THE SYNTHESIS AND IN VITRO PHARMACOLOGICAL EVALUTION OF SOME NOVEL ENKEPHALIN ANALOGUES

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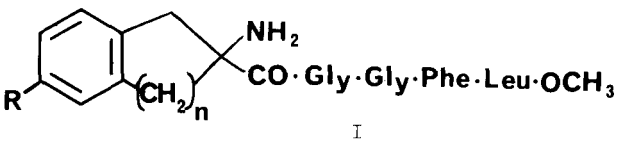

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a: R=H, n=1
b: R=OH, n=1
c: R=H, n=2
d: R=OH, n=2

Structure-activity studies on the enkephalins have revealed the importance of a terminal tyrosine[1] residue for opioid activity (Chang *et al.*, 1976; Morgan *et al.*, 1976). Here we report on the synthesis and *in vitro* opioid activities of some leucine-enkephalin analogues possessing a conformationally restrained tyrosine analogue at position 1 in the enkephalin sequence.

The synthesis of peptides of general structure I employed a strategy previously unreported for enkephalin analogues. The tetrapeptide H-Gly-Gly-Phe-Leu-OCH_3 was built up using classical solution methods from *t*-BOC-glycine by sequential addition of the appropriate amino acid ester and C-deprotection in methanolic sodium hydroxide, coupling being carried out via the dicyclohexylcarbodiimide method. N-Deprotection involved *t*-BOC-cleavage using HCl in glacial acetic acid. The individual tyrosine analogues were synthesised via literature methods, N-protected with carbobenzyloxy chloride in base, O-protected where appropriate with benzyl bromide and coupled with tetrapeptide using dicyclohexylcarbodiimide and 1-hydroxybenztriazole. Hydrogenolysis of the fully protected pentapeptides and purification of the products by gradient buffer elution from carboxymethylcellulose at pH 5.1, afforded compounds Ia-d. All previously unreported compounds were characterised by ir, ^{1}H-nmr, tlc and elemental analysis.

In vitro testing of peptides Ia-Id was carried out by measurement of inhibition of electrically stimulated contractions of the guinea pig ileum myenteric plexus muscle and mouse vas deferens tissue (see Table I). Reversal of this inhibition was demonstrated using naloxone.

Table I: Opioid Activities of Some Leucine-Enkephalin Analogues Possessing a Conformationally Restrained Tyrosine[1] Moiety.

Compound	Relative Potency (Morphine = 1.0)	
	Guinea Pig Ileum	Mouse Vas Deferens
Ia	0.004	0.004[a]
Ib	0.03	0.02[b]
Ic	No inhibition	0.004
Id	1.46 (ID_{50}=61.7nM)	3.01[c] (ID_{50}=322.2nM)
Methionine-enkephalin	1.1 (ID_{50}=86.8nM)	58.2 (ID_{50}=18.2nM)

a: 100% naloxone reversible at 27 µg/ml; b: 37% naloxone reversible at 27 µg/ml; c: 100% naloxone reversible at 27 µg/ml.

The results show that the tyrosine[1] in leucine-enkephalin can be successfully replaced by the conformationally restrained 2-amino-6-hydroxytetralin-2-carboxylic acid analogue to afford a compound with slightly greater opioid activity in the guinea pig ileum test than morphine or methionine-enkephalin, but whose activity in the mouse vas deferens is much lower than that of methionine-enkephalin.

Chang, J.-K., Fong, T.W., Pert, A. and Pert, C.B. (1976) Life Sci. 18, 1473-1482
Morgan, B.A., Smith, C.F.C., Waterfield, A.A., Hughes, J. and Kosterlitz, H.W. (1976) J. Pharm. Pharmac. 28, 660-661