Human β -endorphin: Specific binding in neuroblastoma N18TG2 cells

[opiate receptor/human β -endorphin-(6-31)/morphine/enkephalin]

R. GLENN HAMMONDS, JR., AND CHOH HAO LI

Hormone Research Laboratory, University of California, San Francisco, California 94143

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ABSTRACT The mouse neuroblastoma N18TG2 has specific, saturable binding sites for human β -endorphin (β -EP). The affinity and number of sites are 1.1 nM and 280,000 per cell, respectively. β -EP binding is not inhibited by [Leu]enkephalin or morphine at concentrations up to 0.1 mM; β -EP-(6-31) is a potent inhibitor of binding, and camel β -EP is much less potent. The data suggest the importance of the nonenkephalin segment of the β -EP molecule for interaction with the binding site in N18TG2 cells.

We have previously reported the binding of β -endorphin (β -EP) in NG108-15 cells to be homogeneous by the criterion of Scatchard analysis but clearly heterogeneous by displacement with enkephalins (1). In broadening the scope of our studies to include the two parent cell lines (N18TG2 and C6) that gave rise to NG108-15 cells (2), we have found that the N18TG2 cell (2) has binding sites for β -EP but not for [Leu]enkephalin (EK) whereas the C6 cell (3) does not bind either [Leu]EK or β -EP.

MATERIALS AND METHODS

^3H -Labeled β -EP (^3H - β -EP; 50 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and [$^3\text{H}_2$ -Tyr¹][Leu]enkephalin (^3H -EK) (50 Ci/mmol) were prepared by catalytic tritiation of the corresponding synthetic diiodo derivatives (4). β -EP, EK, and β -EP-(6-31) were synthetic products (5). Morphine sulfate was obtained from Mallinckrodt. Tissue culture supplies were obtained from the Tissue Culture Center of this University.

NG108-15 cells were a gift from Sydney Udenfriend (Roche Institute for Molecular Biology). N18TG2 and C6 cells were obtained from the Tissue Culture Center of this University. All cells were grown in 75-cm² flasks in Dulbecco's modified Eagle's medium (H21 medium) supplemented with 10% fetal bovine serum, 2 mM glutamine, 1 mM pyruvate, penicillin G at 10 units/ml, and streptomycin at 10 $\mu\text{g}/\text{ml}$ in a 90% air/10% CO₂ atmosphere. NG108-15 cells also received 0.1 mM hypoxanthine, 1 μM aminopterin, and 16 μM thymidine. N18TG2 cells received 80 μM 6-thioguanine. For suspension binding assays, cells were harvested, washed with Ca²⁺-, Mg²⁺-free phosphate-buffered saline, and transferred to the assay buffer (290 mM sucrose/25 mM Tris-HCl, pH 7.5/0.1% bovine serum albumin/0.1% bacitracin). Cells ($1-5 \times 10^5/\text{ml}$) and labeled peptides were incubated together for 30 min at 24°C in the presence or absence of unlabeled ligand in plastic tubes. Assays were terminated by filtering and washing on glass fiber filters presoaked in 0.1% myelin basic protein in assay buffer. The filters were assayed after incubation overnight in scintillation fluid.

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FIG. 1. Total and nondisplaceable binding of 1 nM ^3H - β -EP (Left) and 3 nM ^3H -EK (Right) to NG108-15, N18TG2, and C6 cells.

RESULTS AND DISCUSSION

Fig. 1 shows the total binding of ^3H -EK and β - ^3H -EP in C6, N18TG2, and NG108-15 cell lines and that portion of binding remaining in the presence of excess unlabeled EK [Leu]EK and β -EP. No displaceable binding of either peptide was detected in the C6 cell line. No displaceable binding of EK was seen in N18TG2. NG108-15 bound both EK and β -EP as reported (1). Although unlabeled β -EP could completely displace ^3H -EK binding, unlabeled EK could only partially displace β - ^3H -EP binding in NG108-15 cells (1). β - ^3H -EP bound to N18TG2 was not displaced by excess unlabeled EK. Results of repeated Scatchard analysis of binding of β - ^3H -EP to N18TG2 cells were consistent with a single affinity class of binding sites with an apparent K_d of 1.1 ± 0.4 nM and a density of $280,000 \pm 90,000$ sites per cell (three experiments).

Fig. 2 shows the ability of several compounds to displace β - ^3H -EP binding in N18TG2 cells; data from three experiments are presented, and thus this figure shows intraassay as well as interassay variability. Neither morphine nor EK could displace, even at concentrations of 0.1 mM. The 6-31 fragment of β -EP, lacking the enkephalin segment, was of the same order of potency as β -EP. β_c -EP, differing from β -EP in two residue positions, was much less potent than β -EP. One or a combination of these residues may be important in maintaining high affinity to this binding site. N18TG2 is of mouse origin, and the available evidence suggests that mouse β -EP is similar to camel β -EP rather than to human (6). The higher affinity of β -EP is unexpected in this sense.

Abbreviations: β -EP, human β -endorphin; β_c -EP, camel β -endorphin; β - ^3H -EP, β -[$^3\text{H}_2$ -Tyr²⁷]endorphin; ^3H -EK, [$^3\text{H}_2$ -Tyr¹]-[Leu]enkephalin; EK, [Leu]enkephalin.

FIG. 2. Inhibition of ^3H - β_h -EP binding to N18TG2 cells. N18TG2 cells and ^3H - β_h -EP (0.8 nM) were incubated with increasing amounts of the indicated compounds. Percent binding was calculated as $100 \times (B_s - B_n)/(B_0 - B_n)$, in which B_0 is the amount bound in the absence of competing compounds, B_s is the amount bound in the presence of a concentration x of competing compound, and B_n is the nonspecific binding, in this case the binding in the presence of $1 \mu\text{M}$ β_h -EP. The apparent IC_{50} values (concentration for 50% inhibition) are β_h -EP, 0.6 nM; β_h -EP-(6-31), 2.5 nM; β_c -EP, 200 nM; morphine and EK, $>100 \mu\text{M}$.

Although stereospecific opiate receptors in N18TG2 have been reported (7), our results show that the binding site of β_h -EP on N18TG2 cells is probably not an opiate receptor because neither morphine nor EK could reduce binding, and a synthetic peptide [β_h -EP-(6-31)] lacking the enkephalin segment retained high potency. The relationship between the β -EP sites and the opiate sites described previously (7) is not clear. Because binding assays for naloxone used a buffer containing high concentrations of sodium, and assays for dihydromorphine used cell membranes rather than whole cell (7), comparisons with the

present work should be made cautiously. The number of binding sites reported for opiates in N18TG2 is only 20% of that found for β_h -EP and the affinity of naloxone and dihydromorphine is 1/30th to 1/20th the affinity of β_h -EP (7). The presence of β_h -EP-specific binding sites in conjunction with opiate receptors, with an excess of β_h -EP sites to opiate sites, is a feature of both NG108-15 and N18TG2 cells as reported here.

Although specific binding sites for β_h -EP in N18TG2 cells have been demonstrated, their relationship to opiate receptors in the same cells and in other preparations is not known, nor is their function. The possibility that these sites are related to the opiate receptor of rat vas deferens (8-10) remains of interest.

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1. Hammonds, R. G., Jr., Ferrara, P. & Li, C. H. (1981) *Proc. Natl. Acad. Sci. USA* 78, 2218-2220.
2. Minna, J., Glazer, D. & Nirenberg, M. (1972) *Nature (London)* 235, 225-231.
3. Benda, P., Lightbody, S., Sato, G., Levine, L. & Sweet, W. (1968) *Science* 161, 370-371.
4. Houghten, R. A., Chang, W.-C. & Li, C. H. (1980) *Int. J. Pept. Protein Res.* 16, 311-320.
5. Li, C. H., Yamashiro, D., Tseng, L.-F. & Loh, H. H. (1977) *J. Med. Chem.* 20, 325-328.
6. Eipper, B. A. & Mains, R. E. (1979) *J. Biol. Chem.* 254, 10190-10199.
7. Law, P. Y., Herz, A. & Loh, H. H. (1979) *J. Neurochem.* 33, 1177-1187.
8. Lemaire, S., Magnan, J. & Regoli, D. (1978) *Br. J. Pharmacol.* 64, 327-329.
9. Schulz, R., Faase, E., Wuster, M. & Herz, A. (1979) *Life Sci.* 24, 843-850.
10. Huidobro, F., Huidobro-Toro, J. P. & Miranda, H. (1980) *Br. J. Pharmacol.* 70, 519-525.