Enkephalin Antisense Peptides: Design, Synthesis, and Biological Activity[1]

Pravin K. Misra,[2] Wahajul Haq,[2] Seturam B. Katti,[2,4] Krishna B. Mathur,[2] Ram Raghubir,[3] Gyanendra K. Patnaik,[3] and Bhola N. Dhawan[3]

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The antisense mRNA complementary to the sense strand of Met-enkephalin encodes the antisense peptides, His-Glu-Ala-Pro-Ile (compound 88/62). The antisense peptide and its (Gln^1)-analogue (compound 88/63) have synergestic effects on the opioid activity of Met-enkephalin in the GPI test system.

KEY WORDS: antisense peptide; Met-enkephalin; opioid activity.

INTRODUCTION

Blalock and Smith (1) observed an interesting coding pattern of genes in which codons for hydrophobic amino acids on one strand of DNA are complemented by codons for hydrophilic amino acids on the other strand, and vice versa. Subsequently, they demonstrated specific interactions between complementary peptides in the case of the ACTH hormone. Two antisense peptides corresponding to the ACTH 1-24 fragment translated in the same reading frame from 5′ → 3′ and also 3′ → 5′ directions were found to interact with the sense peptide fragment. The specificity of interaction has been shown by the microtiter plate assay method (2,3). This idea has been independently tested by Shai *et al.* (4) using a ribonuclease S-peptide immobilized affinity column. The antisense peptide to the ribonuclease S-region was considerably retarded on the corresponding sense peptide affinity column, suggesting specific interaction between complementary peptides. More recently mild type I antagonist activity for angiotensin II antisense peptide has been reported using a rat uterus assay method (5). However, some studies employing NMR and CD techniques have failed to detect direct interaction between complementary peptides (6). With this in mind, we undertook synthesis of antisense peptides His–Glu–Ala–Pro–Ile (compound 88/62) and Gln–Glu–Ala–Pro–Ile (compound 88/63) to enkephalins and examined their opioid activity.

MATERIALS AND METHODS

Synthesis of Peptides. The peptides have been synthesized by the solution phase stepwise coupling employing DCC/HOBt method. Their homogeneity was checked by reverse-phase HPLC (Waters U.S.A., RP-18 column) using isocratic elution (methanol:water:trifluoroacetic acid (600:400:0.5; flow rate, 1.0 mL/min). All the peptides were found to be more than 98% pure and were characterized by FDMS, NMR, and elemental analyses. The following peptides were synthesized in the present study:

1. Tyr–Gly–Gly–Phe–Met (Met-enkephalin),
2. His–Glu–Ala–Pro–Ile (compound 88/62),
3. Gln–Glu–Ala–Pro–Ile (compound 88/63), and
4. Gly–Gly–Gly–Lys–Ala (compound 89/492).

Isolated Tissue Assay. The guinea pig ileum (GPI) myenteric plexus-longitudinal muscle assay was carried out according to the method described in the literature (7). Graded concentrations of test compounds or predetermined IC_{50} dose of Met-enkephalin and test compounds were added to the organ bath. The percentage inhibition of GPI contraction for each dose was calculated and EC values were calculated by a log plot.

Analgesic Activity. The analgesic activity was measured by the hot-plate method (8). The test compounds, Met-enkephalin, or the combination of Met-enkephalin and test compounds were administered intracerebrally in graded doses to groups of 10 mice each. The response to noxious heat (55 ± 0.5°C) was determined every 5 min after the drug administration, till the basal reaction time was restored. The percentage of animals showing analgesia at each dose level was calculated. Naloxone was used to check the opioid specificity of test compounds in both the assay methods.

In Vitro *Receptor Binding.* Receptor binding studies were carried out using specific radio ligand for each receptor subtype (μ, δ, and κ) and the concentration required to produce 50% inhibition of labeled ligand binding was calculated. These tests were performed by the National Institute of Drug Abuse, Rockville, MD.

RESULTS

The IC_{50} value of Met-enkephalin in the GPI assay was found to be 8.7×10^{-8} *M*, whereas the test compounds did not inhibit electrically induced contractions of GPI at concentrations up to 3×10^{-5} *M*. However, both the compounds produced a concentration-dependent potentiation of Met-enkephalin activity in inhibiting the electrically induced contractions of a longitudinal muscle preparation of GPI (Fig. 1). The concentrations required to produce 25, 50, and 75% potentiation of the Met-enkephalin response in the GPI assay were 1.52×10^{-7}, 3.19×10^{-7}, and 1.32×10^{-6} *M*, respectively, for compound 88/62 (SE, 85 ± 7.6). For compound 88/63 these values were 2.9×10^{-7}, 1.64×10^{-6}, and 2.95×10^{-6} *M* (SE, 80 ± 10.4), respectively. No such effect was observed with peptide 89/492, having the sequence Gly–Gly–Gly–Lys–Ala, which served as control in all our experiments. Further, the opioid activity of enkephalin was reversed by naloxone, and similar results were obtained in the presence of antisense peptides. The concentration of naloxone used in all the experiments was 0.2 μg/mL.

In the hot-plate analgesia test the ED_{50} of Met-enkephalin was 14 ± 1.3 μg by the intracerebral route in mice. Compound 88/62 did not produce analgesia at low

[1] CDRI Communication No. 4765.

[2] Division of Biopolymers, Central Drug Research Institute, Lucknow 226 001, India.

[3] Division of Pharmacology, Central Drug Research Institute, Lucknow 226 001, India.

[4] To whom correspondence should be addressed.

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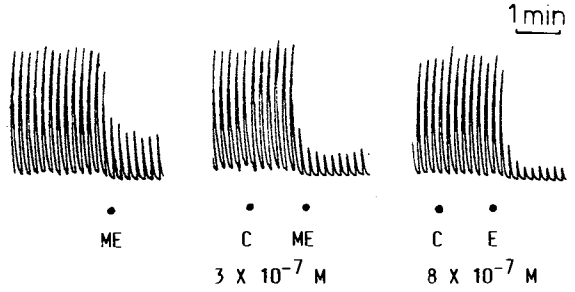

Fig. 1. Concentration-dependent potentiating effect of compound 88/62 (C) on the inhibitory effect of Met-enkephalin (ME; 8×10^{-8} *M*) on electrically induced contractions of guinea pig ileum. E, enkephalin.

doses, whereas 100 μg caused analgesia in 40% of the animals. This effect was antagonized by naloxone (1.0 mg/kg), suggesting involvement of opioid receptors. In contrast, compound 88/63 did not produce analgesia even at the 100-μg dose level, and compound 89/492 thus did not elicit any biological response either *in vivo* or *in vitro* at equal doses. Moreover, pretreatment of animals with various doses of either compounds 88/62 or 88/63 did not affect Met-enkephalin induced analgesia. *In vitro* opioid receptor binding studies showed that the test compounds 88/62, 88/63, and 89/492 have IC_{50} values of more than 1.0 ± 10^{-5} *M*.

DISCUSSION

The complete nucleotide sequence of the gene coding for human pre-proenkephalin has been reported (9). The amino acid sequence of antisense peptide (compound 88/62) was obtained by translating antisense mRNA in the 5′ → 3′ direction in the same reading frame as that of sense mRNA coding for Met-enkephalin.

Met-enkephalin	H–Tyr–Gly–Gly–Phe–Met–OH
+mRNA	5′–UAU–GGG–GGC–UUC–AUG–3′
−mRNA	3′–AUA–CCC–CCG–AAG–UAC–5′
Compound 88/62	HO–Ile–Pro–Ala–Glu–His–H
Compound 88/63	HO–Ile–Pro–Ala–Glu–Gln–H

The Gln^1 analogue (88/63), which is considered to represent the antisense peptide to Leu-enkephalin, was obtained by replacing the codon for leucine by its complementary codon in the above sequence. Although Leu-enkephalin has a different codon, only the leucine codon was replaced so that its antisense peptide (compound 88/63) could be obtained with a single amino acid change His^1 — Gln^1 without changing the overall hydropathy of both the antisense peptides (10). The sequence of control peptide is not related to the coding region of the enkephalin gene.

The data shown in Fig. 1 demonstrate that the antisense peptides produce dose-dependent potentiation of Met-enkephalin response in GPI assay. Reversal of enkephalin activity by naloxone in the presence of test compounds suggests that the response involves opioid receptor. This result is supported by *in vivo* experiments wherein weak analgesic activity of compound 88/62 was inhibited by naloxone. The control peptide, however, does not affect the activity of Met-enkephalin. Results obtained from receptor binding studies rule out the possibility of interaction between the receptor binding site and complementary peptides. Therefore, the mechanism of potentiation of ligand activity could be due to direct interaction between complementary peptides. Furthermore, 2D NOESY studies revealed an additional cross peak due to NOE of the methionine NH with the Cα–H– of alanine in compound 88/62, thereby suggesting some interaction between the two peptides (unpublished data). Our results are opposite to the observation by Johnson and Toress (11) that an arginine vasopressin (AVP) complementary peptide binds AVP and converts it into an antagonist of its own action. At this stage it is difficult to explain these two types of activity profiles.

This is the first report of a complementary peptide enhancing the activity of a ligand. The results presented not only support molecular recognition theory but expand its scope to include enhancement of agonist activity.

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