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Design and synthesis of systemically active enkephalin-like peptides

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Abstract. Following the discovery of enkephalins, it was observed that the mild analgesia evoked by them was completely dissipated within a few minutes even when they were injected directly into active brain sites. As these pentapeptides could serve as the prototypes of an entirely new class of analgesics, studies on their structure-function relationships were undertaken by a large number of investigators to get molecules which would produce profound and long-lasting analgesia even after systemic or oral administration. Various approaches that have helped in the successful achievement of this goal have been briefly reviewed.

Keywords. Enkephalin analogues; analgesic activity; enzymatic deactivation; receptor-binding affinity; systemic administration.

1. Introduction

One of the most practical and logical approaches for obtaining bio-active molecules is to design peptides structurally related to those found in nature and to develop suitable procedures for their synthesis. The main advantage of this approach may well be realised by the fact that congeners of this type would normally be expected to manifest their biological activities by undergoing the same sequence of molecular events in the bio-phase as found in case of the naturally occurring parent peptides. Moreover, attempts of this sort offer unlimited possibilities of getting molecules which may not only exhibit highly improved biological activity and profile, but may also be endowed with marked stability, favourable transport properties and greatly reduced or negligible toxic side effects. Recent achievements in the case of peptides related to enkephalins, LH-RH, somatostatin etc. lend strong support to this view. Some of the highlights of the design and synthesis of systemically active enkephalin-like peptides are presented here.

2. Analgesic activity of enkephalins and their more stable analogues

The isolation and characterisation of Met-* and Leu-enkephalins was the result of an extensive search for endogenous ligands for the stereospecific opiate receptors distributed in the mammalian nervous system (Hughes *et al* 1975). Although both these pentapeptides elicited pharmacological effects of morphine and related alkaloids *in vitro* as well as *in vivo*, they were found to evoke only weak and short-lived analgesia after intracerebroventricular (ICV) administration (Chang *et al* 1976). As it became clear that the analgesia produced by enkephalin was mild mainly due to the rapid cleavage of

*Abbreviations for amino acid and peptide derivatives are according to IUPAC-IUB Commission on biochemical nomenclature, 1972 *Biochemistry* **11** 1726.

its Tyr-Gly bond under the influence of aminopeptidases (Hambrook *et al* 1976), strategies were evolved to protect its N-terminus against such deactivation. Pert *et al* (1976) introduced a D-Ala residue in place of Gly^2 and Bradbury *et al* (1977) methylated the α-NH_2 function of Tyr^1 for stabilisation of the N-terminus and simultaneously converted the terminal carboxy group into an amide for protecting the C-terminus from the attack of carboxypeptidases. The resulting pentapeptide amides, Tyr-D-Ala-Gly-Phe-Met-NH_2 and MeTyr-Gly-Gly-Phe-Met-NH_2, were found to be nearly as potent as morphine on a molar basis after administration by the ICV route.

3. Systemically active enkephalin analogues and the rationale behind their structural design

The initial success in getting more potent analogues of enkephalin encouraged medicinal chemists to take up structure-function relationship studies on a wide scale and a target was set for developing a new class of synthetic analgesics which, inspite of being small peptides, would be able to produce profound and long lasting analgesia after systemic administration. The guiding features for the design of such congeners now included enhancement of receptor-binding affinity, favourable transport properties and ability to cross the blood-brain barrier in addition to their being resistant to enzymatic deactivation.

Belluzzi *et al* (1978) synthesized Tyr-D-Ala-Gly-Phe-D-Leu and the corresponding amide and found that the introduction of D-Leu at position 5 made the peptide systemically active. The amide derivative was nearly one third as active as morphine after IV administration. Frederickson *et al* (1981) recently reported the activity of their pentapeptide in which they kept a D-Ala residue at position 2, but chose to introduce a MeMet-NH_2 residue at position 5 in order to check metabolic deactivation from the C-terminal end. The analgesic potency of this peptide was found to be nearly four times that of morphine when injected by the subcutaneous (SC) route (table 2). Due to its high order of activity this peptide was chosen for clinical trials and has been

Table 1. Morphine-like activities of various enkephalin analogues with *n*- and iso-propylamide residues (Tyr-X-Gly-Y-Z).

				Relative potency	
Sl. no.	X	Y	Z	Hot plate test (ICV)	GPI
1	Gly	Phe	Met-NH·C_3H_7(*n*)	0·47	0·09
2	Gly	Phe	Met-NH·C_3H_7(*iso*)	1·66	0·38
3	D-Ala	Phe	Met-NH·C_3H_7(*n*)	1·43	2·57
4	D-Ala	Phe	Met-NH·C_3H_7(*iso*)	5·00	3·17
5	D-Ala	Phe	Met(O)-NH·C_3H_7(*n*)	0·39	12·12
6	D-Ala	MePhe	Met-NH·C_3H_7(*n*)	13·75	30·00
7	D-Ala	MePhe	Met-NH·C_3H_7(*iso*)	407·40	12·95
8	D-Ala	MePhe	Met(O)-NH·C_3H_7(*n*)	11·59	24·78
9	D-Ala	MePhe	Met(O)-NH·C_3H_7(*iso*)	196·43	21·92
10	Morphine			1	1

Table 2. Relative potencies of some systemically active enkephalin analogues.

Sl. no.	Analogue	Tail flick test				Hot plate test				
		ICV	SC	IV	PO	ICV	SC	IV	IP	PO
1	Tyr-D-Ala-Gly-MePhe-Met(O)-ol	10^3	3·2	6·4	0·32		3·1			0·45
2	MeTyr-D-Ala-Gly-MePhe-Met(O)-ol		16·4	4·9	1·6					
3	Tyr-D-Met-Gly-Phe-Pro-NH_2	78	1·5	5·9		25		1·7		
4	MeTyr-D-Met-Gly-Phe-Pro-NH_2							2·2		
5	H_2N-C(NH)-Tyr-D-Met-Gly-Phe-NH_2	210		4·40						
6	H_2N-C(NH)-Tyr-D-Nle-Gly-Phe-NH_2	20		7·95						
7	Tyr-D-Met-Gly-Phe-D-NleP	0·52		0·17						
8	Tyr-D-Thr-Gly-Phe-Thz-NH_2	27	1·5	4·8	1·7					
9	Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$-N(O)Me_2			7·0						
10	Tyr-D-Ala-Gly-Phe-MeMet-NH_2					238	0·3			
11	Tyr-D-Ala-Gly-Phe-D-Leu-NH_2	3·8		0·3						
12	Tyr-D-Ala-Gly-MePhe-Met-NHC_3H_7(*iso*)					407·4			0·06	
13	Tyr-D-Ala-Gly-MePhe-Met(O)NHC_3H_7(*iso*)					196·43			0·32	0·16
14	Morphine	1	1	1	1	1	1	1	1	1

reported to produce less respiratory depression and physical dependence than morphine. In phase-I clinical trials, this peptide has been found to cause certain toxic side effects such as nasal congestion, dry mouth, heaviness of limbs and changes in emotional state (Gesellchen and Zimmerman 1981).

On the basis of their structure-activity relationship (SAR) studies Bajusz *et al* (1977) concluded that the side chain of the D-amino acid residues, introduced at position 2 of enkephalin, provided an extra binding site for the opiate receptor. Analogues having D-Met at position 2 possessed higher activity than the corresponding D-Ala^2 and D-Nle^2 peptides. Since the introduction of a Pro residue in place of Met^5 or Leu^5 would be expected to stabilise the fourth peptide bond of enkephalins against proteolysis, they prepared Tyr-D-Met-Gly-Phe-Pro-NH_2. The *in vivo* molar potencies of this peptide were quite high, indeed, as compared to morphine. It was 5·9 times and 1·6 times more active than morphine when administered by IV and SC routes respectively. A closely related derivative carrying MeTyr residue in place of Tyr^1 has also been reported (Shaw *et al* 1978). Compared to morphine, it was 2·2 times more potent when administered by IV route suggesting thereby that N-methylation of Tyr^1 further enhances the analgesic activity of Tyr-D-Met-Gly-Phe-Pro-NH_2. Bajusz *et al* (1980) incorporated further modifications into this peptide and obtained very interesting results. As earlier SAR data had indicated, a basic amino or N-methylamino function at the N-terminal end of the enkephalin-like peptides is essential for their biological activity. These investigators believed that replacement of the α-NH_2 function of Tyr^1 with a guanidino function would enhance the interaction between the peptide N-terminus and the receptor, since the guanidinium cation would, apart from establishing electrostatic interactions, also provide an additional hydrogen bonding site.

Considering the earlier reports by Weil and Talka (1957), it could also be expected that the distal shift of the positive charge by approximately 1·2 Å due to the introduction of the guanidino group would also render the Tyr-Gly bond resistant to proteolytic attack. A series of pentapeptides containing the guanidino group at N-termini, D-Met or D-Nle at position 2 and Pro-NH_2 at position 5 was synthesized. Side by side another series of tetrapeptides, where the C-terminal Pro was deleted and the carboxy function of Phe^4 converted into amide, was also obtained. The results of this study showed that guanidination improved the *in vitro* activities of both the pentapeptides as well as tetrapeptides. However, the results of *in vivo* tests were quite different. While the guanidine derivatives of the pentapeptides were less active, the corresponding tetrapeptides, where Pro^5 had been deleted, showed activity several times higher than that of the parent pentapeptides. The tetrapeptide, H_2N-C(NH)-Tyr-D-Nle-Gly-Phe-NH_2, was found to be 7·95 times more active than morphine after IV administration (table 2).

An entirely new type of structural modification was also introduced by Bajusz *et al* (1980) who intended to get novel derivatives of their [D-Met^2]-, [D-Nle^2]- and [D-Ala^2]-enkephalins which would possess a high MVD/GPI potency ratio and thus retain the original enkephalinoid character of the parent peptides. They replaced the fifth amino acid of enkephalin by L- and D-α-aminopentanesulfonic- and α-aminopentanephosphonic acid residues and prepared a series of peptides containing Gly, D-Ala, D-Nle and D-Met at position 2. It could be demonstrated with the help of these peptides that replacement of the terminal –COOH group by $-SO_3H$ and $-PO_3H_2$ provided a nice method for getting carboxypeptidase resistant peptides in which the

acidic character and the L-configuration of the amino acid could be retained as such. The only compound of this series which exhibited analgesic activity after IV administration was Tyr-D-Met-Gly-Phe-D-NleP (table 2).

An important lead for the design of enkephalin analogues with potent morphinomimetic activity came from the work of Yamashiro *et al* (1977). These workers chose to incorporate L-thiazolidine-4-carboxylic acid (Thz) in place of Pro in [D-Met^2, Pro^5]-enkephalinamide on the basis of the favourable results obtained by such replacement of Pro in oxytocin and bradykinin. They also synthesized the corresponding D-Thr^2 analogue and thus obtained a peptide, [D-Thr^2, Thz^5]-enkephalin amide, which produced powerful and long lasting analgesia even after oral administration. The oral activity of this peptide is the highest recorded so far by any of the synthetic congeners of enkephalin.

Roemer *et al* (1977) also reported the activity of two highly potent analogues of enkephalin, Tyr-D-Ala-Gly-MePhe-Met(O)-ol (FK 33-824) and MeTyr-D-Ala-Gly-MePhe-Met(O)-ol. These workers introduced MeTyr and D-Ala residues at positions 1 and 2 respectively for increasing resistance against metabolic deactivation from the N-terminal end and converted the carboxy function at the C-terminus to an aliphatic –OH group. The peptides obtained thus showed enhanced analgesic activity. In a further step, they replaced Phe^4 by MePhe and prepared the sulfoxide derivatives of the peptides with a view to improve the absorption of the compounds from the gut and their passage through the blood-brain barrier. Both the sulfoxide derivatives turned out to be highly active even after oral administration (table 2). FK 33-824 was also selected for clinical trials. It produces effects similar to morphine against electrically induced pain in humans after intramuscular (IM) administration of 1 mg peptide. It has been found to reduce withdrawal symptoms in chronic heroin abusers though it is less effective than morphine. The toxic side effects of this peptide include a feeling of oppression in the chest and heaviness in the muscles (Gesellchen and Zimmerman 1981). Another peptide which has been reported to possess a high order of morphinomimetic activity is Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$-N(O)Me_2. It is seven times more active than morphine when administered by the IV route (Morley 1980).

A few analogues of Met-enkephalin possessing high morphinomimetic activity have also been synthesized recently in our laboratory. Earlier reports (Beddell *et al* 1977) had indicated that a decrease in the lipophilicity around the C-terminus of these pentapeptides was accompanied by a decrease in their opioid activity and a fall in their receptor binding affinity. Based on this finding we decided to synthesize a series of alkyl-amide derivatives of Met-enkephalin, since such a modification at the C-terminus would not only stabilise the pentapeptides against degradation by carboxypeptidases, but would also enhance the binding affinity of the molecules by increasing the hydrophobic environment at this end. In addition, the alkyl chains were also expected to increase the overall lipophilic character of the molecules and enable them to cross the blood-brain barrier. As revealed by the *in vitro* as well as *in vivo* tests, the *n*-propyl- and isopropyl amide derivatives of Met-enkephalin were the most active compounds of this series (table 1) (Raghubir *et al* 1979). In a further step a similar series of [D-Ala^2]-enkephalin-N-alkyl amides was synthesized and here again the *n*-propyl amide and the isopropylamide derivatives were found to be the most active *in vivo*. The *n*-propylamide was also found to be active after IP administration in mice (Mathur *et al* 1979). Highly potent analogues, which are even orally active, have recently been obtained by substituting Phe^4 with MePhe in [D-Ala^2]-enkephalin *n*-propyl- and isopropylamides

and converting them into corresponding sulfoxides (tables 1 and 2) (Raghubir *et al* 1982). Since the Phe residue at position 4 of enkephalins provides a hydrophobic site which is essential for receptor binding and recognition, the introduction of MePhe at this position was expected to check the cleavage of the third peptide bond by enkephalinase A and thereby help in the retention of this site intact in the peptide molecules. Moreover, such a modification was already known to enhance the binding affinity of the pentapeptides (Roemer *et al* 1977).

As shown in tables 1 and 2, although sulfoxide formation reduces the analgesic potency of the peptides, it improves their absorption from the gut and passage through the blood-brain barrier, thereby making them orally active. Detailed biological evaluation of these peptides is being carried out at present.

Thus, it may be noted that by incorporating a set of structural modifications, based on the leads generated in various laboratories, it has been possible to design and synthesize peptides which show analgesic potency several thousand times higher than that of the naturally occurring pentapeptides, enkephalins. Some of these which produce profound and long-lasting analgesia after administration by systemic routes may also find clinical acceptance in due course of time.

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