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Cyclic Dermorphin-Like Tetrapeptides with δ-Opioid Receptor Selectivity. 3. Effect of Residue 3 Modification on *In Vitro* Opioid Activity

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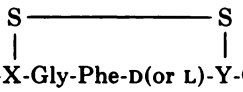
SUMMARY

A series of residue 3-modified analogs of the cyclic, δ-opioid receptor-selective, dermorphin-like tetrapeptide Tyr-D-Cys-Phe-D-Pen and the corresponding residue 4-modified analog of the related δ receptor-selective cyclic pentapeptide [D-Pen^2,D-Pen^5] enkephalin were synthesized and evaluated in opioid receptor binding assays and in the *in vitro* mouse vas deferens (MVD) bioassay. In both series, substitutions that would be expected to alter the orientation of the phenylalanine-substituted aromatic side chain relative to the rest of the peptide, due to changes in the conformation of the peptide backbone, had deleterious effects on binding affinity and MVD potency. In general, these adverse effects were more pronounced in the pentapeptide series, owing, most likely, to the greater rigidity and, therefore, reduced susceptibility to conformational perturbation of the tetrapeptides. Substitution of phenylalanine by *p*-fluorophenylalanine enhances binding affinity in the pentapeptide series, consistent with previous observations in the enkephalins, but is without effect on binding in the tetrapeptide series. Substitution of phenylalanine by homophenylalanine, which alters the relationship of the aromatic phenyl ring to the remainder of the peptide by inserting an additional methylene group between the aromatic moiety and the backbone, greatly reduces binding affinity and MVD potency in the pentapeptide. The corresponding modification in the tetrapeptide series has little effect on δ receptor binding affinity and MVD potency and enhances binding to μ opioid receptors. Several possible interpretations of these results are discussed.

Several years ago, we described (1–4) a series of conformationally restricted, cyclic disulfide-containing, enkephalin analogs of structure:



where: X and Y = Cys or Pen (β,β-dimethylcysteine). Peptides within this series, all of which are conformationally restricted not only by virtue of the cyclization but also as a result of the increased rigidity attributable to the *gem* dimethyls of the penicillamine residue(s), displayed varying selectivity for the δ type of opioid receptor, with the bis-penicillamine-containing analogs DPDPE and [D-Pen^2,L-Pen^5]enkephalin exhibiting extremely high selectivity for this receptor (3, 4). Since these reports, DPDPE, in particular, has assumed a role as a standard δ receptor-selective ligand.

In subsequent studies aimed at exploring the effect of ring size on opioid activity in these cyclic analogs, it was observed that increasing the ring size by utilizing cyclization via dithioether formation rather than via a disulfide, while maintaining the primary sequences of the peptides, had deleterious effects on both binding affinity and δ receptor selectivity (5). In contrast, more compact cyclic systems prepared by disulfide or dithioether formation in tetrapeptides lacking the central glycine residue of the enkephalin pentapeptides, and thus resembling the dermorphin family of opioid peptides (6), displayed variable affinity and selectivity (7).[1] In particular, one

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[1] D. L. Heyl, J. R. Omnaas, F. Medzihradsky, C. B. Smith, and H. I. Mosberg. Opioid receptor affinity and selectivity effects of second residue and carboxyl terminal residue variation in a cyclic disulfide-containing opioid tetrapeptide. Submitted for publication.

ABBREVIATIONS: Pen, penicillamine; *p*-FPhe, *p*-fluorophenylalanine; Hfe, homophenylalanine; Pgl, phenylglycine; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; HPLC, high performance liquid chromatography; MVD, mouse vas deferens.

analog in this series, Tyr-D-Cys-Phe-D-PenOH, was found to display similar δ-opioid receptor binding selectivity as DPDPE, while possessing somewhat higher affinity (7). Unlike the cyclic pentapeptide series, in which various combinations of Cys and Pen in residues 2 and 5 and differing chirality of the carboxy terminal residue result in maintenance of high binding affinity and appreciable δ receptor selectivity, the tetrapeptide series displays a much more stringent dependence upon primary sequence, with only Tyr-D-Cys-Phe-D-PenOH itself exhibiting high opioid binding affinity.[1] This more pronounced dependence on primary sequence can be attributed to the exclusion, in the tetrapeptides, of a central glycine residue, which has been suggested to serve as a hinge in the cyclic pentapeptide series, allowing the appropriate orientations of key structural elements to be maintained throughout the series (8, 9).

Schiller and co-workers (10–12) have published several reports describing a similar series of cyclic dermorphin tetrapeptides of the type Tyr-D-X-Phe-YNH_2, in which cyclization is effected via amide bond formation employing side chain amino and carboxyl functions in residues 2 and 4. Throughout the series, in which ring size is varied from 12- to 15-membered (compared with the 11-membered cyclic structure of Tyr-D-Cys-Phe-D-PenOH), high affinity for the μ-opioid receptor is observed, with maximal μ selectivity being demonstrated by Tyr-D-Orn-Phe-$AspNH_2$ (13-membered ring). Examination of a series of analogs related to Tyr-D-Orn-Phe-$AspNH_2$, but in which residue 3 was modified, uncovered differences in the effects of some of these modifications relative to the same alteration of Phe^4 in enkephalin pentapeptides (12). It was proposed that the Phe^3 residue of the dermorphin analogs interacts with a different subsite of the μ receptor than does the Phe^4 residue of enkephalins, a suggestion consistent with observations in linear analogs as well (13). Observations that specific Phe^3 modifications led to different effects on μ versus δ receptor binding affinity led to the further proposal that the Phe^3 residue of these dermorphin analogs interacts in a different manner with μ compared with δ binding sites (12). Prompted in part by these hypotheses and in part by a desire to further elucidate the structure-activity relations in our δ receptor-selective cyclic tetrapeptide series, we have undertaken an evaluation of the effects of modifications of residue 3 in the δ receptor-selective tetrapeptide Tyr-D-Cys-Phe-D-PenOH and a comparison with the effects of the identical modifications of residue 4 in the δ receptor-selective pentapeptide DPDPE. Although the results reveal significant differences between these two series, these differences do not necessarily indicate that the interactions of the phenylalanine (or substitute) residue in these two series is with different receptor subsites.

Materials and Methods

Syntheses. Peptides newly reported here were synthesized in a sequential fashion by solid phase methods, as described earlier for the parent peptides [D-Pen^2,D-Pen^5]enkephalin (4) and Tyr-D-Cys-Phe-D-Pen (7). Syntheses of all peptides utilized chloromethylated polystyrene (Merrifield) resin cross-linked with 1% divinylbenzene as the solid support. *t*-Butyloxycarbonyl protection of the α-amino function was used throughout. The labile sulfur-containing side chains of the cysteine and penicillamine residues were protected with the *p*-methylbenzyl function. For all peptides, cleavage of the peptide-resin was accomplished by treatment of 1.0 g of peptide-resin with 10 ml of anhydrous HF in the presence of 0.5 g of thiocresol and 0.5 g of cresol. After stirring for 45–60 min at 0°, the solvent was removed under vacuum and the resin was washed several times with dry ether. The peptide was extracted from the resin with three 5-ml washes with *N,N*-dimethylformamide/80% acetic acid (9:1), diluted to 400 ml with 25% acetic acid solution, and lyophilized. Purification of the resulting free sulfhydryl-containing linear peptides was effected by semipreparative HPLC on a Vydac 218TP C-18 column (2.2 cm × 25 cm) with the solvent system 0.1% trifluoroacetic acid in H_2O/0.1% trifluoroacetic acid in CH_3CN, using a 10–50% gradient of organic component. In some cases, lyophilization of the extract was omitted; instead, the *N,N*-dimethylformamide/80% acetic acid washes containing the peptide were combined, diluted 20-fold with aqueous HPLC component, filtered, and subjected to semipreparative HPLC directly, using the same conditions described above. Cyclization to the desired disulfide-containing analogs was effected by treatment with $K_3Fe(CN)_6$ at pH 8.5, and final purification was achieved by HPLC, using the conditions described above. Purity of all peptides was greater than 97%, as measured by analytical HPLC monitored at both 280 and 230 nm. Each peptide was subjected to fast atom bombardment mass spectrometry and in each case the appropriate expected molecular weight was observed.

Receptor binding assays. The binding assays, based on the displacement by the test compounds of radiolabeled sufentanil (μ ligand) or DPDPE (δ ligand) in cerebral membranes from rat brain, were performed as previously described (14, 15). Briefly, the assay mixture, containing membrane suspension in 50 mM Tris buffer (pH 7.4), 150 mM NaCl, the radiolabeled ligand, and the test compound, was incubated to reach binding equilibrium (40 min for assays using 0.5 nM [^{3}H]sufentanil; 60 min for 1.5 nM [^{3}H]DPDPE) at 25°. Subsequently, the samples were rapidly filtered and the radioactivity on the filter was determined by liquid scintillation counting. Inhibition of radiolabeled ligand binding by the test compound was computed from maximal specific binding, determined with an appropriate excess of unlabeled sufentanil or DPDPE. IC_{50} values were obtained by linear regression from plots relating inhibition of the specific binding in probit units to the logarithm of five different ligand concentrations. In every case, the correlation coefficient, r^2, of the log-probit plot was higher than 0.98.

Isolated MVD assay. The MVD assays were performed as previously described (16). Briefly, 1.5-cm vas deferens segments from male albino ICR mice were suspended in organ baths that contained 30 ml of a modified Krebs' buffer (118 mM NaCl, 4.75 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, 11 mM glucose, 25 mM $NaHCO_3$, 0.3 mM pargyline HCl, 0.2 mM tyrosine, 0.1 mM ascorbic acid, 0.03 mM sodium edetate), saturated with 95% O_2/5% CO_2 and kept at 37°. The segments were attached to strain gauge transducers and suspended between two platinum electrodes. After a 30-min equilibrium period, the segments were stimulated once every 10 sec with pairs of pulses of 2-msec duration, 1 msec apart and at supramaximal voltage. Test compounds were evaluated for their ability to inhibit the electrically stimulated smooth muscle contractions in this preparation, and complete concentration-effect relationships were determined. IC_{50} values were determined by probit analysis and values reported are the means of three to nine determinations.

Results

Binding affinities of the residue 3-substituted analogs of Tyr-D-Cys-Phe-D-PenOH and the corresponding residue 4-substituted pentapeptide analogs of DPDPE are presented in Table 1, along with values observed for Tyr-D-Cys-Phe-D-PenOH and DPDPE themselves. Binding affinities for μ and δ receptors are represented by IC_{50} values for the displacement of the μ-selective ligand [^{3}H]sufentanil and the δ-selective ligand [^{3}H]DPDPE, respectively, and the ratio of these IC_{50} values provides a measure of selectivity for the δ receptor. Also shown in Table

TABLE 1
Opioid receptor binding profiles and MVD potencies of X^3 tetra- and X^4 pentapeptides

Analog	Binding IC_{50}[a]		IC_{50} (μ) / IC_{50} (δ)	MVD IC_{50}[b]
	[^{3}H]Sufentanil	[^{3}H]DPDPE		
	nM			nM
Tyr-D-Cys-Phe-D-PenOH	1,210	1.90	637	4.60
DPDPE	7,720	6.40	1,200	5.50
Tyr-D-Cys-Hfe-D-PenOH	62.8	2.71	23.2	2.80
[Hfe^4]DPDPE	>6,000	666	>9	1,380
Tyr-D-Cys-Pgl-D-PenOH	4,520	253	18	723
[Pgl^4]DPDPE	>10,000	5,000	>2	>10,000
Tyr-D-Cys-NMePhe-D-PenOH	>6,000	80.8	>74	243
[NMePhe^4]DPDPE	>6,000	395	>15.2	420
Tyr-D-Cys-pFPhe-D-PenOH	1,020	1.27	803	0.97
[pFPhe^4]DPDPE	1,354	1.90	713	0.52
Tyr-D-Cys-D-Phe-D-PenOH	>6,000	77.0	>78	160
[D-Phe^4]DPDPE	>10,000	2,620	>3.8	>10,000

[a] Binding assays performed with rat brain (cerebrum) membrane preparations at 25° in 50 mM Tris buffer, pH 7.4, in the presence of 150 mM Na^+. Radioligand concentrations were 1.5 nM for [^{3}H]DPDPE and 0.5 nM for [^{3}H]sufentanil. Average range for the μ- and δ-selective binding assays was ±6.5% and ±10%, respectively.
[b] MVD assays performed in a modified Krebs' buffer saturated with 95% O_2/5% CO_2 at 37°. Standard errors for the MVD assays were <20% of the reported mean values.

1 are IC_{50} values for the test compounds in the MVD assay, in which the ability to inhibit electrically stimulated muscle contractions is measured. Opioids that inhibit these muscle contractions are, like the endogenous opioids themselves, agonists, whereas opioids that block these agonist effects are antagonists. The results of this assay, thus, provide a means of distinguishing agonists from antagonists, a distinction not readily apparent from the binding assays. All of the new analogs presented here were found to be full agonists, although some are of very low potency (Table 1). Although actions at the κ-opioid receptor were not assessed in this study, previous results with the parent compounds DPDPE (5) and Tyr-D-Cys-Phe-D-PenOH (7) themselves indicate little, if any, interaction with this receptor.

The data presented in Table 1 indicate similarities as well as differences between the tetrapeptide and pentapeptide series in the effects of phenylalanine residue substitutions. In the tetrapeptide series, those residue 3 substitutions that can be expected to affect the backbone conformation of the peptide are observed to decrease binding affinity, most notably to δ receptors. Thus, substitution of Phe^3 by Pgl, D-Phe, and NMePhe leads to reductions in δ receptor binding affinity of 133- 41- and 43-fold, respectively. These modifications also have a deleterious effect on potency in the MVD assay, resulting in potency losses of 157-, 35-, and 53-fold relative to Tyr-D-Cys-Phe-D-PenOH itself. The magnitudes of the potency losses observed in the MVD assays are in excellent agreement with the reduced binding affinities observed for the δ receptor.

In contrast to the effects observed with the three residue 3 modifications likely to alter backbone conformation, the two Phe^3 substitutions examined whose effects can be expected to be localized to the side chain (*p*-FPhe and Hfe) are accommodated without adverse effects on binding affinity or MVD potency. Of these substitutions, *p*-FPhe results in similar binding affinity as the parent Phe^3 tetrapeptide at both μ and δ receptors but yields a 5-fold increase in MVD potency. The other substitution, Hfe, in which the side chain is lengthened by insertion of a second methylene unit between the backbone and the aromatic ring, results in similar δ receptor affinity and MVD potency as observed for the Phe^3 tetrapeptide. Interestingly, Hfe^3 substitution results in a 19-fold increase in μ receptor affinity, relative to Tyr-D-Cys-Phe-D-PenOH.

In the series of pentapeptides related to DPDPE, some effects similar to those seen in the tetrapeptide series are observed. Again, the three Phe substitutions that can be expected to affect backbone conformation have deleterious effects on binding and MVD potency. Thus, the Pgl^4- (17), D-Phe^4, (17), and NMePhe^4-modified DPDPE analogs experience 780-, 410-, and 62-fold losses, respectively, in δ binding affinity and MVD potency decreases of >1800-, >1800-, and 76-fold, respectively, relative to DPDPE. As reported previously (17), the results for the Pgl- and D-Phe-substituted DPDPE analogs are consistent with observations made for similarly substituted linear enkephalin analogs (18, 19). The observed decreases in δ receptor binding affinity and MVD potency accompanying NMePhe^4 substitution in DPDPE are also consistent with previous reports (20).

The two DPDPE analogs in which the Phe^4 modification can be expected to have effects localized to the side chain, [*p*-FPhe^4]DPDPE and [Hfe^4]DPDPE, display quite different binding and bioassay profiles. The former of these, [*p*-FPhe^4]DPDPE, exhibits enhanced affinity toward both δ- and μ-opioid receptors and a 10-fold increase in potency in the MVD bioassay. These findings are in agreement with recently reported results for this analog (21) and are consistent with the effect of *p*-FPhe modification in linear enkephalins as well (22). Substitution of Phe^4 by Hfe^4 in DPDPE, however, results in δ receptor binding affinity and MVD potency losses of 100- and 250-fold, respectively, in marked contrast to the effects observed for Hfe^3 for Phe^3 substitution in the tetrapeptide series.

It is important to note that, although peptidase inhibitors were not used in either the binding or MVD assays, the affinity and potency differences observed are unlikely to be due to differential sensitivity to peptidase-catalyzed degradation. We have found that peptidase inhibitors have little or no effect on the observed IC_{50} values in either MVD[2] or binding assays (14) with examples of several structural classes of opioid peptides, including cyclic enkephalins such as those studied here.

Discussion

The observed effects on opioid receptor binding affinity and MVD potency of the residue 4 modifications incorporated into

[2] C. B. Smith, Unpublished observations.

the DPDPE analogs listed in Table 1 are consistent with previously reported results for enkephalin analogs in general. The rather stringent dependence of δ-opioid affinity and potency upon the residue 4 side chain reflects the critical role this residue plays in receptor recognition (19, 23, 24). It is generally assumed that the relative orientations of the Tyr[1] and Phe[4] aromatic side chains are a key feature of the binding conformation of the enkephalins and that each of these side chains is involved in favorable binding interactions at the receptor. Thus, modifications that render this relative orientation energetically less favored will result in reduced binding affinity. In this context then, modifications that alter the backbone conformation and/or the orientation of the phenylalanine aromatic ring relative to the peptide backbone generally can be expected to have deleterious effects, as are observed for Pgl, Hfe, and D-Phe substitutions at residue 4, both here and in linear enkephalin analogs. In linear enkephalins, NMePhe[4] substitution is often well tolerated (25), in contrast to the effect observed here in DPDPE. Apparently, in the more constrained cyclic analogs the effect of the *N*-methyl substitution on backbone geometry results in an aromatic side chain orientation that is incompatible with the binding requirements.

In contrast to the results observed with modifications that disturb the orientation of the residue 4 aromatic side chain, substitution in this position by *p*-FPhe, which would not be expected to perturb the side chain conformation relative to Phe itself, leads to enhanced binding affinity at both μ- and δ-opioid receptors and to increased MVD potency. This is presumably due to the altered electronic and/or lipophilic character of the *p*-fluorophenyl ring, which leads to a more favorable binding interaction at the phenylalanine aromatic binding subsite of both the μ and δ receptors. Similar increases in binding affinity and bioassay potency have been observed for *p*-FPhe[4] substitutions in linear (22) and cyclic (21) enkephalin analogs.

Both similarities and differences in the effect of a given modification in the pentapeptide versus the tetrapeptide series can be seen. Substitution of Phe[3] by NMePhe or *p*-FPhe in the tetrapeptide leads to effects generally similar to those found for the analogous residue 4 modification in DPDPE. *p*-FPhe substitution in the tetrapeptide series, like *p*-FPhe[4] substitution in the pentapeptides, results in a significant increase in MVD potency. However, although a concomitant increase in binding affinity is observed in the pentapeptide series, the binding of Tyr-D-Cys-*p*-FPhe-D-Pen to both μ and δ receptors is essentially indistinguishable from that of the corresponding Phe[3] tetrapeptide. Thus, although the altered electronic and/or lipophilic effects accompanying *p*-FPhe substitution in the pentapeptide serve to enhance the binding interaction, this substitution in the tetrapeptide does not significantly affect binding. The similar deleterious effect of NMePhe substitution for Phe[3] in the tetrapeptide series as was observed for Phe[4] substitution in DPDPE indicates that in this series too the *N*-methylation disfavors the side chain geometry required for avid binding. Although it is tempting to ascribe the observed 40–80-fold decreases in δ binding affinity and MVD potency as reflecting the expected increase in *cis-trans* peptide bond isomerism resulting from *N*-methyl substitution, this explanation is highly unlikely. The presence of *cis* and *trans* forms of both peptides is evident in their NMR spectra,[3] in which relatively similar populations *cis* and *trans* forms are observed. Accordingly, *cis-trans* isomerism alone could not account for the very large decreases in binding affinity and potency observed.

Substitution of the Phe residue in the tetrapeptide series by Pgl or D-Phe leads to reduced binding affinity and MVD potency, as was observed in the DPDPE series; however, in the tetrapeptides the magnitude of these reductions is about 10-fold less. More striking is the observation that substitution by Hfe, which reduces δ affinity and MVD potency in the DPDPE series by 100- and 250-fold, respectively, results in only a 1.5-fold decrease in δ affinity and a 2-fold increase in MVD potency in the tetrapeptides. In general, these results indicate that altering the aromatic residue 3/4 side chain length (Pgl, Hfe) or perturbing its orientation relative to the backbone by modifying backbone conformation and/or chirality (Pgl, D-Phe, NMePhe) has a more benign effect on δ-opioid activity in the tetrapeptides than in the pentapeptide series. Three possible explanations suggest themselves. First, these results may reflect a less critical role for this residue in the tetrapeptide series than in the pentapeptides. Given the high binding affinity of members of the tetrapeptide series (equal to or greater than the corresponding pentapeptides) and the important role of the Phe[4] residue in the pentapeptide enkephalins, this would require that different modes of interaction between the two series and the δ receptor be invoked. This different mode of interaction would then compensate for the lack of a binding interaction involving the Phe[3] residue in the tetrapeptides. A second, related explanation, which also requires that different modes of interaction with the receptor occur, is that the Phe[3] residue remains important for binding but interacts with a different receptor subsite with less stringent geometrical requirements than the subsite with which the Phe[4] residue of the pentapeptides interacts. Thus, conformation-altering substitutions in the tetrapeptides would lead to less drastic losses in binding affinity, as is observed. A third possibility, and a more parsimonious one because it does not necessarily require different binding modes between the two series, is that modifications leading to reduced binding affinity and bioassay potency have less pronounced effects in the tetrapeptide series because the modifications perturb the tetrapeptide conformations less than they do the pentapeptide conformations. In this case, some greater contribution of the binding interaction involving the residue 3/4 aromatic moiety (or, alternatively, a lesser contribution of unfavorable interactions at the receptor) can be maintained in the residue 3-substituted tetrapeptide series. We have recently reported that Cys for Pen (and Pen for Cys) substitutions and changes in C-terminal chirality within the Tyr-D-Cys-Phe-D-Pen framework are much less well tolerated than in the corresponding pentapeptide series.[1] We have suggested that this reflects the increased rigidity of the tetrapeptides, which lack the flexible Gly[3] residue that in the pentapeptide series allows the appropriate binding geometry to be assumed. In much the same manner, but with a different result, the rigidity of the tetrapeptides might limit the magnitude of the conformational perturbation resulting from the residue 3 substitutions described here, thereby resulting in a less drastic effect on binding affinity than observed in the pentapeptides.

The MVD and δ receptor binding results observed with the Hfe[3]-substituted tetrapeptide are consistent with any of the three possible explanations discussed above. If, as the first explanation supposes, the residue 3 aromatic group is unimpor-

[3] H. I. Mosberg, K. Sobezyk-Kojiro, Unpublished obervations.

tant for δ binding, then Hfe, which would not be expected to alter backbone conformation, should be a neutral substitution (barring any adverse steric interaction), as is observed. If, as the second explanation hypothesizes, the aromatic ring does contribute to the binding interaction but interacts with a structurally less restrictive subsite, the same result would be expected. If, on the other hand, the tetrapeptides and pentapeptides bind to the same complementary regions of the δ receptor, as would be allowed in the third hypothesis, they likely do so with some differences in orientation of the residue 3/4 aromatic group relative to other key binding elements, particularly the tyrosine residue, owing to the absence in the former and presence in the latter of an intervening glycine residue. Thus, extension of the residue 3 side chain by one carbon atom in Tyr-D-Cys-Hfe-D-Pen may result in a relative positioning of tyrosine and homophenylalanine aromatic rings similar to that in Phe⁴-containing pentapeptide analogs, such as DPDPE. Again, little effect on δ receptor affinity or MVD potency would be expected, as is consistent with observation.

The series of compounds presented here provides fewer insights regarding μ receptor binding requirements, because in almost all cases the analogs examined, in both tetrapeptide and pentapeptide series, display similar poor μ binding affinities. The sole exception, Tyr-D-Cys-Hfe-D-Pen, exhibits an approximately 20-fold improvement in μ binding affinity, compared with the corresponding Phe³ tetrapeptide, and is at least 100-fold higher in μ affinity than [Hfe⁴]DPDPE. This is consistent with previous observations in linear di- and tripeptides related to dermorphin, in which the more extended phenethyl side chain of homophenylalanine was observed to enhance opioid potency in the guinea pig ileum assay, a μ receptor-sensitive assay (26). However, Schiller *et al.* (12) have reported that Hfe for Phe substitution in the potent μ receptor-selective tetrapeptide Tyr-D-Orn-Phe-AspNH_2 has little effect on binding affinity to μ receptors.

Results from other Phe³ modifications in Schiller's cyclic series (12) as well as linear dermorphin-related analogs (13) have led to the suggestion that these generally μ-selective analogs interact with the μ receptor in a different fashion than do corresponding pentapeptides and that, in particular, different subsites exist for the Phe³ versus Phe⁴ aromatic side chain. As discussed above, the observations reported here for the δ receptor-selective tetrapeptide series are also consistent with different aromatic binding subsites, compared with those for DPDPE and related pentapeptides; however, other interpretations are not excluded. Synthetic and conformational studies aimed at addressing this critical question, whose answer will help identify similarities and differences between μ and δ receptor binding sites, are in progress.

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