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Opioid activity of a peptide, β-lipotropin-(61–91), derived from β-lipotropin

(β-endorphin/opiate receptor/enkephalin/prohormone)

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ABSTRACT The pituitary peptide β-lipotropin displays essentially no opioid activity in a preparation of guinea pig ileum or in the opiate receptor binding assay. However, a fragment, β-lipotropin-(61–91), with the enkephalin sequence (Tyr-Gly-Gly-Phe-Met) at its NH_2-terminus, has typical opioid effects in these two assays.

The discovery of pituitary peptides with morphine-like activity (endorphins) (1–3) and the observation that the morphine-like pentapeptide, enkephalin (4), from brain (H-Tyr-Gly-Gly-Phe-Met-OH) is identical with residues 61–65 of the pituitary peptide β-lipotropin (β-LPH) (5, 6) raised the question whether β-LPH or fragments of β-LPH have opioid activity‡. We have examined the opioid properties of native β-LPH, β-LPH-(61–91), and the synthetic fragments β-LPH-(41–58) (β-melanotropin, β-MSH) and β-LPH-(61–65) (methionine-enkephalin).

For bioassay we used a preparation of electrically stimulated myenteric plexus-longitudinal muscle from guinea pig ileum in a 5-ml bath, as described elsewhere (7, 8). Normorphine was used as reference standard. Inhibition of the twitch amplitude, with reversal or blockade by the opiate antagonist naloxone, is a specific property of the opiates. For the opiate receptor binding assay (9) we used a membrane fraction from guinea pig brain homogenate, $[^3H]$etorphine as primary ligand, and levorphanol as standard competing ligand. The stereospecific binding of $[^3H]$etorphine was the difference between bound radioactivity in the presence and that in the absence of levorphanol (10^{-6} M). Inhibition of this stereospecific binding by a test peptide was the measure of opioid activity. The isolation of ovine β-LPH and of β-LPH-(61–91) have been reported elsewhere (10, 11). Synthetic β-LPH-(61–91) was prepared by the solid-phase method (S. Lemaire, D. Yamashiro, and C. H. Li, manuscript in preparation). β-MSH [β-LPH-(41–58)] was also synthesized by the solid-phase method (12). Methionine-enkephalin [β-LPH-(61–65)] was prepared for us by Beckman Instruments Bioproducts Department (Palo Alto).

In the ileum preparation β-LPH was inactive at concentrations up to 10^{-6} M, but at higher concentrations a slowly developing inhibitory effect was observed in most preparations over a period of several minutes. This inhibition was reversed by naloxone. After withdrawal and lyophilization of the bath fluid, the dried residue was desalted on Bio-Gel-P2 in 50 mM ammonium formate, then fractionated on Bio-Gel-P6 in the same solvent, and each fraction was assayed for opioid activity in the receptor binding assay. On this column native β-LPH eluted at the void volume, but the opioid activity recovered from the bath fluid eluted later, at a volume corresponding to the elution of peptides of around 3000 daltons and smaller. This result suggests that the opioid activity seen in the ileum preparation with β-LPH resulted from cleavage to a smaller active fragment, possibly β-LPH-(61–91). Synthetic β-MSH exerted no agonist or antagonist effect on the ileum preparation at concentrations up to 10^{-5} M.

Definite opioid activity in the ileum preparation was displayed by β-LPH-(61–91) and also by methionine-enkephalin (Fig. 1). Spontaneous loss of inhibitory activity of enkephalin occurred to differing extents in different ileum preparations. Whenever considerable loss of enkephalin activity was observed, as in Fig. 1, there was also a slow loss of activity of β-LPH-(61–91), but in other preparations the inhibitory effect of β-LPH-(61–91) was quite stable. Using the procedure described by Kosterlitz and Watt (13), we found no significant antagonist activity with either β-LPH-(61–91) or enkephalin. Although the slopes of the logarithmic concentration-response curves for opiates sometimes differ from one ileum preparation to another, in any given preparation the slopes for normorphine

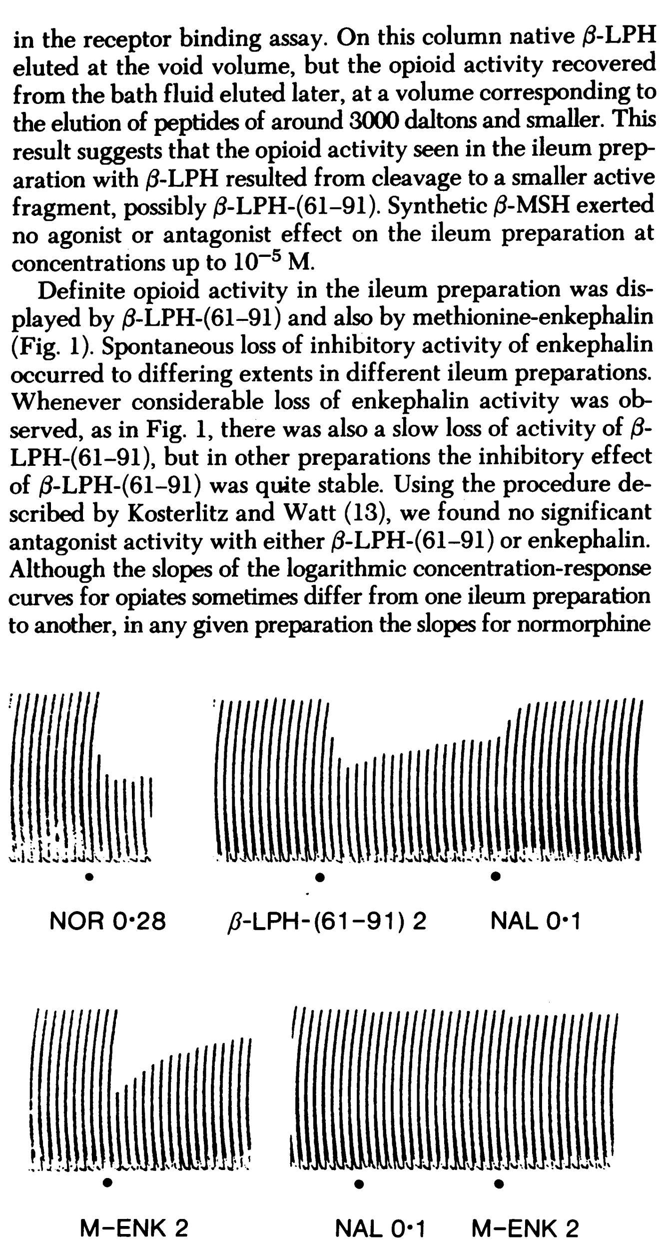

FIG. 1. Effects of β-LPH-(61–91) and of methionine-enkephalin in the ileum preparation. Longitudinal muscle with attached myenteric plexus from guinea pig ileum was mounted in 5-ml bath in bicarbonate Ringer's solution equilibrated with 95% O_2–5% CO_2 and subjected to field stimulation (80 V, 0.5 msec duration) every 6 sec. Details are described elsewhere (7, 8). NOR = normorphine; NAL = naloxone; M-ENK = methionine-enkephalin. Numbers following each symbol represent final bath concentrations (μM).

Abbreviations: β-LPH, β-lipotropin; β-MSH, β-melanotropin; IC_{50}, concentration giving 50% inhibition.

‡ There is now agreement among several groups of investigators to use the term *endorphin* (from *endo*genous and mo*rphine*) as a generic descriptor of peptides with opioid activity. *Enkephalin* refers to a specific pentapeptide isolated from brain; here we are concerned with methionine-enkephalin.

Table 1. Activity of the peptides in the opiate receptor binding assay

Substance	Final concentration (M)	Inhibition of stereospecific [^{3}H]etorphine binding (%)	IC_{50} (M)	Relative potency (normorphine = 1)
Exp. 1				
β-LPH (natural)	3×10^{-7}	1 ± 0.6	—	—
	1×10^{-6}	11 ± 2.9		
β-LPH-(61–91) (natural)	1×10^{-7}	60 ± 0.7	4.5×10^{-8}	5.3
	3×10^{-7}	73 ± 0.6		
	1×10^{-6}	83 ± 0.2		
Methionine-enkephalin (synthetic)	1×10^{-8}	16 ± 1.0	1.2×10^{-6}	0.20
	1×10^{-7}	30 ± 0.2		
	1×10^{-6}	47 ± 1.0		
	1×10^{-5}	66 ± 0.7		
Normorphine	7×10^{-8}	28 ± 0.8	2.4×10^{-7}	1.0
	7×10^{-7}	70 ± 0.9		
Exp. 2				
β-MSH (synthetic)	1×10^{-5}	4 ± 1.2	—	—
Exp. 3				
β-LPH-(61–91) (synthetic)	3×10^{-8}	4 ± 2.1	2.7×10^{-7}	1.7
	1×10^{-7}	37 ± 2.5		
	3×10^{-7}	51 ± 1.3		
Normorphine	3.5×10^{-7}	45 ± 1.6	4.4×10^{-7}	1.0
	7×10^{-7}	58 ± 2.1		
Exp. 4				
β-LPH-(61–91) (natural)	3×10^{-8}	43 ± 0.5	6.6×10^{-8}	5.8
	1×10^{-7}	54 ± 0.4		
	3×10^{-7}	66 ± 0.2		
	1×10^{-6}	76 ± 0.9		
β-LPH-(61–91) (synthetic)	3×10^{-8}	29 ± 0.6	1.5×10^{-7}	2.9
	1×10^{-7}	38 ± 1.3		
	3×10^{-7}	65 ± 1.6		
	1×10^{-6}	76 ± 0.0		
Normorphine	7×10^{-8}	26 ± 2.3	3.8×10^{-7}	1.0
	7×10^{-7}	58 ± 0.7		

Assay was conducted by a modification of the method of Simon *et al.* (9). The particulate fraction of guinea pig brain homogenate was suspended (1% wt/vol) in 0.1 M Tris·HCl buffer (pH 7.4) at 23°. Substances to be assayed were first incubated with the membranes for 5 min. Then [^{3}H]etorphine (2.5×10^{-9} M, 43 Ci/mmol) was added and incubation was continued for another 15 min. Incubation volume was 2 ml. Incubates were filtered through Whatman GF/B glass fiber filters, followed by three washes with 4 ml of ice-cold buffer. Radioactivity on the filters was determined by standard scintillation counting. Nonspecific binding of [^{3}H]etorphine was determined from the residual radioactivity in the presence of 10^{-6} M levorphanol. Stereospecific binding was the difference between radioactivity with [^{3}H]etorphine alone and that in the presence of levorphanol. In this system the (+) enantiomer, dextrorphan, was without effect upon [^{3}H]etorphine binding. Inhibition data are means ± SEM, based, in almost all instances, on triplicate binding assays (quadruplicates in Exp. 4).

and the peptides were similar (Fig. 2). β-LPH-(61–91) and enkephalin were approximately equipotent and were less active than normorphine. In seven preparations the potency of β-LPH-(61–91) relative to normorphine was 0.42 ± 0.13 (normorphine IC_{50} = 88 ± 21 nM; means ± SEM). In eight other preparations the potency of enkephalin relative to normorphine was 0.28 ± 0.04 (normorphine IC_{50} = 69 ± 13 nM), less than one-third that reported by Hughes *et al* (4).

The stereospecific binding of [^{3}H]etorphine was only very weakly inhibited at 10^{-6} M β-LPH (Table 1); it is possible that the brain homogenate, like the ileum preparation, can cleave the peptide, liberating active fragments. β-MSH was without effect at 10^{-5} M. β-LPH-(61–91) was several times more potent than normorphine. The difference between this result and the much lower potency in the bioassay remains unexplained; it could be related to permeability barriers in the ileum preparation. Enkephalin also inhibited the binding, but it was distinctly less potent than normorphine, as in the bioassay. The nonspecific binding of [^{3}H]etorphine, which is measured in the presence of 10^{-6} M levorphanol, was not affected by any of the peptides.

Our results show that native β-LPH-(1–91) has no opioid activity, but that such activity is generated by liberating β-LPH-(61–91), which contains the enkephalin sequence [β-LPH-(61–65)]. By contrast, β-MSH [β-LPH-(41–58)] is completely inactive. These findings indicate that a necessary condition for interaction with opiate receptors (14) may be a free NH_2 group on the tyrosyl residue in the opioid region. This requirement was also observed with a synthetic opioid heptapeptide (15). That this tyrosyl residue is preceded by a basic amino acid pair in β-LPH points to a physiologic mechanism for the generation of endorphin from β-LPH, as with the gen-

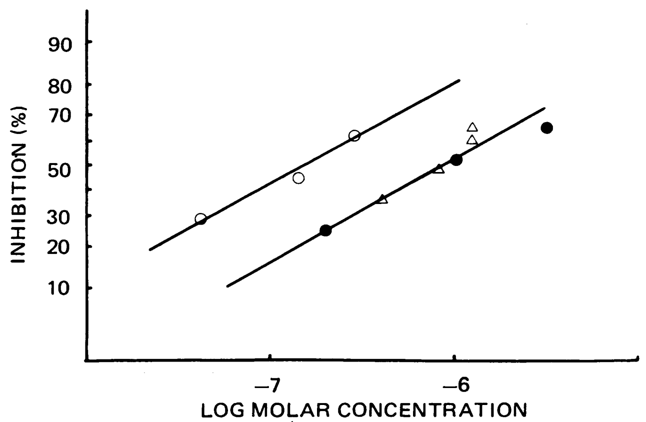

FIG. 2. Logarithmic dose-response curves in the ileum preparation. Naloxone-reversible inhibition of the electrically induced muscle twitch is plotted on a probability scale against $\log_{10}$ of the concentration in tissue bath. ○, normorphine; △, β-LPH-(61–91); ●, methionine-enkephalin. The peptides are approximately one-third as potent as normorphine in this preparation.

eration of insulin from proinsulin (16). Guillemin *et al.* (17) have noted already that β-LPH-(61–76) has opioid activity. It has also been shown (3) that active opioid fragments of several sizes can be generated during purification of native endorphin from pituitary extracts. Evidently, β-LPH is a prohormone that gives rise to endorphin, but the physiologic role of endorphin remains to be elucidated.

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