β-ENDORPHIN: LACK OF CORRELATION BETWEEN OPIATE ACTIVITY AND IMMUNOREACTIVITY BY RADIOIMMUNOASSAY

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SUMMARY: A sensitive radioimmunoassay for human β-endorphin has been developed. When natural human β-lipotropin and various synthetic analogs of β-endorphin were assayed for their immunoreactivity, lack of correlation was found between opiate activity and immunoreactivity. These data suggest that residues 6-15 of β-endorphin are the antigenic determinant.

Among morphine-like peptides from brain and pituitary extracts, β-EP* (1) is the only one to possess analgesic activity by iv injection (2). We have now developed a RIA for β_h-EP (Figure 1), and shown that its immunoreactivity does not correlate with opiate activity.

Experimental

β_h- and β_c-EP were synthetic products as previously described (3,4). β_c-EP-(20-31), β_c-EP-(6-31) and β_h-EP-(1-5)-(16-31) were synthesized by the solid-phase method (D. Yamashiro and C. H. Li, manuscripts in preparation). Opiate activity was measured from the depression of electrically-stimulated contractions of the guinea pig ileum prepared as previously described (5). Met-enkephalin was a gift from Dr. J. Meienhofer of Hoffmann-La Roche.

β_h-EP was conjugated with human γ-globulin by a published procedure (6). Rabbits and guinea pigs were immunized with the conjugate according to the procedure of Vaitukaitis et al. (7). The antigen was dissolved in 0.01 M phosphate buffer of pH 7.5 and emulsified with complete Freund's adjuvant. The emulsion was injected at multiple sites on the backs of the animals, which had also received 0.5 ml of Petrussis vaccine (Eli Lilly Co) two days prior to the administration of the antigen (7). Each animal received a total of one mg of antigen over a period of five weeks, after which they were test bled, by ear vein for rabbits and cardiac puncture for guinea pigs.

*Abbreviations: β-EP, β-endorphin (subscripts 'h' and 'c' indicate β-EP from human and camel pituitaries); β_h-LPH, human β-lipotropin; β_c-EP-(20-31), β_c-EP (20-31)-dodecapeptide; β_c-EP-(6-31), β_c-EP-(6-31)-hexacosapeptide; β_h-EP-(1-5)-(16-31), β_h-EP-(1-5)-(16-31)-heneicosapeptide; RIA, radioimmunoassay; iv, intravenous.

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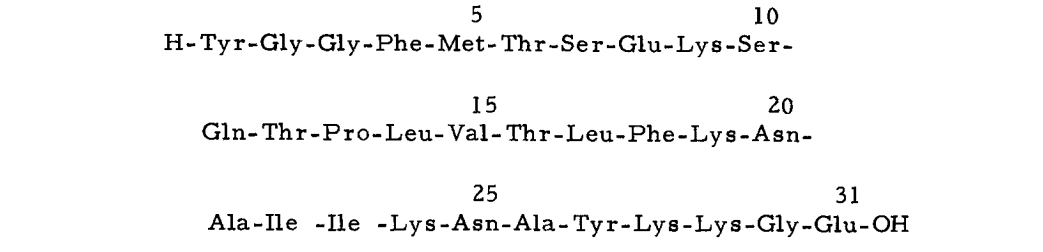

Fig. 1 Amino acid sequence of β_h-EP.

The serum was checked for the presence of antibodies by testing its ability to bind ^{125}I-labeled β_h-EP which was prepared by the lactoperoxidase method as described (8). The specific activity of the iodinated hormone was 85 mCi/mg. Separation of the free iodine and iodinated hormone was done by chromatography on Sephadex G-25 equilibrated with 0.1 N acetic acid.

For RIA, approximately 7000-8000 cpm of [^{125}I]-β_h-EP in 0.1 ml, various quantities of unlabeled β_h-EP, or other related peptides in 0.1 ml were incubated with 0.1 ml of antiserum to β_h-EP in cold at 4°C for a period of 16-20 hr. All dilutions for RIA were carried out in 0.01 M sodium phosphate buffer pH 7.5 containing 0.15 M NaCl, 1% bovine serum albumin and 0.33% EDTA in 12 x 75 glass tubes. Separation of the bound and free hormone was achieved by addition of 0.5 ml of phosphate buffer pH 7.5 containing 20 mg Norit and 10 mg Dextran T-70 (Pharmacia, Uppsala) per ml. The tubes were allowed to stand in cold for ten min after which they were centrifuged for 30 min in cold at 5000 rpm. 0.5 ml of the clear supernatant was mixed with 5 ml of Scintillation fluid (PCS) and counted in a Packard beta counter. The procedure for counting gamma radiation by liquid scintillation spectrophotometry was essentially that of Herscowitz and McKillip (9).

Results and Discussion

Of the three rabbits and five guinea pigs immunized, one rabbit and two guinea pigs responded with production of antibodies. However it was noted that the guinea pig antiserum was generally of higher titer than the rabbit antiserum. For the studies reported herein, antiserum from guinea pig No. 2 was used at a final dilution of 1:3000.

Purification of [^{125}I]-β_h-EP on Sephadex G-25 in 0.1 M acetic acid gave three fractions: the first two represented radioactive β_h-EP as judged by binding tests and the third was found to be free iodine. Only the second fraction

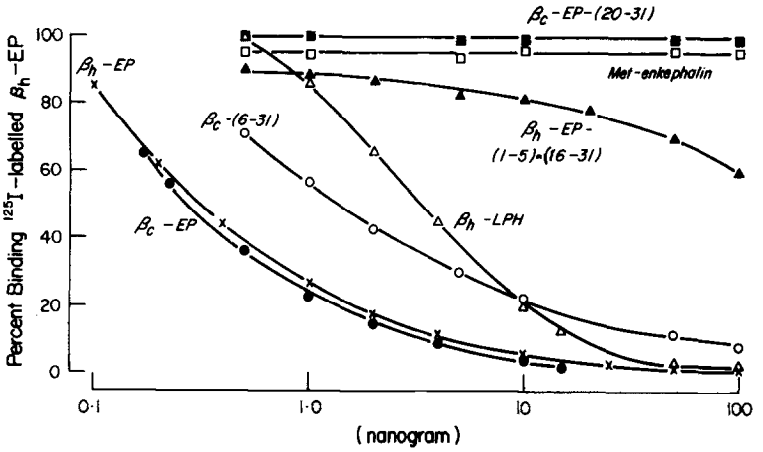

Fig. 2 Competition of β_c-EP, β_c-EP-(6-31), β_h-LPH, β_h-EP-(1-5)-(16-31), β_c-EP-(20-31) and met-enkephalin in the β_h-EP RIA. Final dilution of antiserum was 1:3,000.

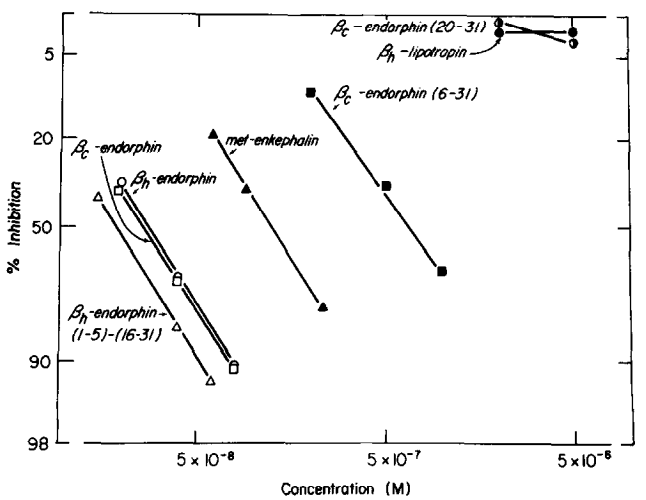

Fig. 3 Guinea pig ileum bioassay of β-EP and its fragments. IC_{50} (50% inhibiting concentration) of β_h- and β_c-EP were 2.70 and 2.69 x 10^{-8}M, respectively. The relative potencies of the peptides were as follows: β_h- and β_c-EP, 1.00; met-enkephalin, 0.26; β_c-EP-(6-31), 0.04; β_h-EP-(1-5)-(16-31), 1.35; β_c-EP-(20-31), < 0.001; β_h-LPH, < 0.001. The inhibition of ileum contraction by β_c-EP-(6-31) appears not to act through the opiate receptor as its effect was of unusually short duration (< 12 sec) and was not blocked by the specific opiate antagonist, naloxone.

was found to be suitable for RIA studies because of the low blank value (<2%) while the first fraction gave blank value up to 25%.

As shown in Figure 2, the sensitivity of RIA for β_h-EP was in the range of 0.1- 10 ng. β_c-EP, which differs from the amino acid sequence of the human hormone in only two residue positions: 27 and 31 (10), gave a completely parallel and almost identical inhibition curve. β_c-EP-(6-31) also exhibited a parallel inhibition curve but had 40% immunoreactivity as compared with β_c-EP. On the other hand, β_c-EP-(1-5)-(16-31) showed very weak cross reaction. It may be noted that β_h-LPH, which contains the complete amino acid sequence of β_h-EP, had only 10% immunoreactivity. Figure 2 also shows that β_c-EP-(20-31) and met-enkephalin, β_c-EP-(1-5), did not show any cross reactivity. Thus, it is probable that residues 6-15 of β_c-EP represent the antigenic determinant of β-EP.

Figure 3 presents logarithmic dose-response curves in the ileum preparation. It is evident that β_c-EP-(1-5)-(16-31) is more active than the parent molecule, yet it shows very weak immunoreactivity by RIA (see Figure 2). Met-enkephalin possesses significant in vitro opiate activity but shows no immunoreactivity. β_c-EP-(6-31) has considerable immunoreactivity but has, perhaps nonspecific, opiate activity. Interestingly, β_h-LPH is immunoreactive but has very low opiate activity. These data clearly show that there is no correlation between opiate activity and immunoreactivity.

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