Vol. 85, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 29, 1978 Pages 795-800

β-ENDORPHIN: COMPLETE PRIMARY STRUCTURE IS REQUIRED FOR FULL ANALGESIC ACTIVITY

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Received October 23, 1978

SUMMARY: The synthesis of β_h-endorphin-(1-30) has been accomplished by the solid-phase procedure and its analgesic potency was assayed by the tail-flick method. Results showed that the synthetic analog had only 56% activity of the parent molecule. Thus, the complete sequence of 31 amino acid residues in β_h-EP is required for full analgesic activity.

Among various fragments [met-enkephalin, β-LPH-(61-69), β-LPH-(61-76), β-LPH-(61-91)] of β-lipotropin (1, 2) having morphine-like activity, only β-endorphin [β-LPH-(61-91)] exhibits potent analgesic activity by intravenous injection (3). In addition, it is the most active peptide when administered directly into the brain (4). In preliminary clinical studies, synthetic β_h-EP (5) exhibited beneficial effects in patients with severe pain (6), schizophrenic behavior and deep depression (7) and narcotic abstinence (8, 9). It is essential to establish the minimal structure of β_h-EP (Fig. 1) for full biological activity. In this communication, we report that the complete amino acid sequence is required for full analgesic activity of β_h-EP.

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Abbreviations: β_h-EP, human β-endorphin; β-LPH, β-lipotropin; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid.

0006-291X/78/0852-0795$01.00/0
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                    5                   10
H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
                   15                   20
  Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
                   25                   31
  Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
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Figure 1. Amino acid sequence of β_h-EP

MATERIALS AND METHODS

Solid phase synthesis (10) was performed on Boc-Gly polymer (0.55 g, 0.25 mmol) in a Beckman model 990 peptide synthesizer. Side-chain protection and coupling was performed as described previously for the synthesis of β_h-EP (5) except that Z protection was used for the side chain of Tyr in position 1 (11). The schedule for synthesis followed that described previously (12), with the following exceptions: (1) in steps 7 and 9, 5% DIEA/CH_2Cl_2 was used; (2) steps 12 and 13 were deleted; (3) in step 16, 0.17 mmol of DIEA in CH_2Cl_2 was delivered. The schedule for symmetrical anhydride coupling was operated in the fully automatic mode by storage of the anhydrides in an ice-bath. The Boc group of the Tyr-1 residue was removed with TFA (13). The yield of protected β_h-EP-(1-30) polymer was 1.729 g. Cleavage and deprotection in HF (14), gel filtration on Sephadex G-10 (0.5 N acetic acid), and chromatography on carboxymethylcellulose were performed as described previously on Sephadex G-50 (15) in 1-butanol:pyridine:acetic acid:water (40:1:10:50) in a 2.21 x 62.2 cm column. This solvent system has been found to be useful for β-EP analogs which have low solubility in the standard system (R. Houghten, unpublished observations). Even in this system, the β_h-EP-(1-30) had limited solubility and chromatography of 107 mg had to be performed in 3 batches, R_f 0.40. The yield was 35.3 mg from 434 mg of protected peptide resin (ca. 14% overall yield based on starting resin); tlc (50 µg), R_f 0.57, single spot. Paper electrophoresis (50 µg samples) at pH 3.7 for 5 h and at pH 6.7 for 6 h, both at 400 V, each gave a single spot (ninhydrin) with R_f^{Lys} of 0.60 and 0.41, respectively. Amino acid analyses of 24-h HCl hydrolysate and a total enzymic hydrolysate are shown in Table 1. Synthetic β_h-EP-(1-15), -(1-21), -(1-26), -(1-28), -(1-29) were obtained as described by Yeung et al., (16).

The analgesic activity of synthetic peptides was assessed in mice by the tail-flick method (17). Male ICR mice weighing 25-28 gm were used (Simonsen Lab, Gilroy, CA). The peptide was dissolved in sterilized saline and injected intracerebroventricularly in a volume of 5 ml according to the method described by Haley and McCormick (18). To evaluate the tail-flick response, a control latency (T_0) was obtained from the mean of two latency determinations. The T_0 value was usually 2-3 sec. Mice with a control latency of more than 3.5 sec were not used for the study. The test latencies (T_1) were determined at 5, 10, 20, 30, 60, 90 and 120 min after the injection of the peptide for each animal. Percent analgesia was calculated as $[(T_1-T_0)/(T_2-T_0)] \times 100$,

Table 1

Amino Acid Analyses of Synthetic β_h-EP-(1-30)[a]

Acid Hydrolysate[b]		Enzyme Hydrolysate[d]	
Amino Acid	β_h-EP(1-30)	Amino Acid	β_h-EP(1-30)
Lys	5.07(5)	Lys	4.95(5)
Asp	1.98(2)	Asp	0.26(0)
Thr	2.72(3)	Thr+Ser	
Ser	1.90(2)	+Asn+Gln	7.35(8)
Glu	2.05(2)	Glu	1.18(1)
Pro	0.93(1)	Pro	1.00(1)
Gly	3.04(3)	Gly	3.16(3)
Ala	2.03(2)	Ala	2.12(2)
Val	1.01(1)	Val	1.11(1)
Met	0.94(1)	Met	0.94(1)
Ile	1.21(2)[c]	Ile	1.93(2)
Leu	2.16(2)	Leu	2.25(2)
Tyr	1.78(2)	Tyr	1.98(2)
Phe	1.92(2)	Phe	1.92(2)

[a]Theoretical values in parentheses

[b]6 M HCl

[c]Low values accounted for by the acid resistant Ile-Ile moiety

[d]For detail, see (5)

where the cut-off time (T_2) was 7 sec. Mice were considered to be analgesic if the percent analgesia was 50% or more, observed at 10, 20 or 30 min after injection. Groups of at least 8 mice were injected with different doses of the peptide. The median antinociceptive dose (AD_{50}) and 95% confidence limits were calculated according to Litchfield and Wilcoxon (19).

RESULTS AND DISCUSSION

β_h-EP caused a dose-dependent inhibition in intensity and duration of tail-flick response when administered in doses of 0.05-0.43 µg per mouse. The AD_{50} of β_h-EP was calculated to be 0.15 µg per mouse. As shown in Figure 2 and Table 2, the removal of one amino acid at a time starting with the COOH-terminus

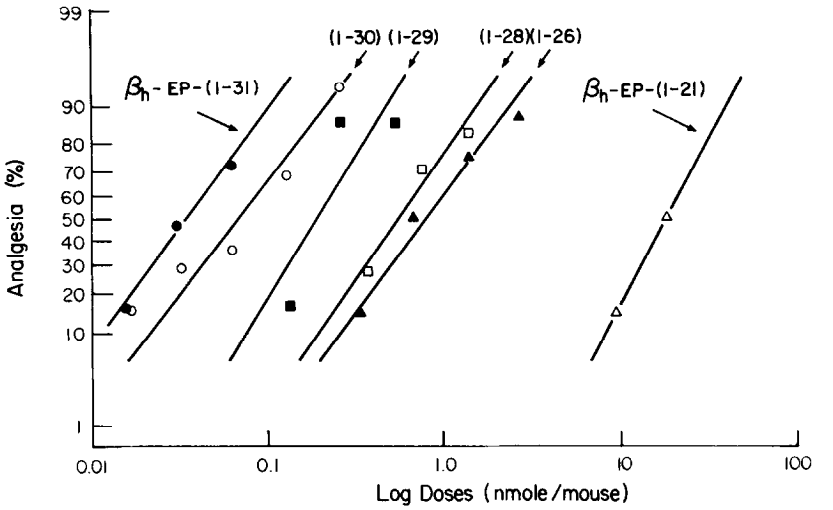

Figure 2. Log dose-response curves for antinociceptive effect produced by intracerebroventricular injection of β_h-EP and its fragments. After control latency was obtained (before drug injection) groups of 8-16 mice were injected with various doses of β_h-EP or its fragments, and the tail-flick response was determined at 10, 20 and 30 min after injection. Mice were considered analgesic if the percent analgesia was 50% or more (see text for calculation).

Table 2

Median Antinociceptive Dose (AD_{50}) of β-Endorphin and its Fragments after Intracerebroventricular Injection in Mice

Synthetic peptides	AD_{50} (μg/mouse)	Relative potency[a]
β_h-Endorphin (β_h-EP)	0.15 (0.12-0.20)[b]	100
β_h-EP-(1-30)	0.20 (0.15-0.43)	71.8
β_h-EP-(1-29)	0.70 (0.39-1.26)	20.1
β_h-EP-(1-28)	2.13 (0.97-4.68)	6.4
β_h-EP-(1-26)	1.57 (0.92-2.70)	8.0
β_h-EP-(1-21)	38.25 (22.24-65.79)	0.3
β_h-EP-(1-15)	>85	<0.1

[a]The potency ratios of the peptides are compared with β-EP on molar base

[b]95% confidence limits in parentheses

of β_h-EP reduced the potency of analgesic activity of the β_h-EP in a stepwise fashion. Thus, removal of COOH-terminus -Glu reduced analgesic activity by 28%, removal of -Gly-Glu reduced analgesic activity by 80%, and removal of -Lys-Gly-Glu reduced analgesic activity by 94%.

Geisow et al., (20) reported that analgesic activity of β-EP depended on the carboxyl terminal tetrapeptide; removal of the terminal tetrapeptide caused a severe loss of potency. Our results indicated that all 31 amino acid residues are essential for the expression of full analgesic activity. It is possible that deletion of one or more amino acid residues in positions 2 to 30 of the β_h-EP structure may not diminish the analgesic activity. Studies of this possibility are being investigated.

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