Vol. 120, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1984 Pages 305-310

EVIDENCE OF A PEPTIDE BACKBONE CONTRIBUTION TOWARD SELECTIVE RECEPTOR RECOGNITION FOR LEUCINE ENKEPHALIN THIOAMIDE ANALOGS*

Kim Clausen and Arno F. Spatola
Department of Chemistry, University of Louisville, Louisville, Kentucky 40292

Carole Lemieux and Peter W. Schiller
Clinical Research Institute of Montreal
Montreal, Quebec H2W 1R7 Canada

Sven-Olov Lawesson
Chemical Institute
Department of Organic Chemistry, University of Aarhus
DK-8000 Aarhus C Denmark

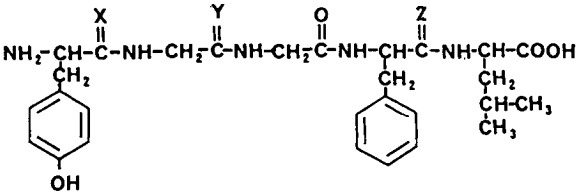
Received March 12, 1984

SUMMARY: The in vitro opioid activities of a series of leucine enkephalin analogs containing a thioamide linkage in place of the peptide bond at various positions of the backbone were determined in μ- and δ-receptor-selective bio- and binding-assays. Thioamide substitution in the 1-2 position resulted in an inactive compound, whereas the same modification in the 2-3 and 4-5 position produced potency enhancement. Most interestingly, the 2-3 modified analog showed a 3 to 5 times higher preference for δ- over μ-receptors than natural leucine enkephalin. These results suggest that subtle backbone modifications can have a profound effect on receptor affinity and selectivity of biologically active peptides.

Variations of both side chain and backbone elements in peptides have been shown to result in a great dichotomy of biological consequences (1-3). In particular, the use of amide bond replacements of many designs has assisted the search for possible functional roles, or contribution to receptor selectivity, for the normal -CONH- peptide linkage. Of particular interest are those replacements that involve subtle perturbations of the molecule while permitting an assessment of these changes on the resulting biological activity profiles. Other than isotopic substitions, the least disruptive changes are those which by replacement of a single atom nevertheless leave intact most of the structural and geometric features of the linkage. Replacement of an amide by a thioamide function fulfills the latter criterion, and has been exploited

* A preliminary account of this work was presented at the Eighth American Peptide Symposium, Tucson, Arizona, May 22-27, 1983.

0006-291X/84 $1.50



I. X=Y=Z=O III. Y=S, X=Z=O

II. X=S, Y=Z=O IV. Z=S, X=Y=O

Figure 1. Structures of Leucine enkephalin (I) and thioamide analogs II-IV.

in both peptide side chains (4,5) and, more recently, as an amide bond surrogate (6-8). We now report the biological consequences of incorporating this modification within three of the four possible isomeric analogs of leucine enkephalin. The structures of these analogs are shown in Figure 1. Compounds were tested in the guinea pig ileum (GPI) and mouse vas deferens (MVD) assay which are representative for μ- and δ-opioid receptor interactions, respectively, and in binding assays based on displacement of [^{3}H]naloxone (μ-receptor-selective) and [^{3}H][D-Ala^2,D-Leu^5]enkephalin (δ-receptor-selective) from rat brain homogenates (9).

METHODS

The synthesis and characterization of the peptides used in this study have been reported elsewhere (6). All products were purified by reversed phase high performance liquid chromatography and fully characterized; purity and structure were authenticated by NMR spectroscopy, amino acid analysis, and fast atom bombardment mass spectrometry, as well as by tlc on at least three solvent systems. The GPI- and MVD-assay and the binding displacement experiments (incubation for 2 hr at 0°C) were performed as described elsewhere (10).

RESULTS AND DISCUSSION

The results of the bio- and binding assays performed with the three thioamide analogs of leucine enkephalin are presented in Tables 1 and 2. A fourth compound, [Glyψ[CSNH]Phe$^{3-4}$]-leucine enkephalin, could not be obtained in high yield by analogous synthetic routes and thus will be prepared and assayed separately.

Substitution of the first peptide bond with a thioamide linkage results in an analog (II) which is inactive both in the GPI and in the MVD assay. On the other hand, a three- to four-fold potency enhancement relative to leucine enkephalin is observed with anaolgs III and IV on the ileum preparation.

TABLE 1. Biological activities of leucine enkephalin and thioamide analogs in guinea pig ileum (GPI) and mouse vas deferens (MVD) assays

Compound number	Structure	GPI		MVD		GPI/MVD
		IC_{50}[nM]	Relative Potency	IC_{50}[nM]	Relative Potency	IC_{50} Ratio
I	Leucine Enkephalin	246±39	1	11.4±1.1	1	21.6
II	H-Tyrψ[CSNH]Gly-Gly-Phe-Leu-OH	inactive	--	>57,000	<0.0002	--
III	H-Tyr-Glyψ[CSNH]Gly-Phe-Leu-OH	61.5	4.0	0.826±0.096	13.8±1.6	74.4
IV	H-Tyr-Gly-Gly-Pheψ[CSNH]Leu-OH	74.1±2.0	3.32±0.09	5.23±0.84	2.18±0.35	14.2

Similarly, analog IV is about twice as potent as leucine enkephalin in the MVD assay. Most interestingly, however, analog III is 14 times more potent than leucine enkephalin in the latter assay. Comparison of the GPI/MVD IC50 ratios (Table 1) indicates that analog III has a more than three times higher preference for δ-receptors over μ-receptors than leucine enkephalin.

Very similar results were obtained in the μ- and δ-receptor selective binding assays (Table 2). Since in these binding experiments all incubations were performed at 0°C, enzymatic degradation of leucine enkephalin or analogs

TABLE 2. Inhibitory effects of leucine enkephalin and thioamide analogs on the binding of [^{3}H]naloxone and [^{3}H][D-Ala^2,D-Leu^5]enkephalin in rat brain homogenates[a]

Compound number	Structure	[^{3}H]naloxone		[^{3}H][D-Ala^2,D-Leu^5]enkephalin		K_i^μ/K_i^δ
		K_i^μ[nM]	Potency Ratio	K_i^δ[nM]	Potency Ratio	
I	Leucine Enkephalin	27.7±5.7	1	8.05±0.95	1	3.44
II	H-Tyrψ[CSNH]Gly-Gly-Phe-Leu-OH	>1,380	<0.02	>805	<0.01	-
III	H-Tyr-Glyψ[CSNH]Gly-Phe-Leu-OH	11.7±4.0	2.36±0.81	0.618±0.214	13.0±4.5	18.9
IV	H-Tyr-Gly-Gly-Pheψ[CSNH]Leu-OH	7.78±2.22	3.55±1.02	2.97±1.29	2.71±1.18	2.62

[a] Mean of three determinations ± SEM.

can be excluded (11) and the obtained potencies reflect true opioid receptor affinities. In excellent agreement with the results of the bioassays, analog III was found to be 13 times more potent than leucine enkephalin in the δ-receptor selective binding assay and the ratio of its bindings inhibition constants (K_i^{μ}/K_i^{δ}) indicates a more than five times higher selectivity for δ-receptors in comparison to leucine enkephalin.

It thus appears that replacement of the second peptide bond by a thioamide moiety selectively strengthens binding to the δ-receptor. In contrast, the 4-5 modified analog shows very little difference in selectivity as compared to leucine enkephalin, thereby suggesting that the C-terminal portion of the peptide backbone may have a minor contribution toward influencing relative receptor affinities. It is interesting to compare our results regarding receptor selectivity with those reported for other linear and cyclic analogs. Structure (conformation)-activity relationships of linear analogs have in general been more difficult to interpret because of the likelihood of multiple conformers and the lack of knowledge concerning the receptor-active conformation (12). However, biological activity data obtained with numerous linear (cf. ref. 1 and 2), cyclic (10,13,14) and dehydro (15) analogs of enkephalin suggest that each receptor class has specific structural and conformational requirements for maximal potency and neither receptor type appears to discriminate a priori against cyclic analogs. On the other hand, it has been suggested (16) that open-chain analogs may prefer the δ-receptor, while the μ-receptor might preferentially interact with more constrained or β-turn containing structures. It is interesting to note that a classical 5→2 hydrogen-bonded β-turn is disfavored in our analog III by virtue of the poor hydrogen bond tendencies of thiocarbonyl versus carbonyl (17), whereas the thioamide linkage at the 4-5 position might actually enhance an intramolecular hydrogen bond. While few literature results are compatible with an absolute requirement for a turn feature in order to elicit the biological response, it cannot be ruled out that the lack of such a feature might be partially responsible for the enhanced δ-receptor selectivity observed with analog III.

Alternatively, the possibility that the peptide backbone participates in intermolecular peptide-receptor hydrogen bonding that might lead to selective receptor discrimination has also to be considered.

The results described above indicate that thioamide replacement of amide bonds in the backbone of biologically active peptides can have a drastic effect on receptor affinity and selectivity. The only other thioamide peptide hormone whose biological activity has been reported is [1-deamino,9-thioglycinamide]oxytocin (18) which showed only 6% the potency of deamino oxytocin in the uterotonic assay. The present enkephalin structures III and IV represent the first examples of thioamide analogs possessing enhanced potency and--in the case of analog III--higher receptor selectivity than the parent peptide.

In summary, it would appear that the utility of the thioamide modification in peptide design is considerable. Among advantageous characteristics are 1) minimal distortion of amide bond geometry as a result of considerable isosterism (19) between amides and thioamides including normal bond lengths and angles (20); 2) suitability as a probe for intra- and intermolecular hydrogen bonding features that introduces fewer distortions than ψ[CON(Me)] (N-methyl) or ψ[COO] (depsipeptide) replacements; 3) altered donor properties of the thioamide NH group (17) which can be an exploitable feature of drug design; and 4) possible increased resistance to enzyme degradation; the latter property is currently being assessed in our laboratory using these bioactive analogs.

ACKNOWLEDGEMENT

Grants from the Medical Research Council of Canada (MT-5655) and the Quebec Heart Foundation are gratefully acknowledged by one of us (PS). We also acknowledge the support of the Danish Natural Science Research Council (KC), NIH grant #AM/CA31364 (AFS), and NATO grant 0599/82.

REFERENCES

(1) Morgan, B. A. (1977) Amino-Acids, Peptides, and Proteins, Vol. 10, pp. 474-489, Royal Society of Chemistry, London.
(2) Schiller, P. W. (1979) ibid. Vol. 12, pp. 558-579.
(3) Spatola, A. F. (1983) Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins (Weinstein, B., ed.), Vol. 7, pp. 267-357, Marcel Dekker, New York.
(4) Ressler, C., and Banerjee, S. N. (1976) J. Org. Chem. 41, 1336-1340.

(5) Saneii, H., and Spatola, A. F. (1982) Tetrahedron Lett. 23, 149-152.
(6) Clausen, K., Thorsen, M., Lawesson, S.-O., and Spatola, A. F. (1984) J. Chem. Soc., Perkin Trans. I (in press) and references cited therein.
(7) Clausen, K., Yde, B., and Lawesson, S.-O. (1983) Peptides 1982 (Blaha, K. and Malon, P., eds.,), pp. 207-210, Walter de Gruyter & Co., Berlin.
(8) Lajoie, G., Lepine, F., Maziak, L., and Belleau, B. (1983) Tetrahedron Lett. 24, 3815-3818.
(9) Lord, J. A. H., Waterfield, A. A., Hughes, J. S., and Kosterlitz, H. W. (1977) Nature (London) 267, 495-500.
(10) DiMaio, J., Nguyen, T.M.-D., Lemieux, C., and Schiller, P. W. (1982) J. Med. Chem., 25, 1432-1438.
(11) DiMaio, J., and Schiller P. W. (1980) Proc. Natl. Acad. Sci. USA, 77, 7162-7166.
(12) Schiller, P. W. (1983) The Peptides (Udenfriend, S., and Meienhofer, J., eds.), Vol. 7, Academic Press, New York (in press).
(13) Mosberg, H. I., Hurst, R., Hruby, V. J., Galligan, J. J., Burks, T. F., Gee, K., and Yamamura, H. I. (1982) Biochem. Biophys. Res. Commun. 106, 506-512.
(14) Berman, J. M., Goodman, M., Nguyen, T. M.-D., and Schiller, P. W. (1983) Biochem. Biophys. Res. Commun. 115, 864-870.
(15) Shimohigashi, Y., Stammer, C., Costa, T., and von Voigtlander, P. F. (1983) Int. J. Pept. Protein Res. 22, 489-494, and references cited therein.
(16) Fournie-Zaluski, M. C., Gacel, G., Maigret, B., Premilat, S., and Roques, B. P. (1981) Mol. Pharmacol. 20, 481-491.
(17) Walter, W., and Voss, J. (1970) The Chemistry of Amides (Zabicky, J. ed.), pp. 383-475, Interscience Publishers, London.
(18) Jones, Jr., W. C., Nestor, Jr., J. J., and du Vigneaud, V. (1973) J. Am. Chem. Soc. 95, 5677-5679.
(19) Marshall, G. R. (1982) Chemical Regulation of Biological Mechanisms (Cubitt, A. G., and Creighton, A. M., eds.), pp. 279-292, Royal Society of Chemistry, London.
(20) La Cour, T. F. M., Hansen, H. A. S., Clausen, K., and Lawesson, S.-O. (1983) Int. J. Pept. Protein Res. 22 , 509-512.