.; Kosterlitz, H. *Eur. J. Pharmacol.* **1979**, *58*, 11.
(26) Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. *Nature (London)* **1977**, *17*, 1047.
(27) Chavkin, C.; James, I. F.; Goldstein, A. *Science* **1982**, *215*, 413.

ligands [^{3}H]DSLET and [^{3}H]DPDPE. As shown in Table II, compounds **5–8** display only moderate selectivity for the δ-receptor. Interestingly, compound **5**, which shows weak activity in the MVD assay, displays good δ-receptor binding affinity, comparable to **1** and **2**. We are presently evaluating **5** as an antagonist in the MVD. Analogues **7** and **8** have binding affinities similar to **3** and **4** despite their higher potency in the MVD assay.

The biological data indicate that the substitution of D- or L-Apmp for Pen at position 2 reduces opioid activity in both functional assays. This is also accompanied by a loss of δ-receptor selectivity. This suggests that at position 2 additional steric bulk and/or hydrophobicity at the β carbon has a deleterious effect on δ-receptor selectivity and activity. However, as determined in the binding assay, the effects of this substitution are not as pronounced on δ-receptor affinity and selectivity. Substitution of D- or L-Apmp for Pen at position 5 results in enhanced potency in the MVD preparation with little change in δ-receptor selectivity. These analogues are among the most potent compounds reported to date in this assay system with IC_{50} values similar to that of the highly potent δ-agonist H-Tyr-D-Cys-Gly-Phe(*p*-NO_2)-D-Cys-NH_2(IC_{50} = 0.0187).[28] Compared to the same substitution at position 2, they are 5 orders of magnitude more potent. Surprisingly, this increased δ-receptor potency is not reflected by an increase in δ-receptor affinity in the binding assay. This may be the result of a higher efficacy of these analogues at the δ-receptor. Alternatively, since the MVD preparation in addition to δ-receptors also contains μ-receptors, it may be that the additional interaction with the latter receptors could explain the higher than expected IC_{50} values. This discrepancy may also reflect differences between rat brain and MVD δ-receptors.[29]

Experimental Section

Protected amino acids were purchased from Peninsula Laboratories. The *N*-(*tert*-butyloxycarbonyl)-*S*-(4-methylbenzyl)-cysteine-4-(oxymethyl)phenylacetamidomethyl-resin (Boc-Cys-(4-MeBz)-PAM-resin, 2% cross-lined S-DVB, 200–400-mesh, 0.22 mmol/g of resin) was purchased from Vega Biochemicals and hydroxymethyl-resin (1% cross-linked S-DVB, 200–400-mesh, 0.75 mmol/g of resin) was purchased from Peninsula Laboratories. SM-2 (polystyrene–divinylbenzene) was purchased from Bio-Rad. *N*-(*tert*-Butyloxycarbonyl)-*S*-(4-methylbenzyl)-β,β-pentamethylenepropionic acid[19] (Boc-Apmp(4-MeBz)) was prepared by an improved procedure.[20] Solvents and reagents were reagent grade. Methylene chloride (CH_2Cl_2) was HPLC grade and dimethylformamide (DMF) was stored over 4-Å molecular sieves and filtered prior to use. The purity of all peptides was routinely checked by HPLC using a 4.5 mm × 25 cm Altex Ultrasphere 5-μm ODS column with UV detection at 220 nm and thin layer chromatography (TLC) on EM silica gel plates with visualization with 10% Clorox/1% KI–starch. For preparative HPLC a 10 mm × 25 cm Altex Ultrasphere 10-μm ODS column was employed. For analytical HPLC a linear gradient of water–acetonitrile containing 0.1% trifluoroacetic acid (TFA) was used (80:20 to 50:50 over 30 min at 1.5 mL/min) and preparative HPLC was performed isocratically with 25:75 acetonitrile–water containing 0.1% TFA at 4.0 mL/min. Elemental analyses were performed on a Perkin-Elmer 240 apparatus. Where analyses are reported by symbols of elements, results were within 0.4% of the calculated values. For amino acid analyses, after oxidation for 4 h at 0 °C with performic acid, peptides were hydrolyzed in 6 N HCl at 110 °C for 24 h. Aliquots of the hydrolysis solutions were buffered to pH 10.2 with 1 M boric acid and derivatized with 9-fluorenylmethyl chloroformate. Following extraction with hexane/ethyl acetate (80:20 v/v) the aqueous layer was removed and analyzed by HPLC using a Beckman Ultrasphere-Octyl column (4.6 × 250 mm, 5 μm) at 28 °C with detection at 265 nm employing linear gradients of 75 nM sodium acetate (pH 4.20)–acetonitrile 72:28 to 40:60 over 20 min, 40:60 to 10:90 over 2 min and 10:90 to 0:100 over 3 min at 1.5 mL/min.[30] Fast atom bombardment (FAB) mass spectrometry was performed by the Physical and Structural Chemistry Department of Smith Kline and French Laboratories on a VG ZAB high resolution spectrometer.

General Peptide Synthesis. Peptides **5** and **6** were synthesized on 1.0 mmol of Boc-Cys(4-MeBz)-PAM resin. Peptides **7** and **8** were prepared on 1.0 mmol of hydroxymethyl-resin. Couplings were performed with 3 equiv of the Boc-protected amino acid by using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in DMF for 2–12 h. Couplings of Boc-D,L-Apmp(4-MeBz) were performed by using 1.5 equiv of the amino acid, (dimethylamino)pyridine (DMAP), and DCC in CH_2Cl_2 for 12 h. The Boc group was removed with a single 20-min treatment with 50% TFA in CH_2Cl_2. The resulting amine TFA salt was neutralized with two 2-min treatments of 7% diisopropylethylamine (DIEA) in CH_2Cl_2. Protected peptide resins were cleaved and deprotected with anhydrous hydrogen fluoride (HF) with 10% anisole at 0 °C for 1 h. After removal of the HF in vacuo at 0 °C the residue was washed with diethyl ether and the peptide extracted with acetic acid (HOAc) and DMF into 1 L of distilled, degassed water. The pH was adjusted to 7.4 with ammonium hydroxide and a solution of 0.01 M potassium ferricyanide was added dropwise with stirring until a faint yellow color persisted for 20 min. The pH was then adjusted to 4.5 with acetic acid and the entire solution passed through an SM-2 column. The column was washed with water and the peptide eluted with 70% acetonitrile, 30% water, and 0.1% TFA. This eluant was evaporated and the peptide lyophilized from HOAc.

[L-(and D)-Apmp^2,Cys^5]Enkephalin (5, 6). The crude, oxidized peptides (136 mg) resulting from HF cleavage of 0.5 mmol of peptide-resin were separated and purified by preparative HPLC to yield 22.6 mg of one isomer [HPLC, k' = 11.02; TLC 1-butanol/ethyl acetate/acetic acid/water 1:1:1:1 v/v (BEAW) R_f = 0.77, 1-butanol/pyridine/acetic acid/water 15:10:3:12 v/v (BPAW) R_f = 0.76; FAB mass spectrum, m/z 658 $(M + H)^+$, amino acid analysis Gly 1.13, Phe 1.00, Cys(SO_3H) 0.94. Anal. ($C_{31}H_{39}N_5O_7S_2 \cdot 2.8H_2O \cdot C_2F_3HO_2$) C, H, N] and 18.7 mg of the other diastereomer [HPLC, k' = 12.57; TLC (BEAW) R_f = 0.77, (BPAW) R_f = 0.76; FAB mass spectrum, m/z 658 $(M + H)^+$; amino acid analysis Gly 1.02, Phe 1.00, Cys(SO_3H) 0.82. Anal. ($C_{31}H_{39}N_5O_7S_2 \cdot 3H_2O \cdot 1.25C_2F_3HO_2$) C, H, N].

[D-Cys^2,L-(and D)-Apmp^5]Enkephalin (7, 8). There was obtained 170 mg of crude, oxidized peptides from the HF cleavage of 0.5 mmol of peptide-resin. Separation and purification of 100 mg of this mixture by preparative HPLC yielded 25 mg of one isomer [HPLC, k' = 9.51; TLC (BEAW) R_f = 0.67, (BPAW) R_f = 0.66; FAB mass spectrum, m/z 658 $(M + H)^+$; amino acid analysis Gly 1.06, Phe 0.92, Cys(SO_3H) 1.00. Anal. ($C_{31}H_{39}N_5O_7S_2 \cdot 3H_2O \cdot 1.13C_2F_3HO_2$) C, H, N] and 25 mg of the other diastereomer [HPLC, k' = 11.23; TLC (BEAW) R_f = 0.65, (BPAW) R_f = 0.64; FAB mass spectrum, m/z 658 $(M + H)^+$; amino acid analysis Gly 1.00, Phe 1.01, Cys(SO_3H) 0.85. Anal. ($C_{31}H_{39}N_5O_7S_2 \cdot 4.5H_2O \cdot 1.3C_2F_3HO_2$) C, H, N].

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Registry No. **5**, 117439-60-8; **6**, 117439-61-9; **7**, 117439-62-0; **8**, 117439-63-1; BOC-DL-Apmp(4-MeBz)-OH, 117439-64-2.

(28) Schiller, P. W.; DiMaio, J. In *Peptides, Structure and Function*; Hruby, V. J., Rich, D. H., Eds.; Pierce: Rockford, IL, 1983; p 269.

(29) Shimohigashi, Y.; Costa, T.; Pfeiffer, A.; Herz, A.; Kimura, H.; Stammer, C. H. *FEBS Lett.* **1987**, *222*, 71.

(30) Under these conditions Tyr was destroyed and we were unable to dctect Apmp in peptide hydrolysates or in standards of Apmp.