COMPARISON OF THE ANALGESIC PROPERTIES OF LIPOTROPIN C-FRAGMENT AND STABILIZED ENKEPHALINS IN THE RAT

A.F. Bradbury, D.G. Smyth, C.R. Snell,
J.F.W. Deakin and S. Wendlandt

Laboratory of Peptide Chemistry and Division of
Physiology and Pharmacology
National Institute for Medical Research,
Mill Hill, London NW7 1AA

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SUMMARY

The C-Fragment of lipotropin (LPH 61-91) was shown to produce strong and long-lasting analgesia on intraventricular infusion in the rat. Its analgesic properties were compared with those of three synthetic derivatives of methionine enkephalin (LPH 61-65) which had been stabilized against enzymic degradation by blocking one or both termini with N-methyl and C-amide groups. C-Fragment was approximately 50 times more potent on a molar basis than N-methyl methionine enkephalin amide. The singly blocked pentapeptides, like methionine enkephalin, produced little more than transient analgesia. It was concluded that the analgesic properties of C-Fragment depend on the length and nature of the peptide chain rather than on the resistance of its N-terminal pentapeptide to degradation.

INTRODUCTION

The N-terminal pentapeptide of lipotropin C-Fragment, known as methionine enkephalin (met-E, LPH 61-65), has been isolated from brain and found to behave like morphine in peripheral assays.[1] However, the analgesic action of met-E is very much weaker than that of morphine: injected into the cerebral ventricles of the cat[2], rat[3] or mouse[4], it produces at most a weak and transient effect. In contrast the C-Fragment of lipotropin (LPH 61-91), which was isolated from pituitary[5,6] and later found in brain[7],

is about 100 times more potent than morphine: when injected into the third ventricle of the cat C-Fragment produces profound analgesia lasting several hours[2].

It has been suggested that the inability of met-E to produce significant analgesia might be related to its susceptibility to destruction by brain enzymes[8] but it could be that the difference in potency between met-E and C-Fragment is simply a reflection of their affinities for brain opiate receptors. Indeed, C-Fragment displaces naloxone from the brain receptors in vitro about 30 times more strongly than met-E[9]. The results of the present communication show that the slight analgesic properties of met-E are greatly enhanced in C-Fragment by the sequence of amino acids that extends from its N-terminal pentapeptide. In addition, we report the synthesis and analgesic properties of a derivative of met-E which is protected against enzymic destruction; the analgesic effects produced by this derivative last longer than the effects of met-E.

MATERIALS AND METHODS

The protected pentapeptides, t-BOC* N-methyl O-benzyl tyrosylglycylglycylphenylalanylmethionine (t-BOC N-methyl met-E) and t-BOC O-benzyl tyrosylglycylglycyl-phenylalanylmethionine (t-BOC met-E) were synthesized by the solid phase method[10] on a Beckman 990 peptide synthesizer. Removal from the solid support and deprotection were carried out by exposure to hydrogen

*Abbreviation: t-BOC = t-butyloxycarbonyl

bromide in trifluoracetic acid in the case of met-E and N-methyl met-E, and by ammonolysis in dimethylformamide and ethanol followed by treatment with hydrogen bromide in acetic acid for the corresponding amides. The peptides were purified by gel filtration on Sephadex G-15 in 50% acetic acid and were chromatographed on SP Sephadex C25; where appropriate further chromatography was performed on DEAE Sephadex A25. Purity was established by amino acid analysis and by digestion with aminopeptidase M and carboxypeptidase A.

Testing for analgesic effects in the rat was carried out by the hot plate method[11] with the temperature maintained at 55.5°C ± 0.5°C. Hind paw lick latency was recorded together with the latency of the first sign of any reaction of pain, usually flicking of the hind paws or licking of forepaws. If no reaction was observed within 20 sec, the animal was removed from the hot plate. Three baseline tests preceeded each drug administration. Drugs were injected in 10 µl of saline vehicle into the lateral ventricle via chronically implanted cannulae. With N-methyl met-E the poor solubility necessitated the use of a larger injection volume (20 µl); in a control experiment the same volume of saline vehicle injected either before or after the peptide was without effect. Latencies were recorded at five minute intervals in the tests of the C-Fragment and, to ensure detection of transient effects, at intervals of three minutes for the synthetic peptides. Tests were repeated until baseline latencies returned. Analgesia was defined as a hind paw lick latency of greater than 20 sec or twice the baseline latency. The other reactions to pain were also increased in latency in animals showing analgesia. It was observed that hind paw flicking disappeared more slowly and reappeared more quickly than hind paw licking, perhaps reflecting differences in actions on pain reflexes mediated at different levels of the nervous system. Analgesia was usually but not always associated with catatonia.

RESULTS AND DISCUSSION

A series of experiments was carried out at two dose levels (Table 1 and Fig.1). At the low dose, met-E amide did not induce analgesia while the N-methyl met-E produced a fleeting effect lasting about 15 min with minimal signs of catatonia in one animal. The N-methyl

Table 1. Duration of analgesia as assessed by the hot plate method following intraventricular administration of met-E, synthetic derivatives of met-E and C-Fragment in rats

	Low dose			high dose		
	No. of rats	Dose μmole	Mean duration min ± S.E.	No. of rats	Dose μmole	Mean duration min ± S.E.
Met-E				1	0.17	10
Met-E amide	3	0.05	0	2	0.21	27 ± 2.1
N-Methyl Met-E	4	0.06	14.8 ± 2.8		Insoluble	
N-Methyl Met-E amide	10	0.05	33.1 ± 6.1	3	0.17	106 ± 25.7
C-Fragment	6	0.0015	27.3 ± 11.1	6	0.003	100 ± 16.3
Lipotropin	1	0.003	0	2	0.015	0

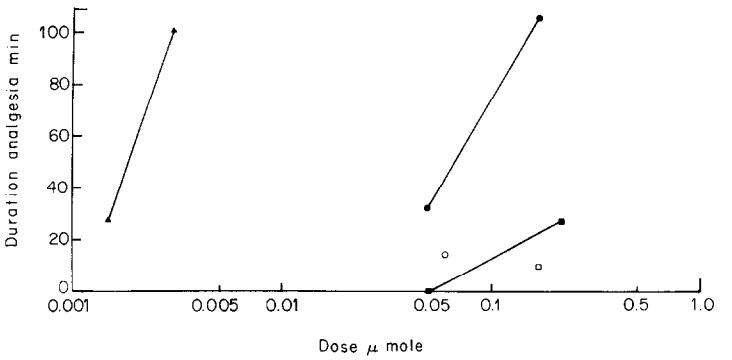

Fig. 1. ▲——▲ C-Fragment, ●——● N-methyl met-E amide, O N-methyl met-E, ■——■ met-E amide, □ met-E.
For C-Fragment and the N-methyl met-E amide, half the animals had received another dose of the same drug or morphine more than six days previously. The response of these animals did not differ from naive animals. Animals showing no response to an injection were tested with an effective dose of morphine or protected pentapeptide to eliminate negative results due to faulty cannulation.

met-E amide produced analgesia and catatonia lasting about 30 min. At the high dose, the analgesic action of the N-methyl met-E amide persisted for more than 100 min and was accompanied by marked catatonia. The effects of a similar dose of met-E amide, on the other hand, were detectable for less than 30 min, with minimal catatonia. Met-E (100 μg, 0.17 μmole) gave in one animal a transient analgesic effect of 10 min duration with no catatonia.

The C-Fragment was able to produce analgesia and catatonia at much lower doses than any of the penta-peptides (Fig.1) whereas the parent polypeptide lipotropin

showed no analgesic action. Both the analgesic effects and catatonia produced by C-Fragment or by N-methyl met-E amide were rapidly and completely reversed by intraperitoneal injection of naloxone (1 mg/kg).

The results show that modification of met-E can lead to increase in the duration of analgesic action. This may be related in part to increase in affinity for the brain opiate receptors since the modified peptides appear to bind *in vitro* a little more strongly than met-E[12]. However, the most prolonged analgesia was produced by N-methyl met-E amide, though its affinity is no greater than that of the pentapeptide amide. Thus protection at the N-terminus of met-E prolongs the analgesic action.

It is important to note that C-Fragment has an analgesic potency in the rat about 50 times greater than that of met-E, even when both termini of the pentapeptide are protected against enzymic degradation. It is clear, therefore, that the analgesic properties of C-Fragment depend on the length and nature of the peptide chain rather than on the resistance of its N-terminal pentapeptide to degradation.

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