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β-ENDORPHIN-(1-27): ACETYLATION OF ALPHA-AMINO GROUPS ENHANCES IMMUNOREACTIVITY BUT DIMINISHES ANALGESIC AND RECEPTOR-BINDING ACTIVITIES WITH NO CHANGES IN CIRCULAR DICHROISM SPECTRA

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SUMMARY

Four synthetic β_h-endorphin analogs have been assessed for their immunoreactivity, receptor-binding activity and analgesic potency as well as their α-helical content by circular dichroism. These synthetic analogs are: β_h-endorphin-(1-27), [Gln^8]-β_h-endorphin-(1-27), [Ac-Tyr^1]-β_h-endorphin-(1-27) and [Ac-Tyr^1,Gln^8]-β_h-endorphin-(1-27). Results show that acetylation caused 3.8-8.8 X increase of immunoreactivity in comparison with the parent analog but abolished receptor-binding and analgesic activities. In addition, acetylation does not alter circular dichroism spectra in 90% methanol. Thus, there is no correlation of α-helix content with biological activities of these four synthetic β_h-endorphin analogs.

It has been recently shown (1) that omission of a single amino acid residue in position 14, 15 or 20 of the β-EP structure (see Fig. 1) abolishes the immunoreactivity but retains ileal opiate, receptor-binding and analgesic activities. In an effort to further delineate the structure-activity relationship of β_h-EP, we have synthesized four analogs: β_h-EP-(1-27), [Gln^8]-β_h-EP-(1-27), [Ac-Tyr^1]-β_h-EP-(1-27) and [Ac-Tyr^1,Gln^8]-β_h-EP-(1-27). Biological investigations of these analogs revealed that immunoreactivity of β_h-EP-(1-27) and [Gln^8]-β_h-EP-(1-27) was greatly

Abbreviations: β_h-EP, human β-endorphin; CD, circular dichroism

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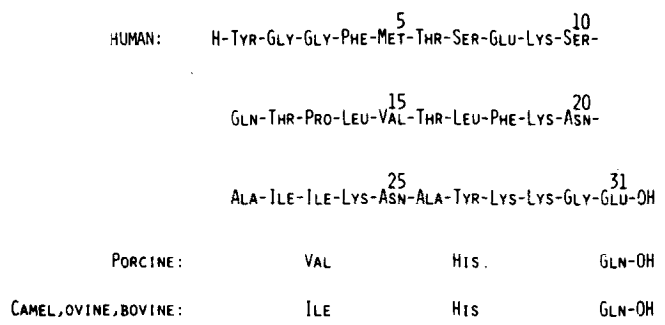

AMINO ACID SEQUENCE OF HUMAN, PORCINE, CAMEL, OVINE, AND BOVINE β-ENDORPHINS

Fig. 1. Amino acid sequence of β_h-endorphin

increased by acetylation while receptor-binding and analgesic activities were nearly abolished. CD spectra of non-acetylated and acetylated peptides indicate no changes in α-helical content. Results of these studies are reported herein.

MATERIALS AND METHODS

β_h-Endorphin, β_h-EP-(1-27) and analogs were synthesized by the solid-phase method as previously described (2,3). Immunoreactivity was assessed by radioimmunoassay using the published procedure (4,5). The opiate receptor-binding assay was performed according to the procedure recently described (6,7) using $^3H_2$-Tyr^{27}]-β_h-EP (8) as the primary ligand and synthetic β_h-EP (2) as standard competing ligand. The analgesic activity in vivo was estimated in mice by the tail-flick method (9) as described (10). Circular dichroism (CD) spectra were obtained on a Cary Model 60 spectropolarimeter equipped with a Model 6002 CD attachment. Details of all procedures and methods of calculation have been described (11). Methanol was of spectral grade.

RESULTS AND DISCUSSION

Biological activity of β_h-EP-(1-27) and analogs as assayed by various procedures is summarized in Table 1. In comparison with β_h-EP, deletion of four amino acids from the COOH-terminus caused a loss of 34% immunoreactivity, 70% of receptor binding activity and nearly 98% analgesic potency. Substitution of glutamine in position 8 (Glu) elevated immunoreactivity and

Table 1

Biological Activity of Synthetic β_h-Endorphin-(1-27) and Analogs

Synthetic Peptides	Immunoreactivity		Receptor-binding Activity		Analgesic Activity	
	IC_{50} [a]	Relative Activity	IC_{50} [b]	Relative Potency	AD_{50} [c]	Relative Potency
β_h-Endorphin	116	100	0.62	100	3.11 (1.40-5.53)	100
β_h-EP-(1-27)	175	66	2.05	30	≥143	≤ 2
[Gln^8]-β_h-EP-(1-27)	141	82	0.68	90	26.8 (14.0-51.1)	12
[Ac-Tyr^1]-β_h-EP-(1-27)	45	252	1655	0.04	>141	< 2
[Ac-Tyr^1,Gln^8]-β_h-EP-(1-27)	16	725	950	0.07	>141	< 2

[a] IC_{50} in 10^{-12} M

[b] IC_{50} in 10^{-9} M

[c] AD_{50} in 10^{-10} M (95% confidence limit)

receptor-binding activity of β_h-EP-(1-27) to values nearly as active as β_h-EP. However, the analgesic potency of [Gln^8]-β_h-EP-(1-27) increased from 2% to 12% but it was still far below the potency for β_h-EP. Earlier studies have already shown that replacement of Glu-8 by glutamine enhances the receptor-binding activity of [Gly^{31}]-β_h-EP-Gly-Gly-NH_2 (12).

As shown in Table 1, acetylation of α-NH_2 group in both β_h-EP-(1-27) and [Gln^8]-β_h-EP-(1-27) increased markedly the immunoreactivity. This is clearly evident as illustrated in Fig. 2. Apparently the presence of the positively charged α-amino group interferes with the antigen-activity interaction and blocking of the α-NH_2 group by acetylation removed the interference. We recently reported that the immunoreactivity of β_h-EP is abolished by omission of a single residue in position 14, 15 or 20 of the 31-amino acid sequence (1). Present data show that modification of a single residue (Tyr-1) in β_h-EP-(1-27) and [Gln^8]-β_h-EP-(1-27) induces impressive elevation of immunoreactivity.

Both receptor-binding and analgesic activities of β_h-EP-(1-27) and [Gln^8]-β_h-EP-(1-27) are drastically decreased by

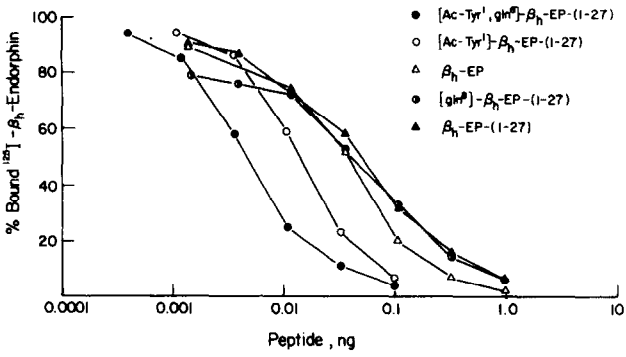

Fig. 2. Immunoreactivity of β_h-EP-(1-27) and analogs relative to β_h-EP based on radioimmunoassay with rabbit antiserum to β_h-EP.

acetylation (Table 1). This is not surprising as it is known that any modification of Tyr-1 in β-EP lowers opiate activities (13,14).

β-Endorphin in water shows little secondary structure as evidenced by CD spectra (15,16). Both methanol (15) and trifluoroethanol (16) promote the formation of α-helix in as much as one-half of the peptide molecule. CD spectra of β_h-EP analogs with various chain lengths in 90% methanol indicated that β_h-EP-(1-30) had nearly full helical structure when compared with β_h-EP (17). On the other hand, β_h-EP-(1-30) had only 72% analgesic activity of the parent molecule (18). It is therefore of interest to examine the CD spectra of [Gln^8]-β_h-EP-(1-27) and [Ac-Tyr^1,Gln^8]-β_h-EP-(1-27) as it is possible that acetylation may cause a change of α-helical content in 90% methanol and, in turn, induce alteration of biological profile.

Figure 3 presents the CD spectra of [Gln^8]-β_h-EP-(1-27) and [Ac-Tyr^1,Gln^8]-β_h-EP in 90% methanol. The α-helical content of these two analogs was estimated, using parameters for aqueous solutions (11), to be 20-25% from $[\theta]_{221\ m\mu}$ and 35-40% from

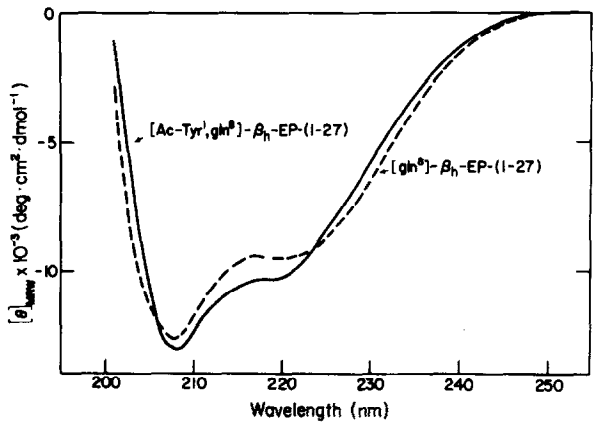

Fig. 3. CD spectra of [Gln^8]-β_h-EP-(1-27) and [Ac-Tyr^1,Gln^8]-β_h-EP-(1-27) in the region of amide bond absorption in 90% methanol.

$[\theta]_{209\ m\mu}$. The latter value may be too high due to contributions from a random-coil band in the same spectral region. At any rate, there is no difference in CD spectra between $[Gln^8]$-β_h-EP-(1-27) and its acetylated analog in 90% methanol. It may be concluded that the enhancement of immunoreactivity and the loss of analgesic and receptor-binding activities by acetylation of α-NH_2 group in these opioid peptides are not due to the change of α-helix forming potential. Although a correlation between α-helix content in trifluoroethanol and biological activity has been reported (19), this study indicates that retention of α-helical comformation is not sufficient to maintain full biological activity.

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REFERENCES

1. Li, C. H., Yamashiro, D., Tseng, L-F., Chang, W-C., and Ferrara, P. (1980) Proc. Natl. Acad. Sci. USA 77, 3211-3214.
2. Li, C. H., Yamashiro, D., Tseng, L-F., and Loh, H. H. (1977) J. Med. Chem. 20, 325-328.
3. Zaoral, M., Yamashiro, D., Hammonds, Jr., R. G., and Li, C. H. Int. J. Peptide Protein Res., in press.
4. Li, C. H., Rao, A. J., Doneen, B. A., and Yamashiro, D. (1977) Biochem. Biophys. Res. Commun. 75, 576-580.
5. Chang, W-C., Yeung, H. W., and Li, C. H. (1979) Int. J. Peptide Protein Res. 13, 278-281.
6. Ferrara, P., Houghten, R., and Li, C. H. (1979) Biochem. Biophys. Res. Commun. 89, 786-792.
7. Ferrara, P. and Li, C. H. (1980) Int. J. Peptide Protein Res. 16, 66-69.

8. Houghten, R. A. and Li, C. H. (1978) Int. J. Peptide Protein Res. 12, 325-326.
9. D'Amour, F. E. and Smith, D. L. (1941) J. Pharmacol. Exp. Ther. 72, 74-79.
10. Loh, H. H., Tseng, L-F., Wei, E., and Li, C. H. (1976) Proc. Natl. Acad. Sci. USA 73, 2895-2898.
11. Bewley, T. A., Brovetto-Cruz, J., and Li, C. H. (1969) Biochemistry 8, 4701-4708.
12. Yamashiro, D., Ferrara, P., and Li, C. H. (1980) Int. J. Peptide Protein Res. 16, 70-74.
13. Yamashiro, D., Tseng, L-F., Doneen, B. A., Loh, H. H., and Li, C. H. (1977) Int. J. Peptide Protein Res. 10, 159-166.
14. Doneen, B. A., Chung, D., Yamashiro, D., Law, P. Y., Loh, H. H., and Li, C. H. (1977) Biochem. Biophys. Res. Commun. 74, 656-662.
15. Yang, J. T., Bewley, T. A., Chen, G. C., and Li, C. H. (1977) Proc. Natl. Acad. Sci. USA 74, 3235-3238.
16. Hollat, M., Kajtar, M., and Gráf, L. (1977) FEBS Letters 74, 185-189.
17. Li, C. H. (1979) In Peptides-Proceedings of the Sixth American Peptide Symposium (Gross, E. and Meienhofer, J., eds.) Pierce Chemical Company, Rockford, Illinois, pp. 823-833.
18. Li, C. H., Tseng, L-F., Yamashiro, D. (1978) Biochem. Biophys. Res. Commun. 85, 795-800.
19. Gráf, L., Hollósi, M., Barna, I., Hermann, I., Borvendég, J., and Ling, N. (1980) Biochem. Biophys. Res. Commun. 95, 1623-1627.