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Biochemistry

β-Endorphin-(1–27) is an antagonist of β-endorphin analgesia

(competitive inhibitor/peptide segment of β_h-EP/mouse)

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ABSTRACT **β_h-Endorphin-(1–27), a naturally occurring fragment of human β-endorphin (β_h-endorphin), diminishes the analgesic effect of β_h-endorphin when coinjected intracerebroventricularly into mice. A parallel shift in the dose–response curve of β_h-endorphin in the presence of β_h-endorphin-(1–27) suggests competition at the same site. The potency of β_h-endorphin-(1–27) in antagonizing analgesia is >4 times greater than that of the opiate antagonist naloxone.**

Discrepancies between analgesic and binding potency in a number of β-endorphin (β-EP) analogs, both synthetic and naturally occurring (1), led us to suggest that these peptides act with differing efficacies (2, 3) and that the ratio of analgesic to binding potency could be used as a relative measure of efficacy. Differences in efficacy would be demonstrated if analogs with greater binding than analgesic potency act as competitive antagonists of analgesia. Of particular interest among β-EP-related peptides is the naturally occurring fragment of β-EP, β-EP-(1–27). This fragment of β-EP is present in the brain as well as the pituitary (4–8). Although immunoreactive material corresponding to β-EP-(1–27) is present in some brain areas in abundance equal to or greater than the parent molecule, this peptide has been regarded as an inactivated form of β-EP (4, 8). The analgesic and binding potencies of human β-EP-(1–27) [β_h-EP-(1–27)], 2% and 30% relative to human β-EP (β_h-EP) (9, 10), make this peptide a good candidate for an inhibitor of β-EP action. A similar relation exists between the analgesic and binding potencies of porcine β-EP and β-EP-(1–27) (11, 12). We describe here competitive inhibition of β_h-EP analgesia by β_h-EP-(1–27) and propose the name β-EP-inhibiting peptide for the 1–27 fragment of β-EP.

MATERIALS AND METHODS

β_h-EP and β_h-EP-(1–27) were synthetic products (9, 13). Naloxone was a gift from Endo Laboratories (New York). Male Swiss–Webster mice weighing 25 g were used to assess the effect of intracerebroventricularly administered peptides and antagonists on heat escape latencies (14, 15). Groups of 9–10 mice were used for each dose. At 5, 15, 30, 45, and 60 min after injection, each mouse was loosely restrained in a gloved hand with its tail centered on an opaque platform over an aperture to a high-intensity lamp. The time elapsed between turning the lamp on and tail motion was measured automatically to the nearest 0.1 sec. Light intensity was adjusted to give a 1.5- to 2.0-sec base-line latency, and the lamp was turned off automatically after 10 sec. Percentage of analgesia was calculated as described (15). Median antinociceptive dose (AD_{50}), 95% confidence intervals, and the slope of the dose–response curves were calculated by nonlinear least-squares regression to a two-parameter logistic equation. Binding assays were performed with washed rat brain membranes and tritiated β_h-EP (3, 16). Glass fiber filters were treated with polyethylenimine as described (17).

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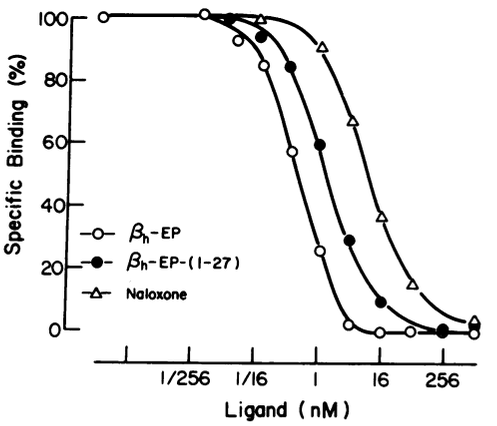

FIG. 1. Inhibition of [^{3}H]β_h-EP binding to rat brain membranes. Membranes (0.5 mg of protein) and [^{3}H]β_h-EP (3 nM) were incubated with increasing concentrations of the indicated compounds. The percentage of specific binding was calculated as $100(B_x - B_n)/(B_0 - B_n)$, in which B_x is the amount bound in the presence of a concentration x of competing compound, B_0 is B_x for $x = 0$, and B_n is B_x for $x = 1$ μM β_h-EP.

RESULTS AND DISCUSSION

As reported (9, 10), β_h-EP-(1–27) retained 30% of the potency of β_h-EP in displacing [^{3}H]β_h-EP from rat brain membranes (Fig. 1 and Table 1). In the same assay, naloxone was 1/10th as potent. β_h-EP-(1–27) retained just less than 2% of the potency of β_h-EP in producing analgesia (Fig. 2 and Table 1). The high ratio of binding to analgesic potency allows the prediction that β_h-EP-(1–27) will act as an antagonist.

Injection of various doses of β_h-EP together with a fixed dose of β-EP-inhibiting peptide [β_h-EP-(1–27)] produced a parallel shift of the dose–response curve. Larger doses of β-EP-inhibiting peptide resulted in larger shifts. Similar results were obtained by injecting β_h-EP together with naloxone. Linear regression of the slope of the dose–response curve vs. AD_{50} gave a correlation coefficient of −0.05, indicating no detectable change in slope in the presence of antagonist. A noncompetitive antagonist would be expected to produce progressively flatter agonist dose–response curves as the dose of antagonist increases. From dose–response curves obtained for β_h-EP in the presence of either β-EP-inhibiting peptide or naloxone, apparent AD_{50} and corresponding dose ratios were calculated (Table 2). Because a parallel shift of the dose–response curve of a potent agonist in the presence of an antagonist is evidence for competitive inhibition (18, 19), results were analyzed by a pA_x plot as defined in Fig. 2 (18). Fig. 2 *Inset* shows the results with either β_h-EP-inhibiting peptide or naloxone as antagonist and β_h-EP as agonist. Linear relationships result in both cases. The slope and pA_2

Abbreviations: β-EP, β-endorphin; β_h-EP, human β-EP; AD_{50}, median antinociceptive dose.

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Table 1. Analgesic potency and binding affinity of β_n-EP, β_n-EP-inhibiting peptide (βEIP), and naloxone

	Analgesic activity			Binding affinity	
	AD_{50},* pmol/mouse	Slope†	Relative potency	IC_{50}‡ × 10^{-9} M	Relative potency
β_h-EP	27 (22–34)	1.32	1.00	0.33 (0.28–0.40)	1.00
βEIP	1500 (1300–1700)	1.33	0.017	1.1 (0.99–1.3)	0.30
Naloxone	—	—	—	12 (8.9–16.6)	0.027

*Dose giving half-maximal analgesia; numbers in parentheses are 95% confidence limits.
†Slope of the log-probit dose–response curve.
‡Concentration giving 50% inhibition of [^{3}H]β_h-EP-specific binding; numbers in parentheses are 95% confidence limits.

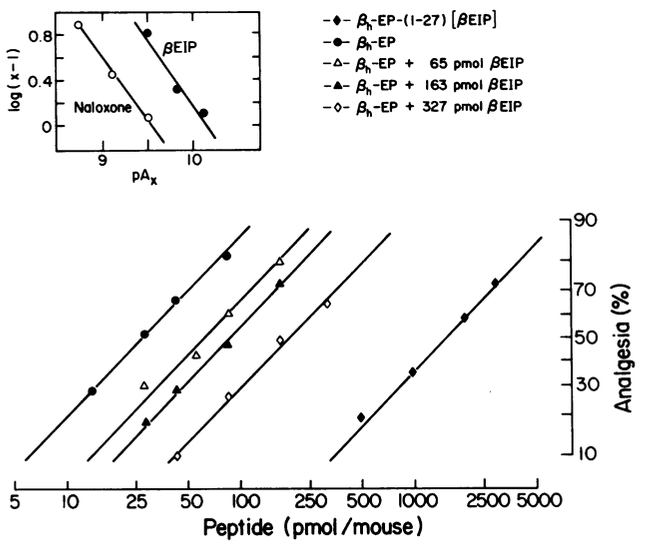

FIG. 2. Analgesic effect of β_h-EP and β_h-EP-inhibiting peptide and antagonism of β_h-EP by β_h-EP-inhibiting peptide (βEIP). Log (dose) vs. probit (% analgesia) for β_h-EP alone or in the presence of increasing doses of βEIP. Peak analgesic effect was observed at 30 min after injection, and the percentage of inhibition in the presence of antagonist was constant from 15 to 60 min after injection. (*Inset*) pA_x plot for antagonism of β-EP's analgesic effect by βEIP or naloxone. Abscissa = pA_x = −log(mol of antagonist per mouse) at which the dose ratio (see below) is x. Ordinate = log(x − 1), where x is the dose ratio (ratio of AD_{50} of β_h-EP in the presence of antagonist to that in its absence). Lines intersect the pA_x axis at a point corresponding to pA_2, the negative logarithm of the antagonist dose required to reduce the effect of β_h-EP by half. Least-squares regression gave for βEIP pA_2 = 10.2, slope = −0.96, and $pA_2 - pA_{10}$ = 0.94; for naloxone pA_2 = 9.55, slope = −1.06, and $pA_2 - pA_{10}$ = 0.92.

Table 2. Antagonism of β_h-EP by β_h-EP-inhibiting peptide (βEIP) or naloxone

Antagonist	Dose of antagonist, pmol/mouse	AD_{50},* pmol/mouse	Slope*	Dose† Ratio
βEIP	0	27 (22–34)	1.32	1.0
	66	62 (33–99)	1.27	2.3
	164	86 (65–120)	1.26	3.2
	328‡	196 (116–296)	1.30	7.3
Naloxone	0	28 (17–39)	1.31	1.0
	290	60 (35–87)	1.29	2.1
	870	107 (78–121)	1.31	3.8
	1740	204 (159–339)	1.27	8.6

AD_{50}, slope, and dose ratio of log-probit dose–response curves for β_h-EP in the absence and presence of increasing fixed doses of either naloxone or βEIP.
*As in Table 1.
†Ratio of the AD_{50} of β_h-EP in the presence of antagonist to that in its absence.
‡Corrected for intrinsic analgesic activity of antagonist (9%).

− pA_{10} were close to those expected for competitive antagonism; slope = −1.0 and $pA_2 - pA_{10}$ = log(9) = 0.954. Apparent pA_2 values provide estimates of antagonist potency. β_h-EP-inhibiting peptide is 4.5 times more potent than naloxone in antagonizing analgesia and 10 times more potent than naloxone in competing for tritiated β_h-EP binding.

Other naturally occurring β-EP derivatives having good binding affinity and low analgesic activity may also be a source of inhibitory action and regulation of opioid systems in the brain. Previous studies (20) have demonstrated that β-EP-(6–31) can produce a slight inhibition of β-EP analgesia when injected at a high dose. However, β-EP-(6–31) has not been found in brain or pituitary, and it retains only 1/500th of the opiate receptor binding potency of β-EP. Inhibition of a peptide hormone by a segment of the same hormone may be a general phenomenon and of great significance in the physiology of biologically active peptides.

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1. Li, C. H. (1981) in *Hormonal Proteins and Peptides*, ed. Li, C. H. (Academic, New York), Vol. 10, pp. 1–34.
2. Hammonds, R. G., Jr., Nicolas, P. & Li, C. H. (1982) *Int. J. Pept. Protein Res.* **19**, 556–561.
3. Nicolas, P., Hammonds, R. G., Jr., & Li, C. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2191–2193.
4. Smyth, D. G. (1983) *Br. Med. Bull.* **39**, 25–30.
5. Smyth, D. G., Snell, C. R. & Massey, D. E. (1978) *Biochem. J.* **75**, 261–270.
6. Ng, T. B., Chung, D. & Li, C. H. (1981) *Int. J. Pept. Protein Res.* **18**, 443–450.
7. Akil, H., Veda, Y., Lin, H. L. & Watson, S. J. (1981) *Neuropeptides* **1**, 429–446.
8. Zakarian, S. & Smyth, D. G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5972–5976.
9. Zaoral, M., Yamashiro, D., Hammonds, R. G., Jr., & Li, C. H. (1981) *Int. J. Pept. Protein Res.* **17**, 292–296.
10. Li, C. H., Tseng, L.-F., Jibson, M. D., Hammonds, R. G., Jr., Yamashiro, D. & Zaoral, M. (1980) *Biochem. Biophys. Res. Commun.* **97**, 932–938.
11. Geisow, M. J., Deakin, J. F. W., Dostrovsky, J. O. & Smyth, D. G. (1977) *Nature (London)* **269**, 167–168.
12. Feldberg, W. & Smyth, D. G. (1977) *Br. J. Pharmacol.* **60**, 445–454.
13. Li, C. H., Yamashiro, D., Tseng, L.-F. & Loh, H. H. (1977) *J. Med. Chem.* **20**, 325–328.
14. D'Amour, F. E. & Smith, D. C., Jr. (1941) *J. Pharmacol. Exp. Ther.* **72**, 74–79.
15. Loh, H. H., Tseng, L.-F., Wei, E. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2895–2898.
16. Ferrara, P., Houghten, R. & Li, C. H. (1979) *Biochem. Biophys. Res. Commun.* **99**, 786–792.
17. Johnson, N., Houghten, R. & Pasternak, G. W. (1982) *Life Sci.* **31**, 1381–1384.
18. Arunlakshana, O. & Schild, H. O. (1959) *Br. J. Pharmacol.* **14**, 48–58.
19. Takemori, A. E., Kupferberg, H. J. & Miller, J. W. (1969) *J. Pharmacol. Exp. Ther.* **169**, 39–45.
20. Lee, N. M., Friedman, H. J., Leybin, L., Cho, T. M., Loh, H. H. & Li, C. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5525–5526.