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β-ENDORPHIN: REPLACEMENT OF GLUTAMIC ACID IN POSITION 8 BY GLUTAMINE INCREASES ANALGESIC POTENCY AND OPIATE RECEPTOR-BINDING ACTIVITY

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SUMMARY

A β-endorphin analog with replacement of glutamic acid in position 8 by glutamine has been synthesized by modified procedures of the solid-phase method. The analgesic potency of the synthetic analog was increased to nearly three-fold with a concomitant increase of opiate receptor-binding activity in neuroblastoma x glioma hybrid cells. This is the first instance in which a replacement of a single amino acid causes an increase of analgesic potency of β-endorphin.

β-Endorphin (1) is a potent analgesic agent by intracerebral (2) or intravenous (3) injections. So far, it has not been possible to obtain a synthetic β-EP analog with an analgesic potency more than twice that of the parent molecule (4). We report here a nearly 3-fold increase in the analgesic potency of β_h-EP (see Fig. 1) by replacement of a single amino acid glutamic acid in position 8 with a concomitant increase in

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Abbreviations: β_h-EP, human β-endorphin; Z, benzyloxycarbonyl; Bzl, benzyl; Boc, tert-butyloxycarbonyl; tlc, thin-layer chromatography

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opiate receptor-binding activity. In addition, immunoreactivity of the synthetic analog decreases as revealed in the β_h-EP radioimmunoassay system.

MATERIALS AND METHODS

Synthesis of [Gln^8]-β_h-EP was carried out by modified procedures of the solid-phase method (5) as previously described (6). It was performed on Boc-Glu(Bzl)-brominated polymer (163 mg, 52 μmole) with side-chain protection as follows: 2-Clz for Lys (7); 2-BrZ for Tyr-27 (8) and Z for Tyr-1 (9); Bzl for Thr and Ser. The completed protected peptide resin (459 mg) was treated with liquid HF (10) and the product isolated by gel filtration on Sephadex G-10 (0.5 N acetic acid) and chromatographed on carboxymethylcellulose (6). Final purification was effected by partition chromatography on Sephadex G-50 in a 2.21 x 58 cm column in the solvent system 1-butanol:pyridine: 0.1% aqueous acetic acid (5:3:10) (11). The product eluted with R_f 0.25 and its yield was 84 mg (81% peptide content by spectral analysis; 38% overall yield based on starting resin); tlc (1-butanol:pyridine:acetic acid:water, 5:5:1:4) on 50 μg gave a single spot with R_f 0.65 by ninhydrin and Cl_2-toluidine detection; paper electrophoresis on 50 μg samples at pH 3.7 (pyridine acetate) and pH 6.7 (γ-collidine acetate) gave a single spot in each case with R_f^{Lys} of 0.60 and 0.45, respectively (375 V, 4-5 hr, ninhydrin and Cl_2-toluidine detection). Amino acid analysis of a 24-hr HCl hydrolysate gave (theoretical values in parenthesis): Lys, 4.97 (5); Asp, 1.98 (2); Thr, 2.87 (3); Ser, 1.63 (2); Glu, 3.00 (3); Pro, 1.05 (1); Gly, 3.12 (3); Ala, 1.99 (2); Val, 0.99 (1); Met, 1.04 (1); Ile, 1.34 (2); Leu, 2.06 (2); Tyr, 1.94 (2); Phe, 2.02 (2). Low value for Ile is accounted for by the acid resistant Ile-Ile sequence. Amino acid analysis of a total enzyme digest (trypsin and chymotrypsin followed by leucine aminopeptidase M) gave: Lys, 5.08 (5); Asp, 0.18 (0); Thr + Ser + Asn + Gln, 8.65 (9); Glu, 1.04 (1); Pro, 0.95 (1); Gly, 2.93 (3); Ala, 1.92 (2); Val, 1.12 (1); Met, 0.93 (1); Ile, 1.79 (2); Leu, 2.05 (2); Tyr, 2.18 (2); Phe, 2.20 (2).

The analgesic potency was estimated in mice by the tail-flick method (12) as described (2,3). The opiate receptor binding assay was carried out with rat brain membrane preparation (13) or neuroblastoma x glioma hydrid cell NG108-15 (14) as described using [$^3H_2$-Tyr^{27}]-β_h-EP (15) as the primary ligand and synthetic β_h-EP (6) as standard competing ligand. Immunoreactivity was assessed by radioimmunoassay using the published procedure (16,17).

RESULTS AND DISCUSSION

Biological activity of [Gln^8]-β_h-EP as assayed by various procedures is summarized in Table 1. Figure 2 presents the analgesic potency of the analog as assayed by the tail-flick test in mice. In comparison with the parent molecule replace-

Table 1

Biological Acitivity of Synthetic [Gln^8]-β_h-Endorphin

	Opiate receptor-binding activity		Analgesic potency		Immunoreactivity	
	IC_{50}[a]	Relative Potency	AD_{50}[b]	Relative Potency	IC_{50}[c]	Relative Potency
β_h-Endorphin	0.22 ± 0.05[d]	1.0	0.098 (0.058-0.170)[f]	1.0	8.0 ± 0.7	1.0
	0.69 ± 0.07[e]	1.0	0.028 (0.020-0.039)[g]	1.0		
[Gln^8]-β_h-EP	0.13 ± 0.03[d]	1.7	0.036 (0.020-0.052)[f]	2.7	14.3 ± 0.7	0.6
	0.21 ± 0.02[e]	3.3	0.013 (0.009-0.018)[g]	2.2		

[a] IC_{50} in nM ± standard error

[b] AD_{50} in nM ± (95% confidence limit)

[c] IC_{50} in fM ± standard error

[d] Assayed with rat brain membrane preparation

[e] Assayed with neuroblastoma x glioma hybrid cells

[f] Carried out in Wisconsin

[g] Carried out in California

HUMAN:	H-TYR-GLY-GLY-PHE-MET-THR-SER-GLU-LYS-SER-		
	GLN-THR-PRO-LEU-VAL-THR-LEU-PHE-LYS-ASN-		
	ALA-ILE-ILE-LYS-ASN-ALA-TYR-LYS-LYS-GLY-GLU-OH		
PORCINE:	VAL	HIS	GLN-OH
CAMEL, OVINE, BOVINE:	ILE	HIS	GLN-OH

Figure 1: Amino acid sequence of human, procine, camel, ovine, and bovine β-endorphin.

ment of Glu^8 by glutamine caused an increase of 2.2-2.7 fold analgesic potency and 1.7-3.3 fold of opiate receptor-binding activity but a loss of 40% immunoreactivity. Earlier studies (18) have shown that substitution of Glu^8 by glutamine elevated the immunoreactivity and opiate receptor-binding activity of β_h-EP-(1-27) to values nearly as great as β_h-EP but the analgesic potency of [Gln^8]-β_h-EP-(1-27) increased from 2% to only 12% in comparison with the potency of β_h-EP. In addition, replacement of Glu^8 by glutamine increases the opiate receptor-

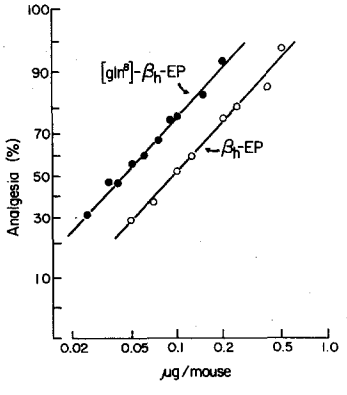

Figure 2: Comparison of antinociceptive effects of β_h-EP (o) and [Gln^8]-β_h-EP (●) in tail-flick test; groups of mice, 10-13 in each, were injected intracerebroventricularly. Percent analgesia as function of dose.

binding activity of synthetic [Gly^{31}]-β_h-EP-Gly-Gly-NH_2 (19) but reduces analgesic potency (20).

There are 2 Glu residues at positions 8 and 31 in the β_h-EP structure (Fig. 1). The analgesic potency of β_h-EP appears to be unchanged by the replacement of Glu^{31} by Gly (21). In addition, camel β-EP with glutamine in position 31 (1; see Fig. 1) exhibits nearly identical analgesic potency (20). These observations, together with the data herein reported, suggest that glutamic acid in position 8 plays an important role in both opiate receptor-binding activity and analgesic potency of the opioid peptide. The data in Table 1 also confirm the lack of correlation between immunoreactivity and opiate receptor-binding activity and analgesic potency as reported previously (22).

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