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Structure-activity relationships of methionine-enkephalin

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The amino acid sequence of methionine–enkephalin has been found to correspond to residues 61–65 of pituitary β-lipotropin (Hughes, Smith & others, 1975b). More recently, it has been shown that not only methionine–enkephalin but also the lipotropin fragments 61–68, 61–69, 61–76, 61–87, 61–89, 61–91 interact with the opiate receptors in brain homogenates, guinea-pig ileum or mouse vas deferens (Bradbury, Smyth & others, 1976; Cox, Goldstein & Li, 1976; Gráf, Rónai & others, 1976; Guillemin, Ling & Burgus, 1976; Waterfield, Hughes & Kosterlitz, unpublished observations).

$Lipotropin_{61-64}$ has only 3% of the potency of methionine–enkephalin to inhibit [^{3}H]dihydromorphine binding in brain homogenates (Bradbury & others, 1976) and only 1% of its potency to depress the electrically induced contractions of the guinea-pig ileum and mouse vas deferens (Table 1). Methionine–enkephalin, together with leucine–enkephalin (Hughes & others, 1975b), therefore appears to be the smallest peptide capable of interacting significantly with opiate receptors. Although it is not a potent antinociceptive agent, even after injection into the cerebral ventricles (Belluzzi, Grant & others, 1976; Feldberg & Smyth, 1976), its possible physiological significance is indicated by its ability to reduce the rate of spontaneous and evoked firing of neurons in the brain stem of the rat and cat (Bradley, Briggs & others, 1976; Gent & Wolstencroft, 1976) and the cortex, thalamus and medulla of the rat (Hill, Pepper & Mitchell, personal communication). Since this effect has a fast onset of action and declines rapidly after turning off the iontophoretic current, degradation to a much less potent tetrapeptide may play an important role in bringing about the termination of action after the release of methionine–enkephalin, and also of leucine–enkephalin. It was therefore important to study the structure-activity relationship of methionine–enkephalin.

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Table 1. *Structure-activity relationships of methionine–enkephalin.* The peptides have been synthesized by the solution method (Bower, Guest & Morgan, in preparation). The agonist activities of the compounds were determined on the mouse vas deferens (Hughes, Kosterlitz & Leslie, 1975a) and the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum (Kosterlitz & Watt, 1968; Kosterlitz, Lydon & Watt, 1970). None of the compounds showed antagonist activity in the guinea-pig ileum (dose ratio <2). The ID50 values of methionine–enkaphalin were 100 nM in the guinea-pig ileum and 12·5 nM in the mouse vas deferens.

Compound	Relative agonist activity (Methionine–enkephalin = 1) Mouse vas deferens	Guinea-pig ileum
Tyr–Gly–Gly–Phe–Met	1	1
Tyr–Gly–Gly–Phe–Gly	0·03	0·02
Tyr–Gly–Gly–Phe	0·01	0·01
Des–NH_2–Tyr–Gly–Gly–Phe–Met	0	0
Phe–Gly–Gly–Phe–Met	0·0003	0·002
Tyr–Gly–Gly–Tyr–Met	0·001	0·003

Removal of methionine at the C-terminus causes a loss of 99% of activity. Since replacement of methionine by glycine also decreases activity by 97–98% (Table 1), it would appear that an amino acid with a hydrophobic side chain at the C-terminus is essential for activity of the pentapeptide. This view is supported by the fact that replacement of methionine by leucine does not decrease the potency in the mouse vas deferens although activity is reduced by 60% in the guinea-pig ileum (Waterfield, Hughes & Kosterlitz, unpublished observations).

Another essential feature is the need for an intact

tyrosine residue at the N-terminus. The pentapeptide with des-NH_2-tyrosine in position 1 is inactive and replacement of tyrosine by phenylalanine reduces activity by more than 99% (Table 1). A surprising finding is the loss in activity when the phenylalanine residue at position 4 is replaced by tyrosine. A reduction in hydrophobic character is probably not responsible for an effect of this magnitude.

From these findings it would appear that modifications at either the N-terminus or the C-terminus would lead to a major loss of activity of methionine–enkephalin; rapid inactivation by enzymatic action has already been shown for natural enkephalin (Hughes, 1975). This biological lability and the associated rapid termination of action make methionine–enkephalin and leucine–enkephalin, the other component of natural enkephalin (Hughes & others, 1975b), good candidates for the role of neurotransmitters or neuromodulators. It has been shown (Feldberg & Smyth, 1976) that, when administered into the third cerebral ventricle, methionine–enkephalin is much less potent as an antinociceptive agent than lipotropin$_{61-91}$. This difference in activity may be at least partly explained by the fact that any degradation of the pentapeptides abolishes activity whereas removal of residues at the C-terminus of lipotropin$_{61-91}$ still leads to active peptides. On the other hand, lipotropin$_{61-91}$ may be protected against enzymatic degradation by its secondary structure; there is, however, so far no information available as to its metabolic fate.

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