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Morphine-like activities of synthetic enkephalin analogues

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Enkephalins are thought to be endogenous opiate-like substances derived from long chain polypeptides and are about 2 to 3 times more active than morphine in in vitro experiments. However, their actions to produce analgesia and to inhibit the guinea-pig ileum twitch response are of short duration because of their rapid degradation mainly by carboxypeptidase and aminopeptidase (Dupont et al 1977). Various attempts have been made to obtain enkephalin derivatives with much longer duration and more potent action by protecting the N- and C-terminals of the molecule (Bradbury et al 1977; Roemer et al 1977; Mathur et al 1979; Roemer & Pless 1979).

We have modified, both the amino and carboxy terminals of the enkephalin structure and in some instances, the glycine at position 2 was also replaced with D-alanine. The synthesis of the peptides has been presented elsewhere (Kiso et al 1979, 1980a, 1980b).

The morphine-like activities of several enkephalin analogues were compared with methionine enkephalin on the guinea-pig ileum twitch response caused by transmural stimulation at 0·1 Hz, 1 ms duration. For construction of dose-response curves, test peptides were added cumulatively to the organ bath. The morphine-like activities of the peptides were expressed as negative logarithms of the molar concentrations producing 50% inhibition of the twitch response. The results are summarized in Table 1.

Conversion of methionine in the enkephalin structure to the corresponding methioninol (Tyr–Gly–Gly–Phe-Metol, Metol: methioninol residue) did not increase the morphine-like activity on the guinea-pig ileum although this modification has been shown to increase the analgesic activity (Roemer & Pless 1979) by preventing the proteolysis of 4th peptide bond. The combination of the amino-terminal methylation (MeTyr) and carboxy-terminal modification to methioninol produced a surprisingly potent enkephalin analogue, MeTyr–Gly–Gly–Phe–Metol (Me-enk-ol), about 66 times more potent than the original methionine enkephalin. The pD_2 value increased from 7·94 to 9·21.

On the other hand, methylation of the phenolic hydroxy moiety in the tyrosine of Me-enk-ol resulted in a dramatic decrease of morphine-like activity. The pD_2 value was 6·15. This suggests that the hydroxy group of tyrosine plays an important role in enkephalin activity and may be involved in binding to the opiate receptors.

To protect the peptide bond against the action of peptidases and enhance the affinity to the opiate receptors (Pert et al 1976), the glycine at position 2 was replaced by D-alanine. In the case of Tyr–Gly–Gly–Pheol (Pheol: L-phenylalaninol residue), this modification (Tyr–D–Ala–Gly–Pheol) resulted in an increase in activity of about 60 times. The pD_2 value increased from 5·76 to 7·53. This suggests that methionine at position 5 is not always necessary for activity since Tyr–D–Ala–Gly–Pheol and methionine enkephalin were approximately equipotent. However, in the case of MeTyr–Gly–Gly–Phe–Metol (pD_2 = 9·21) this modification did not show any increase in activity (MeTyr–D–Ala–Gly–Phe–Metol, pD_2 = 8·27), despite the fact that in Tyr–Gly–Gly–Phe–Metol this modification resulted in increase in analgesic activity (Roemer & Pless 1979).

To confirm the morphine-like specificity of these derivatives, antagonism by naloxone was tested. Inhibitions produced by enkephalin derivatives were antagonized by naloxone in concentrations of 10^{-8} M to 10^{-7} M. The mean pA_2 values of naloxone for Me-enk-ol and for MeTyr–D–Ala–Gly–Phe–Metol were 9·40 ± 0·38 and 9·21 ± 0·18, respectively, whereas those for methionine enkephalin and morphine were 9·13 ± 0·04 and 9·14 ± 0·09, respectively. When the twitch response was completely abolished by treatment with 10^{-8} M Me-enk-ol or MeTyr–D–Ala–Gly–Phe–Metol, addition of naloxone at 10^{-7} M readily restored the response to almost the same level as before opiates.

The analgesic activities of Me-enk-ol and MeTyr–D–Ala–Gly–Phe–Metol were assessed in the rat by the hot

Table 1. Morphine-like activity of enkephalin derivatives on guinea-pig isolated ileum twitch response. Values are expressed as negative logarithms of ED50 values. Values (means ± s.e.) were obtained in ilea from 20 animals. Metol: L-methioninol residue, Pheol: L-phenylalaninol residue.

	pD_2	Relative potency
Morphine	7·39 ± 0·06	1·00
Tyr–Gly–Gly–Phe–Met	7·49 ± 0·11	1·26
MeTyr–Gly–Gly–Phe–Met	7·90 ± 0·11	3·24
Tyr–Gly–Gly–Phe–Metol	7·37 ± 0·13	0·96
MeTyr–Gly–Gly–Phe–Metol	9·21 ± 0·07	66·07
MeTyr(Me)–Gly–Gly–Phe–Metol	6·15 ± 0·03	0·06
MeTyr–D–Ala–Gly–Phe–Metol	8·27 ± 0·12	7·59
Tyr–Gly–Gly–Pheol	5·67 ± 0·14	0·02
Tyr–D–Ala Gly Pheol	7·53 ± 0·12	1·39

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plate test. Male rats, about 250 g, were used in groups of 5. All were conditioned to the hot plate (60 °C) by more than four exposures. Those jumping from the hot plate after 10 s or before 2 s were not used. The opiates were administered by injection into the cerebroventricles and an equal amount of 0·9% NaCl solution was also given to the control group. The reaction time on the hot plate was determined at various times up to 150 min after the injection to assess the analgesic activity.

Me-enk-ol at a dose of 0·05 mg kg^{-1} gave a peak analgesia (20 s) between 20 and 40 min falling away to a 10 s response at 90 min. The peak analgesia for morphine, 0·1 mg kg^{-1}, was observed between 10 and 120 min with a falling away to 17 s at 150 min. Replacement of the glycine at position 2 of Me-enk-ol with D-alanine increased the analgesic activity. MeTyr-D-Ala-Gly-Phe-Metol which is about 1/5 as active as Me-enk-ol in inhibiting the guinea-pig ileum twitch response was more potent than morphine and Me-enk-ol in the analgesic activity. The peak effect persisted for over 150 min at a dose of 0·05 mg kg^{-1} with a fall to 17 s at 170 min. Besides analgesia, catalepsy was seen after treatment with these peptides.

From the present experiments, it is concluded that *N*-methylation of the tyrosine residue and conversion of the methionine residue of methionine enkephalin to methioninol dramatically increased morphine-like activity in vitro. The results also indicate that replacement of the glycine at position 2 of Me-enk-ol with D-alanine increased the analgesic activity.

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Effect of cocaine on contractile responses to noradrenaline of canine isolated mesenteric vein

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The canine anterior mesenteric vein has an inner circular band of muscle and an outer layer of longitudinal muscle bundles (O'Connor & Slater 1972). A two-layer mesenteric vein occurs in other mammals (Loizou & Tindall 1969). It is possible to record the contractions of the muscle layers using ring, helical and longitudinal vein preparations (Sutter 1965; McConnell & Roddie 1970) but these inevitably disrupt the normal relation between the two layers. The contractions of two venous muscle groups can be measured independently by perfusing a vein segment and recording its longitudinal tension. The perfusion pressure monitors the circular muscle contractions and the isometric tension measures the longitudinal muscle contractions (Williamson 1969; Hall & O'Connor 1973).

The contractile activity of noradrenaline in isolated vascular tissue is affected by its uptake into sympathetic nerve terminals (de la Lande et al 1967). Application of noradrenaline (NA) to the intimal and adventitial surfaces often results in differences in the contractile response (de la Lande et al 1966, 1967; Crotty et al 1969).

This paper describes a method for adding noradrenaline to either surface of the canine mesenteric vein whilst recording the contractions of the circular and longitudinal muscles. Veins treated with cocaine were used to determine the extent to which neuronal uptake of noradrenaline affects the response of the two muscle layers.

A 5–8 cm segment of anterior mesenteric vein was removed from mongrel dogs of either sex, 10–20 kg, after death, the tributaries tied off, and all excess tissue was removed. Two circular tissue-baths, one inside the other, within a water-filled jacket at 37 °C, were used and the vein was mounted vertically in the inner bath. A fixed cannula at the base of the bath was inserted into the proximal end of the vein. An inverted

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