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Biochemistry

β-Endorphin-(1–27) is a naturally occurring antagonist to etorphine-induced analgesia

(competitive inhibitor/radioreceptor assay/mouse tail-flick method)

PIERRE NICOLAS* AND CHOH HAO LI

Laboratory of Molecular Endocrinology, University of California, San Francisco, CA 94143

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ABSTRACT **The potent opioid peptide β-endorphin is found in the brain and pituitary with two related fragments, β-endorphin-(1–27) and β-endorphin-(1–26). The fragments retain substantial opioid-receptor binding activity but are virtually inactive analgesically. β-Endorphin-(1–27) inhibits β-endorphin-induced and etorphine-induced analgesia when coinjected intracerebroventricularly into mice. Antagonism by competition at the same site(s) is suggested from parallel shifts of the dose–response curves of etorphine or β-endorphin in the presence of β-endorphin-(1–27). Its potency is 4–5 times greater than that of the opiate antagonist naloxone. β-Endorphin-(1–26) does not antagonize the antinociceptive action of etorphine or β-endorphin in doses up to 500 pmol per animal.**

Recent studies have shown that β-endorphin (β-EP) is found in the pituitaries and the brain of various species together with two related peptides: β-EP-(1–27) and its des-His^{27} or des-Tyr^{27} derivative β-EP-(1–26). Each of these peptides occurs also in an α-*N*-acetyl form (1–5). Studies of the regional distribution and biosynthetic pathways of the β-EP-related peptides in the brain and pituitaries have shown distinctive proportions of these forms in specialized areas arising from differential proteolytic cleavages and acetylation of β-EP (6–8). Of various peptides related to β-EP, only the unmodified hormone possesses potent analgesic properties. The formation of its derivatives is accompanied by loss of analgesic activity (9–12).

Since no particular role had been reported for these truncated and/or acetylated peptides, it was suggested that post-translational inactivation of β-EP might be an important all-or-none metabolic process maintaining a physiological level of bioactive endorphin in the brain (5, 6). However, the diversity and natural abundance of these inert opioid peptides, in regard to such a simple switch mechanism for inactivation, have given the impetus to search for more specific biological functions. We have recently reported on the inhibition of the analgesic activity of β-EP by β-EP-(1–27) (13). This particular fragment retains 30% of the binding potency of the parent hormone for brain opioid receptors and <2% of its analgesic potency. It diminishes the analgesic effect of β-EP when coinjected intracerebroventricularly (icv) into mice in doses that are compatible with those found in the brain. To provide further evidence in support of the hypothesis that natural fragments of β-EP might be implicated in the control of pain perception, we have studied effects of β-EP-(1–27) and β-EP-(1–26) on the modulation of antinociception induced both by β-EP and by the highly potent opiate agonist etorphine in mice.

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MATERIALS AND METHODS

Human β-EP (β_h-EP) (14), β_h-EP-(1–27) (11), and β_h-EP-(1–26) (12) were synthesized as described. Etorphine was a generous gift from E. L. Way. Naloxone was from Endo Laboratories (Garden City, NY). [$^3H_2$-Tyr^{27}]β_h-EP ([^{3}H]β_h-EP) (50 Ci/mmol; 1 Ci = 37 GBq) was prepared as described (15). [^{3}H]Etorphine (933 Ci/mmol) was purchased from New England Nuclear.

Binding assays were performed in Tris·HCl, pH 7.5/0.1% bovine serum albumin/0.01% bacitracin with washed rat brain membranes (0.5 mg of membrane protein per assay) and [^{3}H]β_h-EP (0.3 nM) or [^{3}H]etorphine (0.3 nM) as primary ligand (16, 17).

Effect of icv administered opiates or peptides on heat escape latency was assessed by the tail-flick method (18), using groups of 10 mice (male Swiss Webster, 20–25 g; Simonsen Laboratories, Gilroy, CA) per dose. Percentage analgesia was calculated as described (19). Median antinociceptive dose (AD_{50}), 95% confidence limits and slope of the logarithm of the dose vs. probit (% analgesia) curves were calculated by a nonlinear least-squares regression to a 2 parameters logistic. AD_{50} values were calculated for each peptide or opiate alone and in combination with various fixed doses of putative antagonist. The ratio of the AD_{50} value in the presence of antagonist to that in its absence (dose ratio, *x*) was then calculated for each dose of antagonist (13, 20).

Competitive antagonism was quantified by use of the apparent pA_2 (the negative logarithm of the antagonist dose required to reduce the effect of a dose of agonist by one-half) for agonist–antagonist interactions (21, 22). Log (dose ratio − 1) is plotted against −log (dose of antagonist in mol per mouse). We refer to the corresponding graphs as Schild plots (21). For competitive antagonism to the same receptor, the curve of the Schild plot is a straight line with a slope of −1.0 and intercepts on the abscissa at $pA_x = pA_2$, where pA_x is the negative logarithm of the dose of antagonist injected in mol per mouse. Potency of competitive antagonist was quantified using pA_2 values and the relation $pA_2 = \log K_2$, where K_2 is the apparent equilibrium association constant of the antagonist (22).

RESULTS

Log (dose) vs. probit (% analgesia) curves for the antinociceptive effect produced by icv injection of β_h-EP, β_h-EP-(1–27), β_h-EP-(1–26), and etorphine are shown in Fig. 1 and AD_{50} values are summarized in Table 1. Under similar conditions, naloxone in doses up to 500 nmol per mouse does

Abbreviations: β-EP, β-endorphin; β_h-EP, human β-endorphin; icv, intracerebroventricular; [^{3}H]β_h-EP, [$^3H_2$-Tyr^{27}]β_h-EP; AD_{50}, median antinociceptive dose.

*Permanent address: Groupe de Neurobiochimie Cellulaire et Moleculaire, Université Pierre et Marie Curie, 96 Boulevard Raspail, 75006 Paris, France.

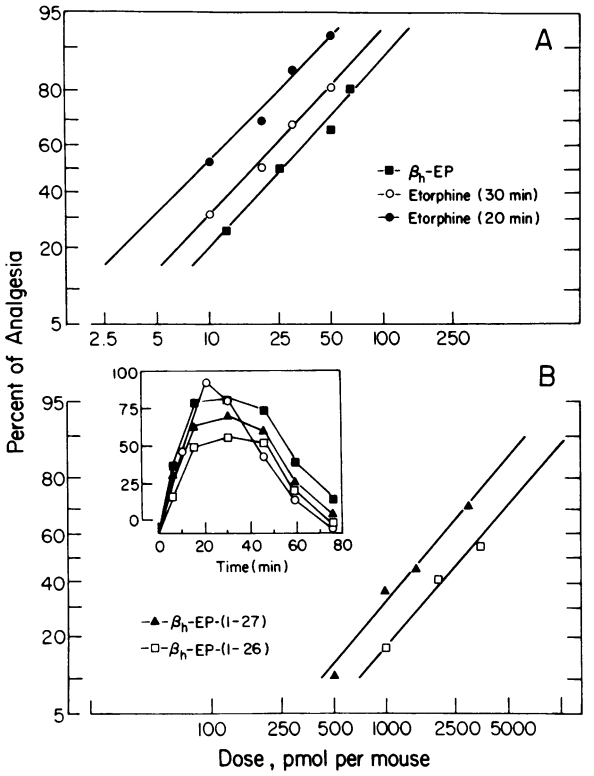

FIG. 1. Analgesic effect of β_h-EP, β_h-EP-(1–27), β_h-EP-(1–26), and etorphine. (*Inset*) Time course of analgesia produced by etorphine (50 pmol), β_h-EP (75 pmol), β_h-EP-(1–27) (3000 pmol), and β_h-EP-(1–26) (3500 pmol).

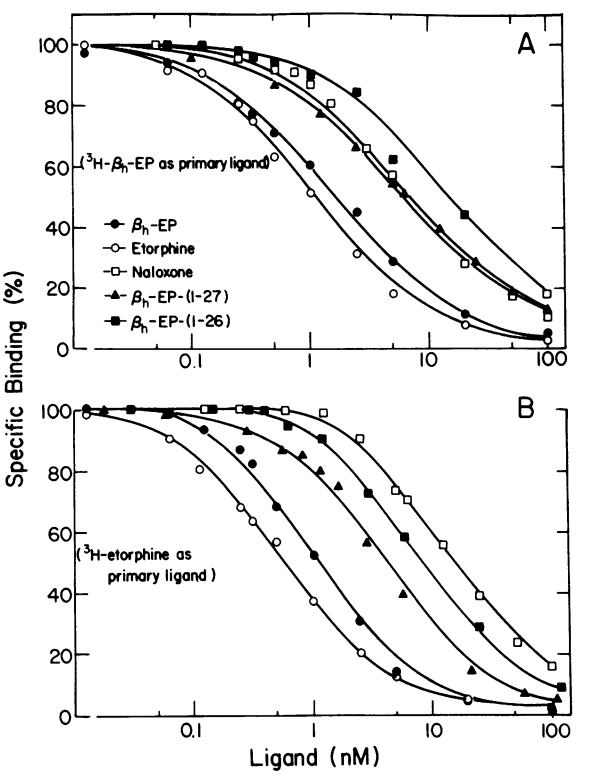

FIG. 2. Inhibition of [^{3}H]β_h-EP binding (*A*) and [^{3}H]etorphine binding (*B*) to rat brain membranes. Percentage specific binding was calculated as $100 \times (B_s - B_o)/(B_o - B_n)$ in which B_s is the amount bound in the presence of competing compound and B_n is the nonspecific binding. In these cases, the binding is in the presence of 0.5 μM etorphine (*A*) or 0.5 μM β_h-EP (*B*).

not elicit an analgesic response in this assay. The duration of analgesia produced by β_h-EP and related peptides was identical (Fig. 1 *Inset*). The antinociceptive effect peaked at 30 min, lasted nearly 45 min, and dissipated after 80 min. Etorphine had a slightly more rapid onset of action and the peak analgesic effect was observed at 20 min after injection (Fig. 1 *Inset*). Nearly parallel dose–response curves were observed, suggesting that opioid peptides and etorphine could produce their pharmacological effect by acting through a similar population of opioid receptor subtypes *in vivo*. Etorphine was nearly 3 times more potent than β_h-EP and, therefore, ≈450 times more potent than morphine (23) on a molar basis. β_h-EP-(1–27) and β_h-EP-(1–26) exhibited, respectively, just less than 2% and 1% the potency of the parent molecule (Table 1).

Competitive inhibition of [^{3}H]β_h-EP or [^{3}H]etorphine binding to rat brain membranes by opioid peptides and opiates is shown in Fig. 2. The corresponding IC_{50} values (concentration that inhibits response by 50%) are reported in Table 1. Etorphine displaced [^{3}H]β_h-EP binding with a slightly higher potency than β_h-EP, while significant decrements in potency occurred on removal of a few COOH-terminal residues of β_h-EP, giving rise to β_h-EP-(1–27) and β_h-EP-(1–26). These fragments retained almost 30% and 12%, respectively, of the potency displayed by the parent molecule. In the same assay, naloxone was 1/15th as potent. The same order in potencies was found for opioid peptides when [^{3}H]etorphine was used as primary ligand (Fig. 2, Table 1).

The time for the peak analgesic effect of etorphine either alone or in the presence of various doses of β_h-EP-(1–27) was

Table 1. Analgesic potency, opioid receptor binding affinities, and relative biological activities of β_h-EP, β_h-EP-(1–27), β_h-EP-(1–26), etorphine, and naloxone

Compound	Analgesic activity			Binding affinity			
				vs. [^{3}H]β_h-EP		vs. [^{3}H]etorphine	
	AD_{50}, pmol per mouse	Slope	RP*	IC_{50}, nM	RP†	IC_{50}, nM	RP
β_h-EP	26.3 [17.2–37.1]	1.3	100	1.10 [1.03–1.25]	100	1.66 [1.50–1.83]	65
β_h-EP-(1–27)	1632 [1081–3100]	1.40	1.61	4.11 [3.60–5.08]	27	5.85 [5.17–7.21]	18.5
β_h-EP-(1–26)	2875 [1020–3620]	1.36	0.90	9.25 [7.45–11.63]	12	15.5 [14.3–16.9]	7
Etorphine, 20‡	9.37 [4.10–14.50]	1.37	281	0.58 [0.50–0.66]	189	1.08 [0.94–1.23]	100
Etorphine, 30‡	18.0 [13.1–23.0]	1.41	146				
Naloxone§	—	—	—	17.6 [12.1–19.2]	6	7.77 [6.82–9.42]	14

Numbers in brackets are 95% confidence limits. Slope means slope of the log (dose) vs. probit (% analgesia) curves. RP, relative potency; IC_{50}, concentration giving 50% inhibition of [^{3}H]β_h-EP or etorphine specific binding.
*Values are calculated on a molar basis using the AD_{50} of β_h-EP as 100.
†Values are calculated using the IC_{50} of either β_h-EP or etorphine when used as primary ligand.
‡Etorphine analgesia was tested 20 min and 30 min after injection.
§Dose up to 500 nmol per mouse does not induce an antinociceptive response in this assay.

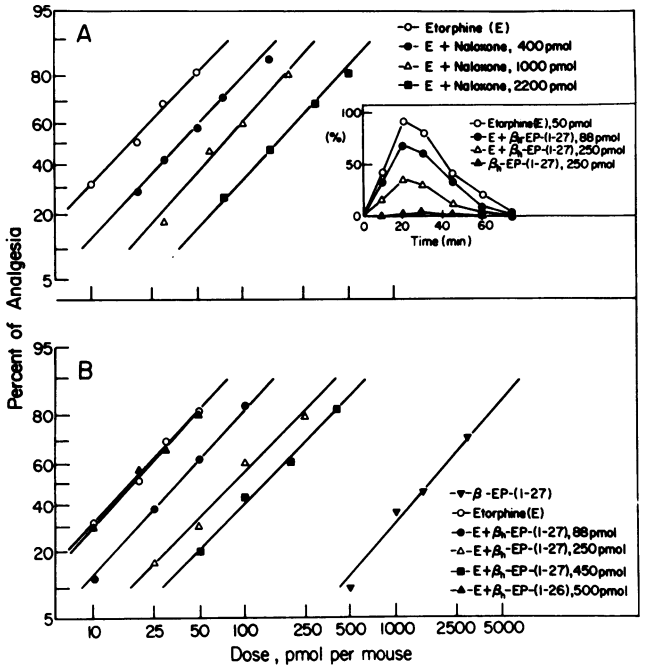

FIG. 3. Antagonism of etorphine-induced analgesia by naloxone and β_h-EP-(1–27) and β_h-EP-(1–26). (*Inset*) Time course of analgesia produced by etorphine (50 pmol) in the absence or in the presence of 88 pmol or 250 pmol of β_h-EP-(1–27). Time course of analgesia elicited by 250 pmol of β_h-EP-(1–27) alone. (*B*) As in *A* but for etorphine (50 pmol) alone or in combination with 88 pmol, 250 pmol, and 450 pmol of β_h-EP-(1–27), or 500 pmol of β_h-EP-(1–26) or β_h-EP-(1–27) alone.

the same (Fig. 3*A Inset*). There was a marked decrease in the analgesic response to etorphine when small doses of β_h-EP-(1–27) were coinjected. The maximal analgesic effect was observed 20 min after injection, but percentage inhibition was nearly constant with time up to 40 min after injection for each dose of antagonist used. Similar data were obtained for antagonism of etorphine by naloxone (not shown).

Dose–response curves obtained for the analgesic activity of etorphine either alone or in the presence of β_h-EP-(1–27) are displayed in Fig. 3*B*. Injection of various doses of etorphine together with fixed doses of β_h-EP-(1–27) produced a parallel shift of the dose–response curve of the opiate agonist to the right with increasing doses of peptide. Linear regression of the slope of the dose–response curves vs. AD_{50} gave a correlation coefficient of −0.037, indicating no detectable difference in slope between agonist alone and in the presence of various amounts of antagonist. A noncompetitive

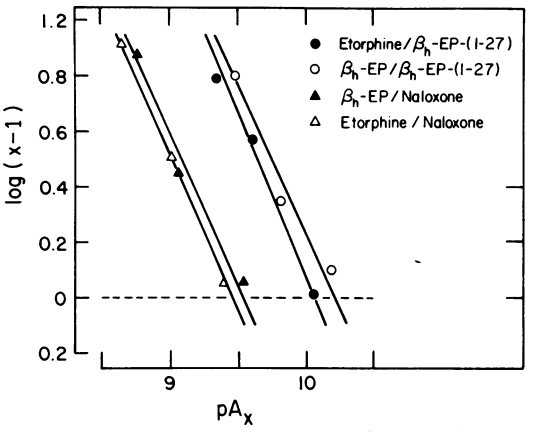

FIG. 4. Schild plots for antagonism of etorphine or analgesic effects of β_h-EP by naloxone or β_h-EP-(1–27).

antagonist would be expected to produce progressively flatter agonist dose–response curves as the dose of antagonist increases (21, 24). Similar results were obtained by injecting etorphine together with naloxone (Fig. 3*A*, Table 2). β_h-EP-(1–26) failed to demonstrate any inhibitory effect on β_h-EP- or etorphine-induced analgesia, even in doses of up to 500 pmol per animal (Fig. 3*B*, Table 2). From dose–response curves obtained for etorphine in the presence of either β_h-EP-(1–27) or naloxone, apparent AD_{50} and corresponding dose ratio x were calculated (Table 2). Since a parallel shift of the dose–response curve of a potent agonist in the presence of an antagonist is presumptive evidence for competitive inhibition, results were further analyzed by Schild plots. Fig. 4 shows the results obtained when using either β_h-EP-(1–27) or naloxone as antagonist and etorphine or β_h-EP as agonist. Linear relationships resulted in all cases. The corresponding apparent pA_2 values obtained from intercept on the abscissa and the slope of the best-fit line are reported in Table 3, which also gives the apparent antagonist potencies taking the potency of β_h-EP-(1–27) against β_h-EP as 100. The slope and $pA_2 - pA_{10}$ values were very close to those (−1.0 and 0.954) expected for competitive antagonism for each pair of agonist/antagonist examined.

DISCUSSION

Results presented herein are consistent with a regulatory role of β_h-EP-(1–27) in the modulation of antinociceptive effects elicited by opiates or opioid peptides. Low doses of β_h-EP-(1–27) reverse the analgesic action of the opiate etorphine and

Table 2. Antagonism of etorphine or β_h-EP analgesia by naloxone, β_h-EP-(1–27), and β_h-EP-(1–26)

Agonist	Antagonist	Dose of antagonist, pmol per mouse	AD_{50}, pmol per mouse	Slope	Dose ratio, x
Etorphine	Naloxone	0	18.0 [13.1–23.0]	1.41	1.00
		400	38.3 [34.2–42.6]	1.35	2.13
		1000	74.7 [45.0–139]	1.44	4.14
		2200	165 [151–179]	1.31	9.15
	β_h-EP-(1–27)	0	18.0 [13.1–23.0]	1.41	1.00
		88	36.8 [31.8–41.2]	1.50	2.04
		250	84.7 [52.7–118]	1.32	4.70
		450*	131 [94.3–189]	1.37	7.27
	β_h-EP-(1–26)	500	20.1 [14.2–26.3]	1.39	1.11
β_h-EP	β_h-EP-(1–26)	0	26.3 [17.2–37.1]	1.30	1.00
		500	24.1 [19.1–30.7]	1.33	0.91

Numbers in brackets are 95% confidence limits. Dose ratio is ratio of the AD_{50} of the agonist in the presence of antagonist to that in its absence.
*Corrected for intrinsic analgesic activity of the antagonist (11%).

Table 3. Relative antagonist potency of naloxone or β_h-EP-(1–27) in etorphine- or β_h-EP-induced analgesia

Agonist	Antagonist	pA_2	$pA_2 - pA_{10}$	Slope	Relative antagonist potency
β_h-EP*	β_h-EP-(1–27)	10.22 [10.03–10.41]	0.94	−0.96	100
	Naloxone	9.55 [9.41–9.68]	0.92	−1.06	21
Etorphine	β_h-EP-(1–27)	10.09 [9.92–10.26]	0.87	−1.12	74
	Naloxone	9.47 [9.35–9.59]	0.82	−1.16	18

Values in brackets are 95% confidence limits. Antagonist potency is relative to the combination of β_h-EP with β_h-EP-(1–27) and is calculated from respective apparent equilibrium association constants K_2 by the relation $\log K_2 = pA_2$.
*From ref. 24.

that of the opioid peptide β_h-EP. This inhibition is dose related and effective during the entire time course of analgesia induced by these highly potent agonists. Quantitative analysis of the inhibition gave linear Schild plots with slope and $pA_2 - pA_{10}$ values close to those expected for competitive antagonism at a single population of receptors. Although naloxone is a pure opiate antagonist, its antagonist potency is 1/4th to 1/5th that of β_h-EP-(1–27) in inhibiting etorphine or β_h-EP analgesia. In contrast, β_h-EP-(1–26) is inefficient in reversing analgesia, although this fragment retains the ability to displace [^{3}H]etorphine or [^{3}H]β_h-EP binding in rat brain membranes with affinity equal or greater than that of naloxone. Since β_h-EP-(1–27) and β_h-EP-(1–26) display no difference in selectivity for the opioid receptor subtypes in the brain, it has to be related to a change in antagonist efficacy upon removal of the tyrosine residue in position 27. In this respect, it may be of note that the nature of the residue in position 27 affects to a large extent the agonist potency of β_h-EP (25–28).

The endogenous inhibitory function of β_h-EP-(1–27) may be supported by the observation of differences in β-EP processing patterns among different brain regions (5–9). β-EP is a major component in rat hypothalamus, midbrain, amygdala, and anterior pituitary. Truncated and/or derivatized forms of β-EP occur as main components in the pars intermedia, the dorsal colliculi, the hippocampus, and the brainstem. However, such differences have to be interpreted cautiously on a physiological basis because the specific neural systems involved in analgesia have not been identified, although opioid receptors involved have been focused on pontine and brain stem loci (29). Moreover, great species variations occur. In human pituitary, the principal form of β-EP is the β-EP itself, with small quantities of β_h-EP-(1–27). The acetylated peptides are virtually absent, and β_h-EP-(1–26) does not exist (5). Nevertheless, our findings suggest a specific function of processing as a regulatory mechanism for opioid activity through generation of fragments that can either inhibit or inactivate antinociceptive properties of opioid peptides *in vivo*. Such a regulation of a peptide hormone by segments of the same hormone may be a general phenomenon and of great significance in the physiology of biologically active peptides.

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