Vol. 89, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 13, 1979 Pages 786-792

β-ENDORPHIN: CHARACTERISTICS OF BINDING SITES IN THE RAT BRAIN

Pascual Ferrara, Richard Houghten and Choh Hao Li

Hormone Research Laboratory
University of California
San Francisco, CA 94143

Received June 7, 1979

SUMMARY: Stereospecific binding of human β-endorphin to rat membrane preparations is described for the first time using [^{3}H-Tyr^{27}]-β_h-endorphin as the ligand. The binding is time dependent and saturable with respect to β_h-endorphin with an apparent dissociation constant of 0.3 nM. Sodium ion (100 mM) elevates this value to 2.5 nM but has no effect on the total number of binding sites present in the membrane preparation. The ability of certain β-endorphin analogs, opiate agonists as well as antagonists to inhibit the binding of β_h-endorphin, is presented.

INTRODUCTION

Among various opioid peptides, β-endorphin (1) is the most active peptide when injected directly into the brain (2) and is the only peptide which exhibits potent analgesic activity by intravenous injection (3). There are no reports heretofore to describe the stereospecific interaction between this peptide and receptors in the central nervous system using β-endorphin as the primary ligand. This communication presents some characteristics of β-endorphin-binding sites in the rat brain.

MATERIALS AND METHODS

Membrane fractions from rat brain homogenates were prepared as described (4) with some modifications. Briefly, male Sprague-Dawley rats (180-220 g) were decapitated and their brains were removed. After removal of the cerebellum, each brain was homogenized in 30 ml buffer A (50 mM Tris buffer of pH 7.4) at 4° C by 10 strokes of a motor driven teflon plunger; the homogenate was

ABBREVIATIONS: β_h-EP, human β-endorphin; β_c-EP, camel β-endorphin; Met-EK, methionine enkephalin; BSA, bovine serum albumin; MBP, myelin basic protein

0006-291X/79/150786-07$01.00/0

centrifuged at 750 x g for 5 min and the supernatant was then centrifuged for 20 min at 25,000 x g. The pellet was resuspended in 35 ml of buffer A and incubated at 37° C for 30 min and re-centrifuged. This pellet was resuspended in 30 ml of buffer A and stored at -20° C until used. Binding was performed on 0.5 ml aliquots of the prepared homogenate in a plastic tube (12 x 75 mm, polystyrene). All determinations were performed in triplicate at 24° C at a final volume of 2 ml of buffer A plus 0.1% BSA. Membranes (0.6 mg of protein) were first incubated for 5 min with test drugs or peptides and with appropriate amounts of tritiated β_h-EP for an additional 25 min. The incubation was terminated by filtration under vacuum through glass fiber filters (Whatman GF-B) which had been previously soaked in buffer A plus 0.1% MBP at 24° C for 20 min. The filters were washed thrice with 5 ml cold buffer A plus 0.1% BSA and placed in 5 ml of PCS (Amersham/Searle: Phase-Combining-System). After standing at 24° C for 24 h, radioactivity was determined by liquid scintillation spectrometry. All values are expressed as specific tritiated β_h-EP bound obtained by the difference between the binding in the absence and presence of 2.5 µM of cold β_h-EP. Triplicate determinations were performed with a variation of less than 7%. The protein concentration was determined by the method of Lowry *et al.* (5) using BSA as standard.

[^{3}H-Tyr^{27}]-β_h-EP (50 Ci/mmol) was prepared as previously described (6). β_h-EP and β_c-EP-(6-31) were synthetic products as described (7,8). Met-enkephalin was a gift from Dr. J. Meienhofer. β_h-LPH was isolated from human pituitaries as described (9). Naloxone, levorphanol and dextrorphan were gifts from Dr. H. H. Loh. BSA was purchased from Sigma. Myelin basic protein was isolated from ox brains as previously described (10).

RESULTS AND DISCUSSION

The use of plastic tubes and 0.1% BSA in the incubation buffer prevented the adsorption of β_h-EP to the tube walls. Under these conditions, it was possible to recover nearly 100% of the tritiated β_h-EP added to the reaction mixture, when an aliquot was counted after an incubation of 30 min. The nonspecific binding of the radioactive peptide to the filters was avoided by soaking the filters, prior to use, in buffer A plus 0.1% MBP. It was found that this basic protein was very effective in preventing the nonspecific binding of the tritiated β_h-EP to the filters (Table I).

The binding of tritiated β_h-EP to brain membrane preparation was detected at very low concentrations (10^{-10} M) of the

TABLE I

Nonspecific Binding of
[^{3}H-Tyr^{27}]-β_h-Endorphin in the Filter

Preincubation	cpm
none	22,500 ± 350
0.1% BSA	21,500 ± 215
1.0% BSA	17,373 ± 205
0.1% MBP	183 ± 12

2 ml of tritiated β_h-EP with 35,000 cpm passed through Whatman GF-B fiber glass filters before washing thrice with cold buffer A plus 0.1% BSA. Values in mean ± SE from 5 determinations.

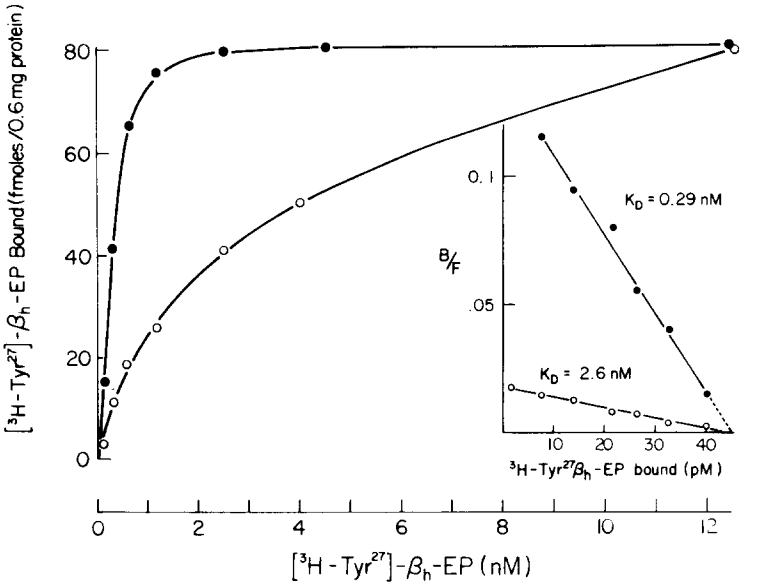

Figure 1. Specific binding of [^{3}H-Tyr^{27}]-β_h-EP to rat brain membrane preparations as a function of [^{3}H-Tyr^{27}]-β_h-EP concentration in absence (●—●) and presence (o—o) of 100 mM NaCl. Binding assay was performed as described in Materials and Methods. Values are means of triplicate incubations. Inset: Scatchard plot of [^{3}H-Tyr^{27}]-β_h-EP binding to rat brain membranes preparation. The slope of the plot, which gives a K_D value, was determined by linear regression analysis.

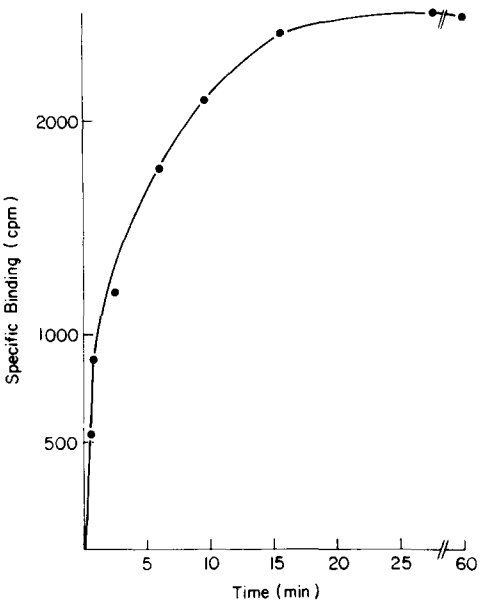

Figure 2. [^{3}H-Tyr^{27}]-β_h-EP binding to rat brain membranes preparation as a function of time. [^{3}H-Tyr^{27}]-β_h-EP (0.58 nM) was incubated with membranes (~0.6 mg of membrane protein) for the indicated times at 24° C and specific binding was measured. Each value was the mean of triplicate determinations.

peptide (Fig. 1). The β-endorphin binding sites in the membrane (0.6 mg of protein) are saturated at 2.5 x 10^{-9} M with half maximal saturation at 0.3 x 10^{-9} M. When analyzed by Scatchard plot, a value for K_D was found to be 0.29 x 10^{-9} M with a maximal binding capacity of these membrane preparations of .15 pmole of β_h-EP per mg of membrane protein. In the presence of sodium ion (100 mM), the Scatchard plots of data (Fig. 1) gave the same maximal binding capacity but gave a K_D value of approximately 2.5 x 10^{-9} M.

The specific binding of tritiated β_h-EP to the membranes is a time dependent process which reached a plateau in 20 min (Fig. 2). The concentration of receptors was calculated to be 0.045 nM based upon the amount of tritiated β_h-EP specifically bound at saturation. From these data, it was possible to compute

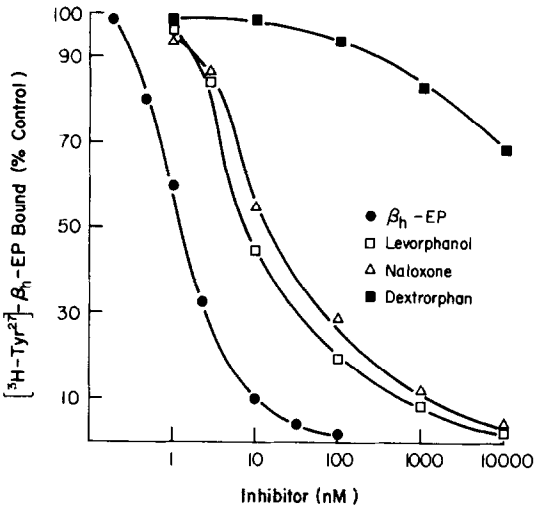

Figure 3. Competition of the binding of [^{3}H-Tyr^{27}]-β_h-EP to rat brain membrane preparation by β_h-EP (●—●), levorphanol (□ —□), dextrorphan (■ —■) and naloxone (Δ—Δ). Membranes were incubated with different amounts of inhibitors for 5 min; [^{3}H-Tyr^{27}]-β_h-EP (0.58 nM) was added. After 25 min, the membranes were collected on Whatman glass fiber filters (GF-B) and washed 3 times with 5 ml of cold buffer A, plus 0.1% BSA and the binding was measured as described under Materials and Methods.

the rate constant (k_1) of β_h-EP-receptor association to be 0.34 nM^{-1} min^{-1} at 24° C.

The inhibition of tritiated β_h-EP binding by naloxone, levorphanol and dextrorphan was shown in Fig. 3. Levorphanol had an activity of 18% when compared with β_h-EP, but had at least 10,000 times more potency than dextrorphan (Table II). Naloxone was 7.5% as active when compared with β_h-EP.

Fig. 4 presents the displacement curve of tritiated β_h-EP obtained with β_h-EP, Met-EK, β_c-EP-(6-31) and β_h-LPH. As shown in Table II, Met-EK had only 5% potency in comparison with β_h-EP, and the activities of β_h-EP-(6-31) and β_h-LPH were minimal. These results correlate well with that obtained in vivo for their analgesic potencies (2,8). It is proposed that the stereospecific

TABLE II

Relative Potency of Opiates and Opioid Peptides by Receptor Binding Assay using Tritiated β_h-Endorphin as the Primary Ligand

Compound	IC_{50} [a]	Relative Potency
β_h-Endorphin	0.6×10^{-9}	100
Levorphanol	3.4×10^{-9}	18
Dextrorphan	1.5×10^{-5}	<<0.01
Naloxone	8.0×10^{-9}	7.5
Met-EK	1.0×10^{-8}	5.0
β_c-EP-(6-31)	4.0×10^{-6}	0.01
β_h-LPH	1.0×10^{-7}	0.06

[a] 50% inhibiting concentration in M (see Figures 3 and 4).

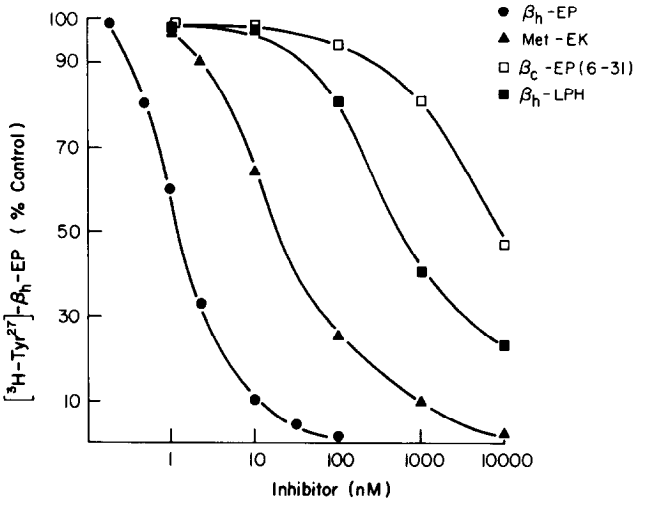

Figure 4. Competition of the binding of [^{3}H-Tyr^{27}]-β_h-EP to rat brain membrane preparation by β_h-EP (●—●), Met-enkephalin (▲—▲), β_c-EP-(6-31) (□ —□) and β_h-LPH (■ —■). Details are described in Figure 3.

high affinity binding sites for tritiated β_h-EP in rat brain membrane preparations may be used as radioreceptor assay for β-endorphin and other opioid peptides.

ACKNOWLEDGEMENT: We thank J. Ramachandran and P. Y. Law for advice. This work was supported in part by the National Institute of Mental Health Grant MH-30245.

REFERENCES

1. Li, C. H. and Chung, D. (1976) Proc. Nat. Acad. Sci. USA 73, 1145-1148.
2. Loh, H. H., Tseng, L-F., Wei, E. and Li, C. H. (1976) Proc. Nat. Acad. Sci. USA 73, 2895-2898.
3. Tseng, L-F., Loh, H. H. and Li, C. H. (1976) Nature 263, 239-240.
4. Pasternak, G. W., Wilson, H. A. and Snyder, S. M. (1975) Mol. Pharmacol. 11, 380-351.
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
6. Houghten, R. A. and Li, C. H. (1978) Int. J. Peptide Prot. Res. 12, 325-326.
7. Li, C. H., Yamashiro, D., Tseng, L-F., Loh, H. H. (1977) J. Med. Chem. 20, 325-328.
8. Li, C. H., Yamashiro, D., Tseng, L-F. and Loh, H. H. (1978) Int. J. Pept. Prot. Res. 11, 154-158.
9. Li, C. H. and Chung, D. (1976) Nature 260, 623-624.
10. Swann, R. and Li, C. H. (1979) Int. J. Peptide Prot. Res. in press.