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β-ENDORPHIN: DELETION OF A SINGLE AMINO ACID RESIDUE ABOLISHES IMMUNOREACTIVITY BUT RETAINS OPIATE POTENCY

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SUMMARY: Two synthetic analogs of camel β-endorphin, one with omission of Leu-14 and the other with omission of Asn-20, have been assayed for immunoreactivity by radioimmunoassay, opiate activity in the guinea pig ileum preparation and analgesic potency in mice. It was found that the omission analogs had no immunoreactivity, but retained significant biological activities. As far as we are aware, this is the first instance in which deletion of a single amino acid residue in a biologically active peptide abolished immunoreactivity.

We have recently reported that the complete primary structure of β-endorphin is required for full analgesic activity (1). It is now found that deletion of a single amino acid residue in position 14 or 20 in the β_c-EP structure (see Fig. 1) does not alter its opiate potency, but causes a complete loss of immunoreactivity.

MATERIALS AND METHODS

β_h-EP and β_c-EP were synthetic products as previously described (2, 3). The solid phase synthesis (4) of Des-Leu^{14}-β_c-EP and Des-Asn^{20}-β_c-EP was briefly described (5) and is detailed as follows. Synthesis was performed on Boc-Gln brominated polymer (0.50 mmole/g) (6) in a Beckman model 990 peptide synthesizer with a fully automated symmetrical anhydride program (1). In one synthesis Leu^{14} of the β_c-EP sequence was omitted and in the other, Asn^{20}. After removal of the last Boc group with trifluoroacetic acid and treatment with HF, the peptides

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Abbreviations: β_c-EP, camel pituitary β-endorphin; β_h-EP, human pituitary β-endorphin; RIA, radioimmunoassay.

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H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
5

Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
15 20

Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH
25 31

Figure 1. Amino acid sequence of β_c-EP. Amino acids underlined indicate those omitted in synthesis of omission analogs.

were purified by gel filtration on Sephadex G-10, chromatography on carboxymethylcellulose, and partition chromatography on Sephadex G-50 by procedures detailed previously (2). The R_f values in the preparative partition columns were 0.14 for Des-Leu^{14}-β_c-EP and 0.42 for Des-Asn^{20}-β_c-EP. The ready separability of these peptides from β_c-EP by the partition method has been demonstrated (5). From 100 mg (50 µmoles) starting resin there was obtained 24.0 mg of Des-Leu^{14}-β_c-EP and 23.0 mg Des-Asn^{20}-β_c-EP. The peptides were homogeneous on tlc (silica gel) in 1-butanol-pyridine-acetic acid-H_2O (5:5:1:4): Des-Leu^{14}-β_c-EP, R_f 0.44; Des-Asn^{20}-β_c-EP, R_f 0.46. They were homogeneous in paper electrophoresis on Whatman 3MM (400 V, 5 hr, ninhydrin detection) at pH 3.7 (Des-Leu^{14}-β_c-EP, R_f^{Lys} 0.68; Des-Asn^{20}-β_c-EP, R_f^{Lys} 0.66) and at pH 6.7 (Des-Leu^{14}-β_c-EP, R_f^{Lys} 0.48; Des-Asn^{20}-β_c-EP, R_f^{Lys} 0.46). Amino acid analysis of a 24-hr HCl hydrolysate gave for Des-Leu^{14}-β_c-EP (theoretical values in parenthesis): Lys, 4.91 (5); His, 0.95 (1); Asp, 2.21 (2); Thr, 3.06 (3); Ser, 1.77 (2); Glu, 3.17 (3); Pro, 0.98 (1); Gly, 3.08 (3); Ala, 2.05 (2), Val, 0.92 (1); Met, 0.91 (1); Ile, 1.62 (2); Leu, 1.03 (1); Tyr, 0.96 (1); Phe, 1.97 (2). The values for Des-Asn^{20}-β_c-EP were: Lys, 4.90 (5); His, 0.93 (1); Asp, 1.16 (1); Thr, 3.10 (3); Ser, 1.79 (2); Glu, 3.18 (3); Pro, 0.97 (1); Gly, 3.04 (3); Ala, 1.99 (2); Val, 0.90 (1); Met, 0.86 (1); Ile, 1.55 (2); Leu, 1.96 (2); Tyr, 0.91 (1); Phe, 1.99 (2). Low values for Ile are accounted for by the well-known resistance of the Ile-Ile moiety to acid hydrolysis.

Opiate activity was measured from the depression of electrically-stimulated contractions of the guinea pig ileum preparations (7, 8). For analgesic assay, male ICR mice weighing 25-30 g (Simonsen Laboratories, Gilroy, CA) were used. The analgesic activity of the synthetic products was assessed by the tail-flick method (9) as previously described (1). Rabbit antiserum to β_h-EP was obtained as described (10) and radioimmunoassay was performed according to the procedure of Chang, et al. (11).

RESULTS AND DISCUSSION

As shown earlier (10), β_c-EP gave a completely parallel and almost identical inhibition curve in RIA using the antiserum to β_h-EP. It is evident in Fig. 2 that deletion of Leu or Asn

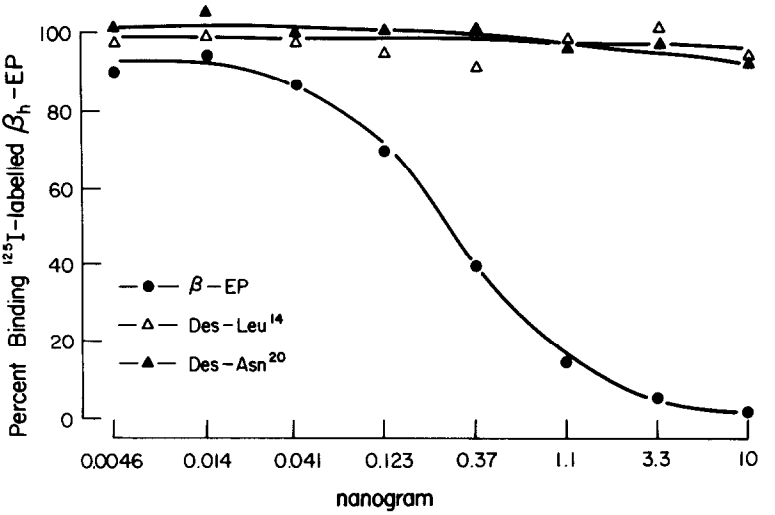

Figure 2. Competition of Des-Leu^{14}-β_c-EP and Des-Asn^{20}-β_c-EP in the β_h-EP RIA. Final dilution of the antiserum was 1:3000.

in position 14 or 20 in β_c-EP abolished completely immunoreactivity in the β_h-EP RIA system.

In spite of its loss of immunoreactivity, the omission analogs possess high opiate activities as assayed *in vitro* by the guinea pig ileum method (7). It may be seen in Table 1 that both analogs are somewhat more active than the parent molecule. When the omission analogs were injected intracerebroventricularly in mice, they exhibited significant analgesic potency but less than that for β_c-EP (Table 2). The lack of correlation between the *in vitro* and *in vivo* assay results has also been observed in earlier studies with other synthetic analogs of β-EP (6, 12). This emphasizes the importance of evaluating the biological activity of an opioid peptide by both *in vivo* and *in vitro* procedures.

The data reported herein clearly show that immunoreactivity of β_c-EP can be dissociated completely from its biological activity. It is remarkable that deletion of a single amino acid

Table 1

Opiate Activity of Synthetic β_C-Endorphin Analogs by Guinea Pig Ileum Assay

Synthetic peptides	IC_{50} [a]	Relative potency
β_C-Endorphin	9.1×10^{-8}	100
Des-Leu^{14}-β_C-EP	6.5×10^{-8}	140
β_C-Endorphin	2.2×10^{-8}	100
Des-Asn^{20}-β_C-EP	2.0×10^{-8}	110

[a] IC_{50} in M

Table 2

Analgesic Potency of Synthetic β_C-Endorphin Analogs in Mice

Synthetic peptides	AD_{50} [a]	Relative potency
β_C-Endorphin	0.043 (0.035-0.075)	100
Des-Leu^{14} - β_C-EP	0.057 (0.033-0.093)	75
β_C-Endorphin	0.026 (0.020-0.032)	100
Des-Asn^{20} - β_C-EP	0.057 (0.042-0.075)	46

[a] AD_{50} in nmol/mouse (95% confidence limit)

residue in β_C-EP destroys its immunoreactivity. As far as we are aware, this is the first time it has been shown that omission of a single amino acid in a peptide is responsible for loss of immunoreactivity.

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