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β-ENDORPHIN: EVIDENCE FOR THE EXISTENCE OF OPIOID AND NON-OPIOID BINDING COMPONENTS FOR THE TRITIATED HUMAN HORMONE IN NG108-15 CELLS

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SUMMARY: Human β-endorphin (β_h-EP) binding on neuroblastoma x glioma hybrid NG108-15 cells using tritiated human beta endorphin (^{3}H-β_h-EP) as a primary ligand was found to have a component which was not displacable with [D-Ser^2]-Leu-enkephalin-Thr^6 (DSLET). The β_h-EP binding on these cells after saturation of the δ opiate sites with 200 nM DSLET was further characterized with synthetic β_h-EP analogs. The non-opioid binding site appears to recognize β_h-EP-(6-31), β_h-EP-(21-31) and β_h-EP-(28-31). Under these conditions, these COOH-terminal segments fully displace the tritiated β_h-EP. However, β_h-EP-(1-27) does not further displace ^{3}H-β_h-EP in the presence of DSLET. The fact that a combination of DSLET and β_h-EP-(6-31) results in a full displacement of ^{3}H-β_h-EP provides direct evidence for the existence of two binding sites for β_h-EP in NG108-15 cells, one recognizing the NH_2-terminal enkephalin sequence and the other the non-opioid COOH-terminal segment.

The neuroblastoma x glioma hybrid cell line NG108-15 nas been used widely in opiate pharmacology (1,2) and was found to possess mainly δ opiate receptors (2). The effects of various opioids and opiates on cAMP levels as well as their binding characteristics have been studied extensively (3,4). In a previous study we reported the characteristics of binding of ^{3}H-β_h-EP to these cells (5) and found that, in contrast to the human hormone, enkephalin and camel β-EP cannot displace bound ^{3}H-β_h-EP completely. It was speculated that there could be a

ABBREVIATIONS: β_h-EP, human β-endorphin; β_h-EP-(6-31), synthetic analog of β_h-EP with the sequence of 6-31 etc.; ^{3}H-β_h-EP, [$^3H_2$-Tyr^{1,27}]-β_h-EP; cAMP, cyclic adenosine monophosphate; DSLET, [D-Ser^2]-Leu-enkephalin-Thr^6; FCS, fetal calf serum; BSA, bovine serum albumin.

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non-opioid interaction with these cells. In view of our recent studies with human glioblastoma and neuroblastoma cells on non-opioid binding sites for β-EP which are specific for the COOH-terminal segment of the human hormone (6,7), we decided to investigate and characterize further the ^{3}H-β_h-EP binding on NG108-15 cells in the presence of a δ-selective opioid ligand [D-Ser^{2}]-Leu-enkephalin-Thr^{6} (DSLET) (8).

MATERIALS and METHODS

β_h-EP, β_h-EP-(6-31), β_h-EP-(1-27), β_h-EP-(1-5)-(16-31), β_h-EP-(21-31) and β_h-EP-(28-31) were synthetic products (9-11), DSLET was purchased from Sigma and ^{3}H-β_h-EP was prepared as described (12). NG108-15 cells were kindly provided by Dr. M. Nirenberg and used from passage 19-25 in this study. The cells were maintained in Dulbecco's modified Eagle medium (DME-H21) supplemented with pyruvate (1.0 mM), pen-strep (0.01 KU), glutamine (2 mM) and 10% FCS at 8% CO_2 in humidified atmosphere.

The binding assay was performed as previously described (6) with cells detached with the aid of Ca^{++}, Mg^{++} free phosphate buffered saline with 0.04 % EDTA. The cells were suspended in 0.025 M Tris-HCl, 0.1% BSA, 10% sucrose and 0.01% bacitracin. Two ml of this suspension which is equivalent to 10^{6} cells were incubated with cold peptide and tracer and separated on polyethylene amine (0.3% in 50 mM Tris-HCl) pretreated glass fiber filters (Whatman GF/B) after 70 min of incubation at 22°C. Each filter was washed with 10 ml of cold 0.05 M Tris-HCl buffer containing 0.1% BSA. After soaking the filters for 8 h in a premixed scintillation cocktail (Hydrofluor, National Diagnostics), they were counted on a Packard liquid scintillation counter.

Computerized curve fitting and relative potency calculation was performed with the help of a HP-85 desk top computer using a program based on statistical methods published elsewhere (13,14).

RESULTS AND DISCUSSION

NG108-15 cells were incubated with β_h-EP, DSLET, β_h-EP-(6-31), β_h-EP-(1-5)-(16-31) and a combination of DSLET (200 nM) plus β_h-EP-(6-31), β_h-EP-(21-31), β_h-EP-(28-31) and β_h-EP-(1-5)-(16-31) or β_h-EP-(1-27). The results are presented in Fig. 1 and summarized in Table 1. They show, that both DSLET and β_h-EP-(6-31) displace about 50% of ^{3}H-β_h-EP from the cells. Only β_h-EP itself and β_h-EP-(1-5)-

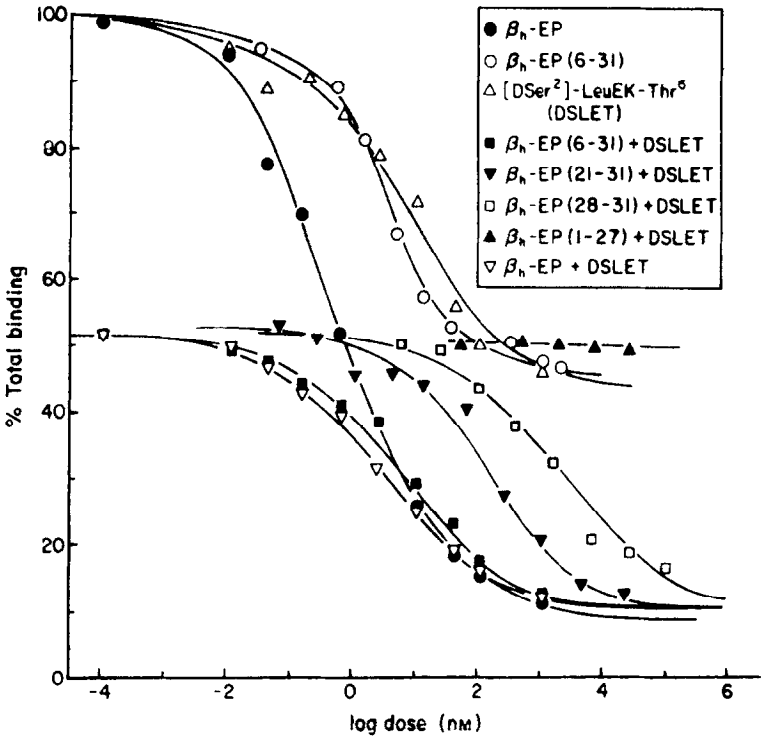

Figure 1. Computerized analysis of ^{3}H-β_h-EP binding data with NG108-15 cells. A 2 ml suspension of 10^6 cells were incubated with 0.5 nM of tritiated ligand and increasing doses of cold peptides for 70 min at 22°C.

TABLE 1

Relative Potency of β_h-EP and Analogues in Displacing Tritiated β_h-EP from NG108-15 Neuroblastoma x Glioma Cells

Synthetic Peptides	IC_{50}†	Relative Potency*
β_h-EP	0.50 (0.45-0.54)	100
β_h-EP-(6-31)	5.50 (4.0-7.9)	9
β_h-EP-(1-5)-(16-31)	0.43 (0.21-0.68)	116
DSLET	4.75	10.5
DSLET (200 nM)		
+β_h-EP	2.2 (1.7-2.7)	100
+β_h-EP-(6-31)	5.0 (3.1-8.4)	44
+β_h-EP-(21-31)	82.6 (47-153)	3
+β_h-EP-(28-31)	420 (230-750)	0.5
+β_h-EP-(1-27)	>2500	nil
+β_h-EP-(1-5)-(16-31)	0.91 (0.73-1.14)	240

†nM (95% confidence limit)
*Relative potency based on the IC_{50} for the portion of ^{3}H-β_h-EP binding which was displaced by these peptides.

(16-31) (not shown) give a full displacement curve. The relative potency of DSLET and β_h-EP-(6-31) alone is based on the ED_{50} from their 50% displacement and compared to the potency of β_h-EP for its complete displacement curve. In the presence of 200 nM DSLET, a full displacement by β_h-EP-(6-31) can be achieved, but not with β_h-EP-(1-27).

The ^3H-β_h-EP binding to NG108-15 cells which remains after the δ-receptors have been saturated with 200 nM DSLET was further characterized with β_h-EP and its COOH-terminal fragments. The relative potency was reduced as the fragments became shorter, but β_h-EP-(28-31) still fully displaces the tritiated ligand (Fig. 1, Table 1). In this set of binding curves the relative potency of the β_h-EP fragments is compared to β_h-EP in the presence of DSLET and this explains why the relative potency for β_h-EP-(6-31) increases about five-fold and is now only half of that of β_h-EP. The relative potency of β_h-EP-(1-5)-(16-31) increases about two-fold. This increase of potency was also observed in human glioblastoma cells (6).

This study shows for the first time that β_h-EP has two distinct binding activities in NG108-15 cells and the binding domains are located at either end of its primary structure. We have previously shown that the non-opioid binding sites for β_h-EP occurs in different human cell lines (6,7) and also in another mouse neuroblastoma cell line N18TG2 (15). Whereas the opioid binding to the NG108-15 cells is linked to inhibition of the adenylate cyclase (3,4), no function has yet been connected with the β_h-EP binding in cells which have COOH-terminally mediated binding site. It is possible that these non-opioid binding sites are related to the immunosystem as similar binding sites were shown on human complement (16)

and are considered to be involved in the lymphoproliferative response (17).

The presence of specific cell surface binding sites for the non-opioid COOH-terminal segment of β_h-EP seems to be a much more common phenomenon than originally thought. It should be noted that the opioid portion of the ^3H-β_h-EP binding varied within this series of experiments and was reduced to 20% in one experiment done at passage 26. It seems likely that the presence of non-opioid sites on the NG108-15 hybrid cells is a much more natural and stable phenomenon than the occurrence of opiate binding sites, particularly in the view of the characterization of similar sites in the parental N18TG2 line (15).

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