



# Characterization of Union Sites for Opioids in Human Lymphocyte Membranes

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## Abstract

The opioid peptides Methionine enkephalin and  $\beta$ -Endorphin are stimulators of Natural Killer cytolytic activity. To know the biochemical mechanisms that explain this action, the binding of Methionine enkephalin and Naloxone in presence or absence of  $\text{Na}^+$  ion and GTP to human Lymphocyte membranes was characterized.

Methionine enkephalin or  $\beta$ -Endorphin in concentrations greater than physiological concentrations delivers stimulations of the order of 25% on the respective control value. For the receptors, a binding assay of the radioligands ( $^3\text{H}$ )-Methionine enkephalin and ( $^3\text{H}$ )-Naloxone was used, with the Scatchard analysis finding only one type of binding site in each case, with  $K_D$  values of 6.8 and 5.9 [nM] and  $B_{\text{max}}$  of 38 and 54 [fmol/mg of protein] respectively. These values are not comparable to those described in nervous, tumor or lymphoid tissue. The displacement tests revealed similarity between Naloxone and Methionine enkephalin binding sites due to their mutual displacement, unlike that observed with  $\beta$ -Endorphin.

In contrast to previous studies, it was found that the effect of the  $\text{Na}^+$  ion (100 mM) did not modify the  $\text{IC}_{50}$  values, whereas GTP markedly reduced the total binding of both ligands: Methionine enkephalin and Naloxone and suggests the participation of a protein with G protein characteristics.

**Keywords:** Methionine Enkephalin;  $\beta$ -Endorphin; Natural Killer Cells; Naloxone; Human Lymphocyte Membranes;  $\text{Na}^+$  Ion; GTP; Displacement Assays

## Introduction

Our immune system has diverse defense mechanisms that can be classified as a humoral and cellular responses, that is, the production of diversity of antibodies by the B Lymphocyte and other cells capable of recognizing and destroying other cells such as Natural Killer (NK) cells, which constitute a subpopulation of lymphocytes with the ability to destroy tumoral or viral infected cells, without prior sensitization or restriction of the Histocompatibility Major Antigen type. The NK cells correspond morphologically to large granular lymphocytes and represent a minor but important proportion of total circulating mononuclear cells [1,2].

This Natural Killer Cell Activity (NKCA) which decreases markedly in pathologies such as leukemia, immunodeficiency and sepsis [2,3] has been the subject of extensive research in recent years, evidencing the existence of several modulators such as several lymphokines, especially IL-2 and IFN ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) [4,5] which promote either the proliferation of NK cells and/or their stimulation [6,7].

Another important group of modulators are the opioid peptides (OP), particularly the enkephalins and endorphins: Methionine

enkephalin (ME), Leucine enkephalin (LE) and  $\beta$ -Endorphin (BE) [8,9].

In humans, BE biosynthesis begins when its  $\beta$ -Lipotrophin precursor is co-incubated with the adrenocorticocotrophin hormone (ACTH), from the cutaneous proopiomelan (POMC) in the corticotrophs of the anterior pituitary gland, then generating  $\gamma$ -Lipotrophin and BE. Because  $\beta$ -Lipotrophin is secreted intact, BE levels are extremely low. However, agents that stimulate the release of ACTH, including hypoglycemia and intense exercise also increase the plasma concentration of BE [10,11]. The enkephalins derived from Proenkephalin A are stored in the adrenal medulla and subsequently released in response to hypoglycemia or renal failure [12,13]. In several species studied they are an important part of the adrenomodular chromaffin granules, mainly in the form of pentapeptides [14,15]. In addition to the actions described for OP, such as the inhibition of the growth of some types of tumors and participation in the control of brain development [16,17], these exert multiple effects on the cells of the Immune system:

- a. In T cells, both peptides decrease antigen-specific cytotoxicity [18,19].
- b. In polymorphonuclear leukocytes, ME stimulates chemotaxis [20], while BE stimulates adhesion to surfaces [20] and the production of superoxides [21,22].
- c. In monocytes stimulation of chemotaxis and inhibition of phagocytosis has been described, by ME, LE and BE [23,24].
- d. In NK cells, it has been described that these three endogenous peptides stimulate cytotoxic activity [8] O'Connor et al, 2006).

Receptors for opioids have been described in the nervous system and peripheral tissues [25,26] and opioid binding sites have been detected in a wide variety of animal and human tumors [27,28]. It is known that the actions of opioids in the nervous system are mediated by at least four different receptor types ( $\mu$ ,  $\delta$ ,  $\kappa$ ,  $\sigma$ ) each of these with ligand selectivity, distribution and different physiological effects [26]. In the IS the existence of binding sites for these neuropeptides has been controversial [29,30].

Binding sites have been described in human T lymphocytes using the antagonist Naloxone [12, 31] and in human and murine leukocytes [32,33], reaffirming the existence of receptors with opioid characteristics [34,35].

Considering the existence of opioid receptors in that would explain the interconnection in nervous/immune system, in this work has been studied and partially characterized the binding sites present in human lymphocyte membranes (HLM) both for the pentapeptide ME due to its possible relationship with the increase of NKCA, as well as for the opiate antagonist Naloxone, capable of reversing this action [17,26]

## Materials and Methods

### Lymphocytes

Obtained from peripheral blood samples from healthy voluntary donors from the blood bank of the José Joaquín Aguirre Hospital of the University of Chile. Cells from the K-562 cell line, derived from human erythroleukemia, were used as target cells.

### Culture and maintenance of target cells

The growth and maintenance of the K-562 cell line RPMI-1640 was added bovine fetal serum (FBS) at a final concentration of 10% and was supplemented with antibiotics and antifungals: 100 U/mL penicillin, 100  $\mu$ g/mL Streptomycin, 1.25  $\mu$ g/mL Fungizone (complete culture medium: CCM). Cell vitality was followed by exclusion of the trypan blue dye. Radiomarking of target cells. K-562 cells ( $10^6$  cells/mL) were incubated in the presence of 100  $\mu$ Ci of sodium chromate ( $^{51}\text{Cr}$ ; Chilean Nuclear Energy Commission) for one hour at 37° C with occasional shaking every fifteen minutes. After the deadline, the cells are washed three times with CCM, finally adjusting to a concentration of  $5 \times 10^4$  cells/mL of CCM. The target cells thus obtained were used immediately in the NKCA assays.

Obtaining lymphocytes (effector cells) [36]. The heparinized blood sample, approximately 40 mL, is divided into 50 mL conical

plastic tubes. To each fraction is added 6.8 mL of a solution composed of dextran (3%) and NaCl (0.9%), inverting the tube three times and leaving it to rest for 30 minutes at room temperature. The supernatant is transferred to another conical tube with the same characteristics. Then, 27 mL of Ficoll-Hypaque solution ( $d=1.077$ ) (Sigma Chemicals Co. (St. Louis MO USA) are added and the tubes are centrifuged at 500xg for 15 minutes at room temperature. The layer of cells formed in the interface is extracted and transferred to 15 mL glass tubes. Cells were washed with 10 mL of PBS solution (15 mM  $\text{KH}_2\text{PO}_4$ , 8 mM  $\text{Na}_2\text{HPO}_4$ , 3 mM KCl, 140 mM NaCl, 5 mM  $\text{MgCl}_2$  and 150  $\mu$ M  $\text{CaCl}_2$ ) centrifuged at 250 xg, for 3 minutes at room temperature and repeating twice the washed. Finally, the cells were resuspended in CCM at a concentration of  $2 \times 10^6$  cells/mL and used immediately in the cytotoxicity assays.

### Treatment of effector cells with neuropeptides

The effector cells were incubated with ME and BE. Thus, lymphocytes ( $2 \times 10^6$  cells/mL) were preincubated in Eppendorf tubes in the presence of ME ( $10^{-6}$ - $10^{-10}$  M) or BE ( $10^{-8}$ - $10^{-12}$  M). The lymphocytes were centrifuged at 250xg for three minutes and resuspended in CCM and the centrifugation is repeated. Finally, the cells are resuspended and adjusted to a concentration of  $2 \times 10^6$  cells/mL in CCM and the cytotoxicity assay is carried out. Lymphocytes incubated only in CCM are used as control. All the stimulation tests were performed with an effector: target ratio of 40 (40:1).

### NKCA assay

To achieve this objective, 0.1 mL of effector cells ( $2 \times 10^6$  cells/mL) were added to 0.1 mL of target cells ( $5 \times 10^4$  cells/mL) in multi-titration plate wells (Nunc), performing this operation in quadruplicate. The plates were centrifuged at 15xg for 10 minutes and immediately incubated for four hours at 37° C and 5%  $\text{CO}_2$ . Then, the plates were centrifuged again, and 0.1 mL of this supernatant was analyzed in a gamma counter model Packard Multi-Prias 1 (Packard Instruments Company).

### Obtaining of HLM

Briefly, the effector cells obtained previously according to section 3, were lysed by sonication for ten minutes at room temperature [37]. Then, they were centrifuged at 30,000 xg for one hour at 4° C. The precipitate was resuspended in 5 mL of Tris.HCl buffer (50 mM, pH: 7.7) supplemented with the antibiotic Bacitracin (50  $\mu$ g/mL) and the amount of total proteins present was determined [38].

### Radioligand binding assays

The HLM obtained were used in binding of 3H-ME and 3H-Naloxone Ogawa et al [39] (Amersham (UK, 49.4 Ci/mmol and 40 Ci/mmol, respectively) which comprises incubating the membranes in Eppendorf tubes (250  $\mu$ g protein per assay) for 30 minutes in increasing concentrations of radioligand (1-40 nM) in volume of 150  $\mu$ L and 25° C with constant agitation The reaction is terminated by dilution at 4° C with five mL of the Tris.HCl buffer and immediate vacuum filtration. The filters (GF/C Whatman) are washed twice with five mL of same buffer and once dry the retained radioactivity determined in them by a liquid scintillation counter, model Delta 300 (Searle Analytic Inc.) having 40% of efficiency for tritium. The

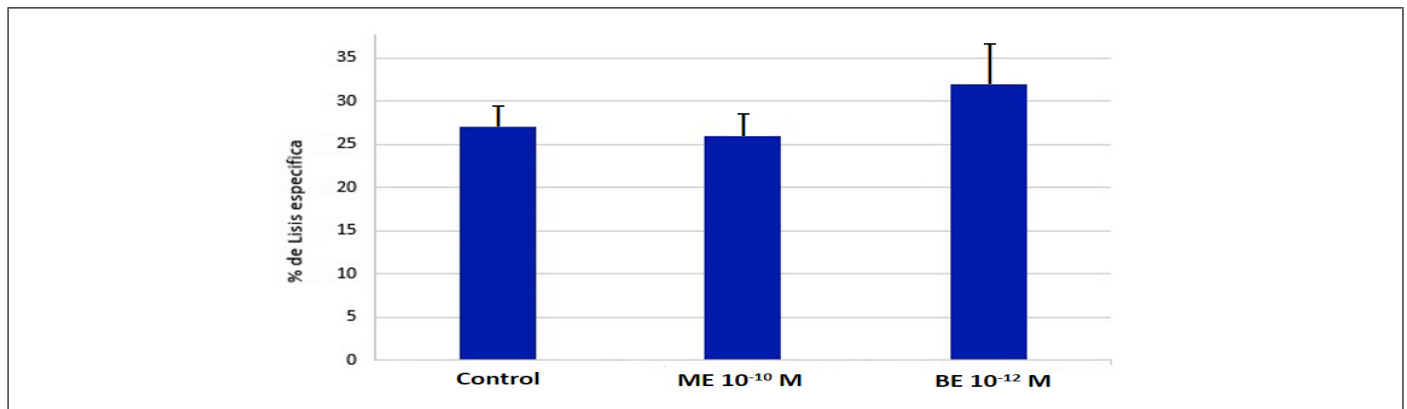
total binding corresponds to the radioactivity retained in the filters. In the non-specific binding, incubation was performed in the presence of unlabelled (Naloxone or ME) at a concentration of  $10^{-5}$  M. Compound Specific binding was determined by the difference between total and non-specific binding. Thus, the concentration of the bound radioligand (B) is plotted as a function of the concentration of the free radioligand (F), obtaining a saturation curve for the equilibrium reached. Scatchard analysis (1949), which is plotted on the ordinate the values of the ratio B/F and the abscissa the value was used to obtain the dissociation constant ( $K_D$ ) and the maximal binding capacity ( $B_{max}$ ) united radioligand (B). A straight line is obtained whose intersection with the horizontal axis determines the value of  $B_{max}$  and the negative reciprocal value of the slope corresponds to the value of  $K_D$ .

**Displacement tests**

Naloxone, ME and BE. Lymphocyte membranes (250  $\mu$ g of protein per assay) were incubated for 30 minutes at 25°C in the

**Results**

**Effect of opioids on NKCA**



**Graphic 1:** Effect of OP in physiological concentrations on NKCA. Determinations in quadruplicate (n = 9), the stimulation with BE was not significant (p > 0.05).

The Graphic 1 shows the NKCA stimulation expressed as a percentage of increase over the control value, in the presence of BE and ME in physiological concentrations. The samples treated with BE achieved a stimulation of around 20% over the control value and

presence of the radioligand ( $^3$ H-ME or  $^3$ H-Naloxone, 20 nM) and the displacer in increasing concentrations ( $10^{-10}$  -  $10^{-2}$  M). Then, both for the end of the reaction and for obtaining the data, which are plotted as percentage of inhibition respect to the control, as a function of the negative value of the logarithm of the concentration of the displacer used and the  $IC_{50}$  values are calculated for each of the displacers. This value corresponds to the concentration of the displacer capable of inhibiting 50% of the binding to membranes.

**Effect of sodium ions and GTP**

Concentrations of 100 mM and 100  $\mu$ M of NaCl and GTP were used, respectively, which were added to the incubation medium and then proceeded in the same way described. in the previous section.

**Statistical analysis**

The data are expressed as averages, standard error (S.E.M) and analyzed by the paired Student's "t" test.

only present in 70% of the samples. The samples incubated in the presence of ME, no stimulation of the NKCA was observed.

The Table 1 show the NKCA in non-physiological concentrations of ME an BE.

**Table 1:** Stimulation of NKCA mediated by ME and BE in non-physiological concentrations, expressed as percentage of specific lysis.

AGE/SEX	CONTROL	BE (M)		ME (M)	
		$10^{-10}$	$10^{-8}$	$10^{-8}$	$10^{-6}$
<b>GROUP A</b>					
34/F	10.8	25.5	27	20.8	25.3
27/M	35	48	51.8	40	44
32/F	10.5	20.1	19.7	15	17.2
31/M	24	41.1	46.2	49.2	39.4
26/M	5.3	17.2	17.4	20.6	21.9
35/M	15.1	19.2	19.8	18.7	20.1
X $\pm$ S.E.M	16.8 $\pm$ 4.4	28.5 $\pm$ 5.3	30.3 $\pm$ 6.1	27.4 $\pm$ 5.6	28.0 $\pm$ 4.5
<b>GROUP B</b>					
36/F	30	38	34	35	17.2
26/M	11.6	13.3	10.4	16	17.4

39/M	17.2	18.9	19.2	17.2	18
25/M	46.1	49.4	47.2	47.2	51.7
28/M	10.2	12	8.7	12.4	9.7
X+ S.E.M	23.0 + 6.7	26.3 + 7.4	23.9 + 7.3	25.6 + 6.7	22.8 + 7.4

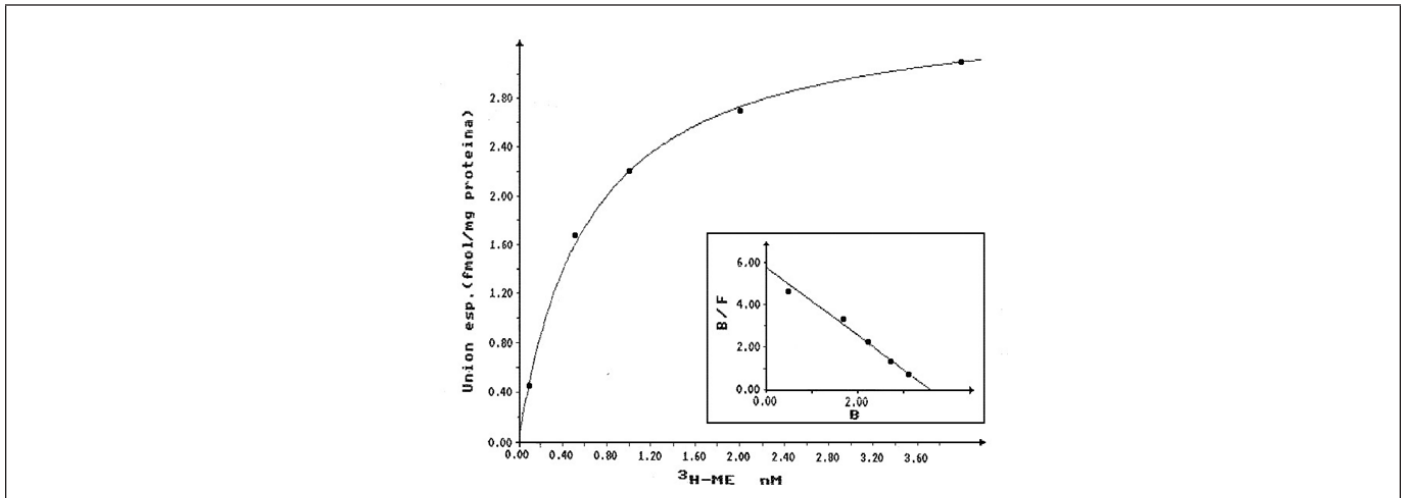
**Group A:** (samples 1-6) an increase in NKCA of at least 20% over the control value is achieved for both concentrations of the peptides studied, however, the stimulation is not significant ( $p > 0.05$ ).

**Group B:** samples 7-11) mixed results are obtained

The group A contains the samples that present stimulation of 25% of the control value, in both concentrations of ME and BE. The group B collects the samples that are not or that present mixed results, both stimulated only by ME (sample 8), only by BE (sample 7), only by a concentration of the opioid studied (sample 11) or

not stimulated by both peptides (samples 9 and 10). Although it was possible to divide the response to opioids into two groups, the stimulation achieved by group A did not turn out to be statistically significant with respect to the control ( $p > 0.05$ ).

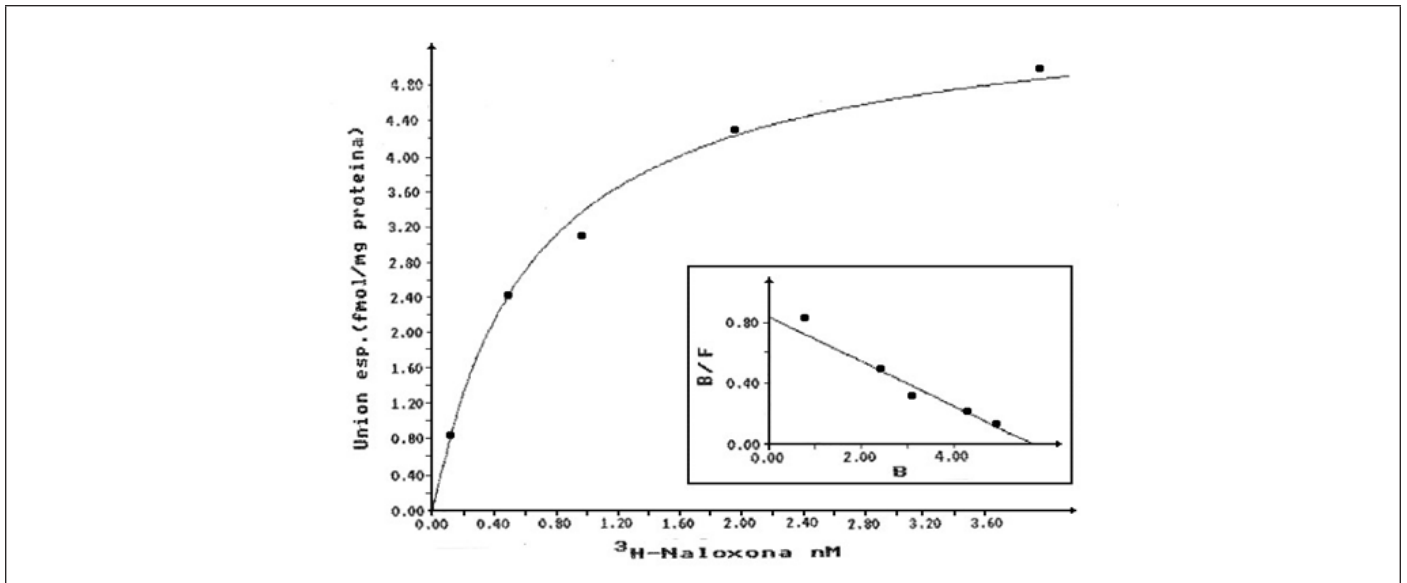
**Binding sites in lymphocyte membranes**



**Graphic 2:** Saturation curve and Scatchard plot (insert) for the binding of 3H-ME to HLM. Each value represents the average value of determinations in duplicate of three different samples.

Saturable specific binding curves reaching equilibrium in 30 minutes were obtained in 70% of the samples for both ligands (ME and Naloxone). Graphic 2 & Graphic 3 show the saturation curve and the Scatchard plot (insert) for ME and Naloxone, respectively,

when using radioligand concentrations between 1 and 40 nM. In both cases, the behavior agrees with the model of a single type of binding site described in the Scatchard analysis. The values of the parameters  $K_D$  and  $B_{max}$  obtained are shown in Table 2.

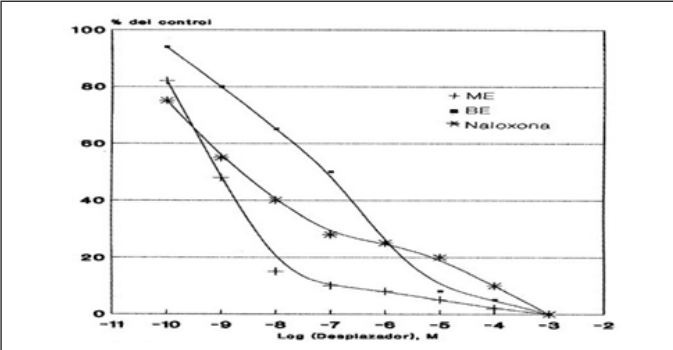


**Graphic 3:** Saturation curve and Scatchard plot (insert) for the binding of 3H-Naloxone to HLM. Each value represents the average value of determinations in duplicate of three different samples.

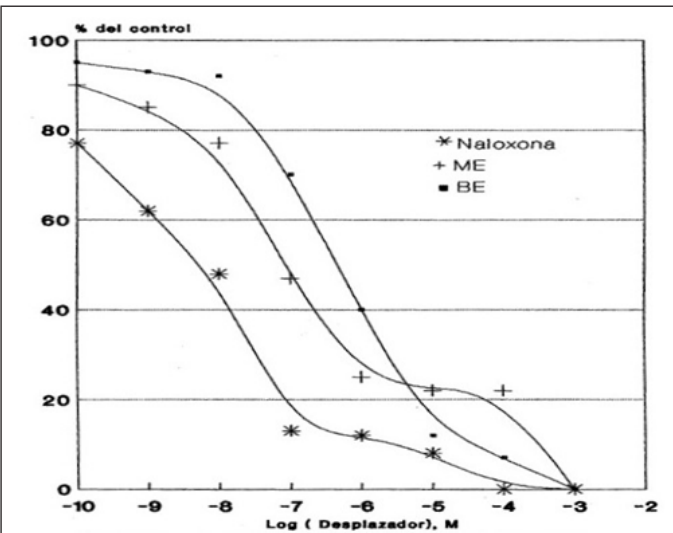
**Table 2:** Values of the parameters KD [nm] and Bmax [fmol/mg protein] obtained in the Scatchard analysis. The parameters are very similar in both cases studied.

Radioligando	KD ± S.E.M	B <sub>max</sub> ± S.E.M
( <sup>3</sup> H) -ME	6.8 ± 0.35	38 ± 2.36
( <sup>3</sup> H) -Naloxone	5.9 ± 0.23	54 ± 3.05

**Displacement of the union to membranes**



**Graphic 4:** Displacement curve of the binding of 3H-ME to HLM mediated by ME, BE and Naloxone. Each value corresponds to the average of determinations in duplicate (n=3).



**Graphic 5:** Displacement curve of <sup>3</sup>H-Naloxone binding to HLM mediated by ME, BE and Naloxone. Each value corresponds to the average of determinations in duplicate (n=3).

**Table 3:** IC<sub>50</sub> values for the displacement of 3H-ME binding to HLM (Graphic 4).

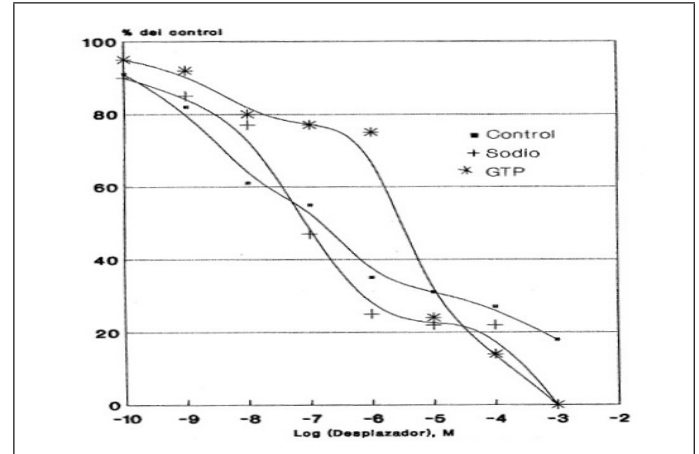
Desplazador	IC <sub>50</sub> (nM)
Metionina encefalina	1
Naloxona	3
B-Endorfina	100

**Table 4:** IC<sub>50</sub> values for displacement of 3H-Naloxone binding to HLM (Graphic 5).

Desplazador	IC <sub>50</sub> (nM)
Metionina encefalina	7
Naloxona	80
B-Endorfina	500

For the binding sites for <sup>3</sup>H-ME and <sup>3</sup>H-Naloxone, a concentration of 20 nM of the radioactive compound was used against a wide range of concentrations of Naloxone, ME and BE (10<sup>-10</sup> to 10<sup>-2</sup> M). Thus, Graphic 4 & Graphic 5 show the displacement of the total binding of <sup>3</sup>H-ME and <sup>3</sup>H-Naloxone, respectively. Whereas Table 3 & Table 4 present the IC<sub>50</sub> values for both studies.

**Effect of Na+ and GTP ions**

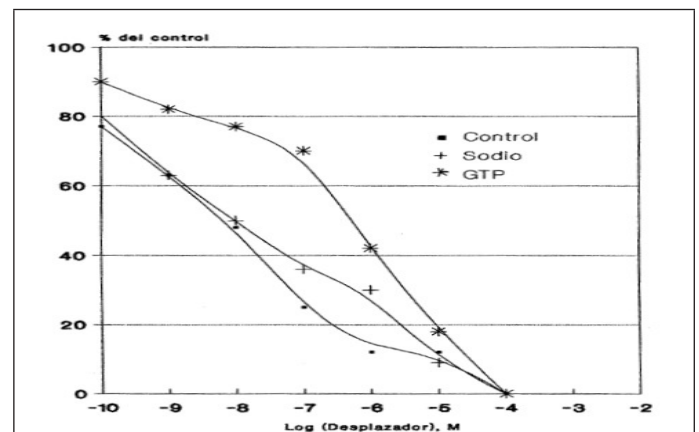


**Graphic 6:** Effect of sodium ions and GTP on the displacement of the binding of 3H-Naloxone in HLM, mediated by ME. Each value corresponds to the average of determinations in duplicate (n=3).

The Graphic 6 shows the displacement in the case of ME, observing that the addition of GTP drastically decreases the ME junction to membranes, which is reflected in the IC<sub>50</sub> value obtained: 88 times greater than that obtained in the absence of GTP. On the other hand, there was no noticeable change in the presence of sodium ions (Table 5).

**Table 5:** IC<sub>50</sub> values for displacement of <sup>3</sup>H-Naloxone binding to HLM mediated by ME, in the presence and absence of NaCl (100 mM) or GTP (100 μM). (Graphic 6).

Condition	IC <sub>50</sub> (nM)
Control	80
Na <sup>+</sup>	100
GTP	7000



**Graphic 7:** Effect of Na<sup>+</sup> and GTP on the displacement of the binding of 3H-Naloxone to HLM, mediated by Naloxone. Each value corresponds to the average of determinations in duplicate (n=3).

The (Graphic 7) exhibits the displacement of the total  $^3\text{H}$ -Naloxone binding mediated by Naloxone in the presence and absence of sodium or GTP ions.

The addition of GTP again drastically altered the displacement of Naloxone expressed in an  $\text{IC}_{50}$  value that was 85 times higher than that obtained in its absence. Finally, the addition of sodium ions did not alter the  $\text{IC}_{50}$  value obtained in its absence (Table 6).

**Table 6:**  $\text{IC}_{50}$  values for displacement of the  $^3\text{H}$ -Naloxone binding to HLM by Naloxone, in the presence and absence of  $\text{NaCl}$  (100 mM) or GTP (100  $\mu\text{M}$ ) (Graphic 7).

Condition	$\text{IC}_{50}$ (nM)
Control	7
$\text{Na}^+$	10
GTP	600

## Discussion

Since the discovery of OP and their endogenous ligands, it has been widely recognized that endogenous OP plays a variety of physiological roles [28,40]. Recently great importance has been given to the possibility that these peptides are involved in the modulation of the IS [41].

It has been shown that OP regulate NKCA [17,42]. NK cells have generated increasing interest due to their potential role in hematopoiesis and in the control of viral infections, as well as in the growth of some types of tumors and in the extracellular destruction of bacteria [43,44]. Although several agents capable of positively or negatively altering NKCA have been described, the mechanism by which this process develops is not completely clear. However, it is possible to divide it into stages: the first step of the lytic pathway involves the recognition of the target cell through receptor-ligand interactions. Next, signals from the target cell promote the activation of the secretory system of the NK cell and thus soluble factors such as NKCF are released to produce the final lysis of the target cell [32,45,46]. Numerous studies related to the NK cytolytic activity and its various modulators, however, not so much work aimed at determining or characterizing the receptors for these same agents. However, it has been described that both Naloxone and  $\beta$ -endorphin inhibit the binding of IL-2 to its receptor expressed in the lymphocyte membrane [47,48] suggesting the participation or existence of opioid receptors or their analogues in immune cells. According to our results, when lymphocytes were incubated in physiological concentrations of ME ( $10^{-10}$  M) and BE ( $10^{-12}$  M) [49,50], no response was obtained. similar in both cases studied. On the other hand, when the lymphocytes were treated with concentrations higher than the physiological ones, a response greater than 25% of the control value was obtained, even though this stimulation is not present in the total of the samples analyzed and no dependence was observed on the age or sex of the individual studied

Probably the individual diversity of the samples as well as to the high heterogeneity existing in the NK cells, since it has been described that they form at least three subpopulations of functionally distinct cells: effector cells capable of binding and destroying the target cell and two non-cytolytic subpopulations, one capable and

the other incapable of joining the target cell [43,51]. Our results and the fact that Naloxone inhibits the increase in NKCA mediated by both IL-2 and IFN- $\alpha$  [47,52] it would be expected to have the existence of opioid receptors in NK cells, both due to the stimulation achieved as per the relationship between OP and Naloxone. In support, the incubation of HML in the presence of  $^3\text{H}$ -ME or  $^3\text{H}$ -Naloxone shows, in 70% of the samples studied, high affinity saturation curves, indicating the presence of specific binding sites. located in the lymphocyte membrane. When performing the Scatchard analysis, a model corresponding to a type of binding site was verified in both ligands studied.

Because ME defines receptors of type ( $\mu$ ,  $\delta$ ) and Naloxone defines receptors of type ( $\mu$ ,  $\delta$  and  $k$ ) [40,53], the existence of  $\mu$ -type and  $\delta$ -type opioid receptors in HLM is suggested. This agrees with the evidence of  $\delta$ -type receptors in cells of the immune system and with the modulation of the immune response mediated by enkephalins, through  $\mu$ -type and  $\delta$ -type receptors [54,55]. Another important use of radioligand binding assays is its contribution to glimpse the molecular mechanism that couples the receptor-transducer-amplifier system with intracellular events. In radioligand binding studies, the binding of agonists and not that of antagonists is generally modulated by ions and nucleotides [56], Newton et al. Thus, there are numerous studies performed in neural tissues that have shown modulation of the receptor-ligand interaction by using  $\text{Na}^+$  and GTP ions (or analogs) it being described that both markedly decrease the specific binding, using ligands that define receptors of the same type. type  $\delta$  and  $k$  as well as the type  $\mu$  and  $k$  [57,58].

When we are using GTP, observed an increase in the  $\text{IC}_{50}$  value and therefore suggests the participation of a protein with G protein characteristics in the binding opioids and opiates to HLM. The G protein participation has recently been confirmed, since a receptor- $\mu$ -opioid-G protein complex has been solubilised from membranes of tumor cells 7315c, which retain the ability to bind agonists and the sensitivity to guanine nucleotides as they are subsequently reconstituted in phospholipid vesicles [59,60]. In general, a G protein associated with cell surface receptors is involved in the regulation of both receptor affinity and the effector system: adenylate cyclase. The binding of GTP to the G protein decreases the affinity of the receptor for agonists, promoting dissociation with the agonist and modulating the activity of the effector system [33,55].

The modulation by  $\text{Na}^+$  ions described in all the previous studies turned out to be of the same nature: a marked decrease in the specific binding is promoted, both individually as in conjunction with GTP analogues Gairin et al [61], suggesting that  $\text{Na}^+$  probably occupies a different subunit within the membrane, which could function in conjunction with GTP to decrease the affinity of the binding. However, in this study a notorious alteration of the specific binding of the radioligand to its receptor was not detected when it was incubated in the presence of  $\text{Na}^+$  ions, indicating that this ion does not affect the affinity of the binding, because it does not bind or not occupies the existing site in the membrane, proposed by the studies. The modulation of radioligand binding to its GTP-mediated receptor suggests that the binding sites for opioids detected in HLM are similar in molecular organization to those described in brain

tissue and tumor cells in culture. The results obtained and presented in this Thesis suggest that cells of the IS may be susceptible to the action of OP present in the circulation, through receptors present in their membrane, although no direct relationship was detected between the existence of binding sites and stimulation of NKCA. That is, there was stimulation of NK cytolytic activity by these peptides both in the presence and in the absence of opioid receptors and in addition in other cases studied receptors were detected, but there was no stimulation by opioids.

Our results suggest some degree of similarity between the ME and Naloxone binding sites by the similarity between the KD and Bmax values obtained as well as by the reciprocal displacement of the membrane binding. This idea is reaffirmed when considering studies that indicate that although there are probably differences in the primary sequence of the receptors, these are products of the same gene, as has been described for the subtypes  $\mu$  and  $\delta$  [62-64]. It should be noted that these results refer to total peripheral lymphocytes. However, the described methodology allows us to deliver the necessary means to continue studies aimed at determining these parameters in fractions enriched in NK cells or in both human and murine NK cell lines.

## Conclusion

The BE and ME opioid peptides stimulate NK cell-mediated cytotoxicity at higher than physiological concentrations. Human peripheral lymphocytes have binding sites with similar characteristics ( $K_D$  and  $B_{max}$ ) for both ligands studied (ME and Naloxone). The binding of radioligands to these binding sites is affected by GTP, which suggests the participation of a type G protein protein. No direct relationship was observed between the stimulation of NKCA and the presence of binding sites for ME.

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