ELSEVIER

Life Sciences, Vol. 60, No. 9, pp. 573-586, 1997

0024-3205/97 $17.00 + .00

PII S0024-3205(96)00640-6

CHARACTERIZATION OF A NALOXONE-INSENSITIVE β-ENDORPHIN RECEPTOR ON MURINE PERITONEAL MACROPHAGES

Jeffrey A. Woods, Nahid A. Shahabi, and Burt M. Sharp

Endocrine-Neuroscience Research Laboratory,
Minneapolis Medical Research Foundation and the
Department of Medicine, Hennepin County Medical Center,
914 South 8th St., D-3, Minneapolis, MN 55404

(Received in final form December 9, 1996)

Summary

Previous studies from our laboratory have characterized a naloxone-insensitive β-Endorphin (β-End) receptor on the human pro-monocytic cell line U937. Since monocytes are macrophage precursors, we sought to identify and characterize this site on fully differentiated effector macrophages. Mice (ICR females, 6-8 wk old) were injected (i.p.) with 1 mL of thioglycollate to induce an inflammatory response. Elicited cells were harvested 3 d later by lavage. Macrophages were enriched by adherence and analyzed via radioreceptor assay (with [^{125}I]β-End, 2,000 Ci·mmol^{-1}) as either intact cells or membrane preparations. Scatchard analysis revealed a single saturable binding site for β-End (K_d = 9.75 ± 2.6 x 10^{-9} M; 8218 ± 2360 sites/cell). Competition studies showed that other opiate receptor ligands including naloxone, DAMGO, U69593, or 2,5 DPDP-enkephalin were ineffective at displacing [^{125}I]β-End when compared to unlabeled β-End. Analysis of competition studies utilizing fragments and analogs of β-End revealed that β-End (6-31) and β-End (1-5,16-31) were equipotent, and N-acetylated β-End was less potent, than β-end (1-31) in displacing [^{125}I] β-End binding. In contrast, β-End (1-27) and β-End (28-31) were ineffective. In summary, we have identified a naloxone-resistant β-End binding site on murine peritoneal macrophages that is similar to one we have previously characterized on U937 cells and cultured murine splenocytes.

Key Words: **macrophage, β-endorphin receptor, naloxone**

Corresponding Author: Burt Sharp, Minneapolis Medical Research Foundation, D-3; 914 South 8th St., Minneapolis, MN 55404, Telephone 612-337-7380, Telefax 612-337-7372

β-Endorphin (β-End) is a 31 amino acid endogenous opiate peptide synthesized in the anterior pituitary and released into the circulation in response to physical or mental stress (1,2). β-End affects a wide variety of processes through receptors located in the central and peripheral nervous system. In the nervous system, β-End inhibits impulse transmission and neurotransmitter discharge by altering membrane permeability to cations and inhibiting adenylate cyclase activity (3). β-End is thought to serve as a neurotransmitter or neuromodulator and plays a role in endogenous control of pain perception (2). Receptors in the central nervous system (CNS) interact with the N-terminal residues of β-End and binding can be antagonized by naloxone (1,4).

In addition to it's effects on the CNS, there is growing evidence demonstrating that β-End binds to and alters the functions of many cells of the immune system (2,5). These effects appear to be mediated through both naloxone-sensitive and naloxone-insensitive mechanisms. Indeed, β-End appears to affect monocyte (Mo) chemotaxis (6,7), macrophage (Mφ) phagocytosis and cytotoxicity (8,9), T cell proliferation (10), neutrophil and Mφ superoxide production (11,12,13), bone marrow Mφ differentiation (14), peripheral blood mononuclear cell (PBMC) interferon(IFN)-γ production (15), natural killer (NK) cell activity (16), and B lymphocyte antibody production (17) in a naloxone-sensitive manner. In contrast, others have reported that β-End's effects including, proliferative responses of murine splenocytes to T-cell mitogens (18,19,20) and interleukin-2 production (18,19), human lymphocyte mitogenic responses (21), human mononuclear cell IFN-γ production (22), B lymphocyte Ia expression (23), Mφ phagocytosis (24), and calcium uptake by rat thymocytes (25) are all resistant to inhibition by naloxone. Based on these functional data, it is tempting to speculate that two separate receptor mechanisms exist (i.e. naloxone-insensitive and -sensitive) that account for the documented effects of β-End on cells of the immune system. Therefore, dependent on receptor affinity, receptor density, and second messenger system, β-End may have different effects on different immune cells or immune cells in different functional states. Although there is an abundance of data regarding the effects of β-End on functional measures of immunity, very little data exists regarding the characterization of β-end binding sites on immune cells.

The idea that β-End can bind to a receptor in a naloxone-insensitive fashion is not new. Indeed, several reports in the late 1970's and early 1980's found that β-End could bind specifically to non-opiate (naloxone-insensitive) receptors on cultured human lymphocytes (26), murine EL-4 thymoma cells (27), human glioblastoma cells (28), and the terminal complex of human complement (29). Likewise, recent binding studies performed in our laboratory (30,31) and others (32,33,34) support the hypothesis that naloxone-resistant β-End receptors exist on immune cells.

Data from these studies has revealed that, unlike neuronal receptors which bind to the N-terminal region of the peptide, binding occurs through the interaction of the receptor with C-

terminal residues (30,31,34). We have recently demonstrated the existence of a naloxone-resistant β-End receptor on the pro-monocytic cell line U937 (31). Since Mo's are precursors to tissue Mφ's, we sought to identify and characterize this site on freshly obtained fully differentiated tissue Mφ's. Demonstration of the existence of this site on freshly obtained, fully differentiated peritoneal Mφ's is important because most functional studies have utilized peritoneal Mφ's in the study of β-End modulation.

Methods

Reagents

The peptides N-acetyl-β-End, β-End 1-27, β-End 6-31, β-End 1-5,16-31, and β-End 28-31 were purchased from Peninsula (Belmont, CA) and β-End 1-31, 2,5 DPDP-enkephalin, and DAMGO were obtained from Multiple Peptide Systems (San Diego, CA). The kappa agonist U69,593 was purchased from Upjohn (Kalamazoo, MI). RPMI-1640, thioglycollate, protease inhibitors, and polyethylenemine were purchased from Sigma Chemical Co. (St. Louis, MO). TM-235 defined serum replacement was purchased from Celox Inc. (Hopkins, MN). All reagents and media were prepared sterile and endotoxin-free.

Animals, cell collection and purification.

Female ICR mice (Harlan, Madison WI) 6-8 weeks old were purchased virus-antibody free and housed in a pathogen free facility on a 12-h light, 12-h dark cycle with ad libitum access to standard chow and water. All animals were acclimated to the facility for at least 1 week prior to experimentation. Animals were sacrificed via over-etherization. Elicited peritoneal cells were obtained 3 d after intra-peritoneal injection (0.033 mL·g. b. wt.$^{-1}$) of thioglycollate broth. These cells were obtained aseptically by lavage of the peritoneal cavity with RPMI-1640 containing 10 U·mL^{-1} heparin. Thioglycollate causes an aseptic inflammation characterized by an initial influx of neutrophils (1-2 d) followed by a large increase in the number of Mo's/Mφ's (3-4 d)(35). We chose to examine inflammatory Mφ's for both scientific and practical reasons. First, inflammatory Mφ's are representative of cells that arrive at the scene of viral and bacterial infections, tissue damage, and tumorigenesis *in vivo* (36). Moreover, recent evidence suggests that β-end and N-acetyl-β-End may be produced in rat spleen and thymus in response to chronic inflammation (37). Therefore, it may be that inflammation recruits cells that possess β-End receptors and/or up-regulates β-End receptors on native cells. A second practical consideration relates to the fact that thioglycollate injection results in a 10-fold increase (vs. untreated animals) in the number of Mφ's obtained by peritoneal lavage.

Cells were washed one time (200 x g, 10 min, 4°C) and then treated with 0.15 M ammonium chloride for 3 min to lyse residual red blood cells. Following this, cells were washed twice in RPMI/heparin and then resuspended in RPMI containing 2% TM-235 defined serum replacement with 1 % penicillin/streptomycin/glutamine. Cell viability and number were determined by trypan blue exclusion and routinely yielded > 95% viable cells. We chose

to analyze both membrane preparations and intact cells because of the unique advantages of each method of preparation (38) and the potential for non-specific uptake and/or degradation of radiolabeled peptide by intact Mϕ's (2).

Intact cell preparation. Enriched intact whole cell macrophage suspensions were obtained by the method of Kumagai (39) with minor modifications. Briefly, cells were adjusted to 1 x 10^6 cells·mL^{-1} and then plated on sterile glass petri dishes (100x15 cm) in RPMI containing 2% TM-235 and allowed to adhere for 3-5 h in a humidified atmoshpere of 5% CO_2 in air at 37°C. After incubation, non-adherent cells (lymphocytes, neutrophils) were removed by 3 rounds of washing with warm RPMI supplemented with 1% penicillin/streptomycin/glutamine. These cells were then incubated in phosphate buffered saline containing 0.5mM EDTA (PBS-EDTA) for 1 h at 37°C and then removed from the glass by gentle repeated pipetting. Cells were washed twice in cold PBS-EDTA and once in radioligand binding buffer (see below), counted, and adjusted to the appropriate concentration for binding assays. Cell viability was always > 90 %. These cells were non-specific esterase positive and phagocytic (as determined by uptake of fluorescent latex beads). Adherent cells were assayed for receptor binding activity immediately or cultured in defined medium (RPMI with 2% TM-235 defined serum replacement) for 18 h. There were no differences seen in receptor binding in assays performed immediately after Mϕ enrichment or after 18 hr of culture.

Membrane preparation. Mϕ membrane preparations were obtained by allowing cell adherence onto glass petri dishes in defined medium for 3-5 h. Cells were then washed three times with warm RPMI supplemented with 1% penicillin/streptomycin/glutamine to remove non-adherent cells and then assayed immediately or cultured in defined medium for 18 h. Binding assays were performed in 10 mM Tris-HCl buffer (pH 7.4) containing 0.32M sucrose and the protease inhibitors bacitracin (50 μg·mL^{-1}), leupeptin (10 μg·mL^{-1}), soybean trypsin inhibitor (10 μg·mL^{-1}), and benzamidine (1mM) and the adherent cells were scraped off the glass with the aid of a teflon cell scraper. These cells were homogenized using a Brinkmann Polytron (Westbury, NY) at a setting of 8 for 30 sec on ice. The homogenate was then centrifuged at 200 x g for 5 min at 4°C. The supernatant was collected and centrifuged at 38,000 x g at 4°C for 20 min. The pellet was resuspended and washed once more followed by resuspension in assay binding buffer. Membrane total protein was determined by the method of Bradford(40).

Radioligand binding assay.

The binding of [^{125}I] β-End (≈2000 Ci·$mMol^{-1}$) was performed as previously described (30,31). Briefly, 50 μL aliquots of either Mϕ cell membrane (1-1.5 μg protein) or a suspension of intact cells (1-2 x 10^6) were incubated with [^{125} I] β-End in a binding buffer consisting of 50 mM Tris-HCl (pH 7.4) containing the protease inhibitors bacitracin (50 μg·mL^{-1}), leupeptin (10 μg·mL^{-1}), soybean trypsin inhibitor (10 μg·mL^{-1}), and benzamidine (1mM). Measurements were performed in triplicate and each experiment was repeated at least 3 times. Non-specific binding was determined in the presence

of 1 x 10^{-5}M unlabeled β-End. After incubation for 90 min at 4°C, the receptor-bound radioactivity was isolated by filtration through glass fibers which had been pre-soaked for at least 2 h at room temperature in 0.1% polyethylenemine. Filters were washed 3 times with 3 mL of cold K_2HPO_4 (50mM, pH 7.4) buffer containing 0.01 % triton x-100. The filters were counted in a Beckman γ-counter for 1 min. Scatchard analysis was performed using the non-linear least squares regression analysis of *Ligand* by McPherson (41).

Results

Intact murine Mφ's were used to analyze the time course of binding at 4, 22, and 37°C (Fig. 1). As can be seen, binding was time and temperature dependent. Results revealed labile specific binding at 37°C, peaking between 5-10 min and rapidly declining thereafter. At 22°C, stable binding was observed between 5 and 30 min, declining thereafter. In contrast, specific binding at 4°C was greater than at 37 or 22°C and stable over a longer period of time (5-180 min). Therefore, subsequent binding studies were performed at 4°C for a period of 90 min.

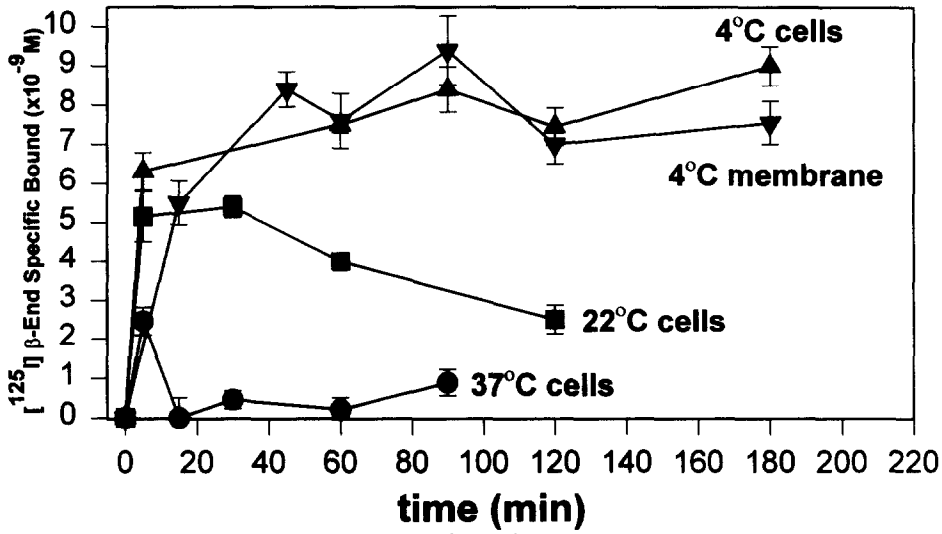

Fig. 1

The effects of time and temperature on [^{125}I] β-End binding to intact macrophages (Mφ's) and Mφ membranes which were prepared as described in Methods. Intact cell pellets and membrane protein were resuspended in binding buffer at 2 x 10^7 cells·mL^{-1} (1 x 10^6 cells·tube^{-1}) or 1-1.5 mg·mL^{-1} protein and incubated at various times and temperatures with 8 nM of [^{125}I] β-End. Non-specific binding was defined in the presence of 10^{-5} M unlabeled β-End. Data are representative of three experiments in which specific binding was between 50-60 % of the total bound for intact cells and 25-35 % for membrane.

Figure 2 shows characteristic titration curves for intact Mφ's and Mφ membrane preparations. Specific binding increased as a

function of intact cell number up to 1.5 x 10^6 cells·tube^{-1}, at which point increasing cell number failed to increase specific binding. Likewise, binding to Mϕ membranes increased as a function of protein content with a plateau occuring around 1 mg protein·mL^{-1}. Based on these results, all of the following binding studies were performed with 1 x 10^6 intact cells or 1-1.5 mg mL^{-1} protein per assay tube.

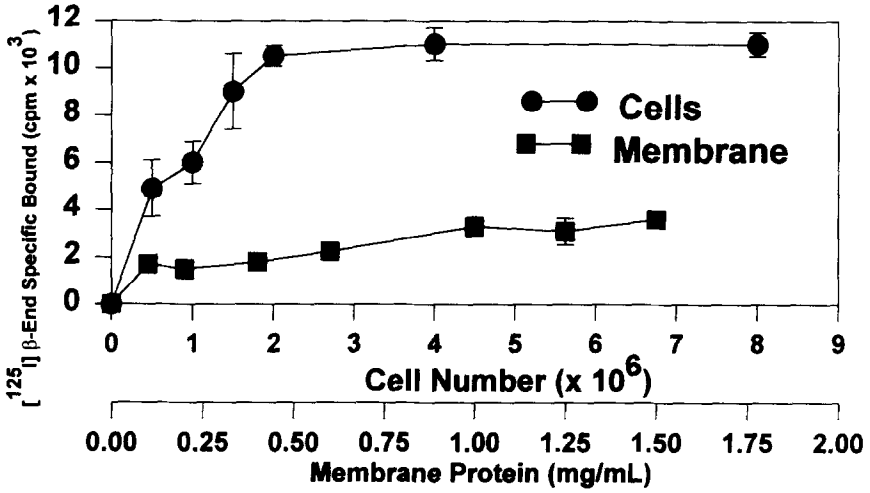

Fig. 2

The effects of Mϕ cell number and Mϕ membrane protein concentration on specific [^{125}I] β-End binding. Cells and membranes were prepared as described in Methods and incubated at 4°C for 90 min with 8 nM of [^{125}I] β-End. Total and non-specific binding, in triplicate, were determined for each data point. These data represent three experiments in which specific binding was between 50-60% and 20-35% of the total bound, for intact cells and membrane preparations respectively.

Unlike other studies that have derived K_d's for β-End receptors on immune cells from competition experiments, we performed saturation experiments using 0.5 to 12 x 10^{-9}M [^{125}I] β-End (Fig. 3). Specific binding reached a maximum at concentrations between 6-8 nM of [^{125}I] β-End, regardless of whether intact cells (Fig. 3a) or membranes were used. It is noteworthy that assays using intact Mϕ's yielded significantly greater specific binding expressed as a percent of total binding (50-65%) than Mϕ membrane preparations (25-35%)(data not shown). This was due largely to lower non-specific binding when intact cells were used. Scatchard plots for intact cells and membranes can be found in Figure 3b. Results for intact cells revealed that a single line best fit the data, yielding a K_d = 10.9 ± 0.75 x 10^{-9} M and a binding capacity of 1.33 ± 0.23 x 10^{-10} moles [^{125}I] β-End·1 x 10^6 cells^{-1} or 7921 ± 1400 receptors·cell^{-1} (based on data from 4 separate experiments). Similarly, a single site with a K_d = 9.08 ± 3.22 x 10^{-9} M and a binding capacity of 1.39 ± 0.49 x 10^{-10} moles [^{125}I] β-End 1 x 10^6 cells^{-1} or 8397 ± 2945 receptors·cell^{-1} was determined by Scatchard analysis of data obtained using Mϕ membranes (based on data from 5

separate experiments). Statistical analysis (t-tests) yielded no significant differences between intact cells or membrane preparations ($p = 0.45$ and $p = 0.81$, for K_d and binding capacity respectively).

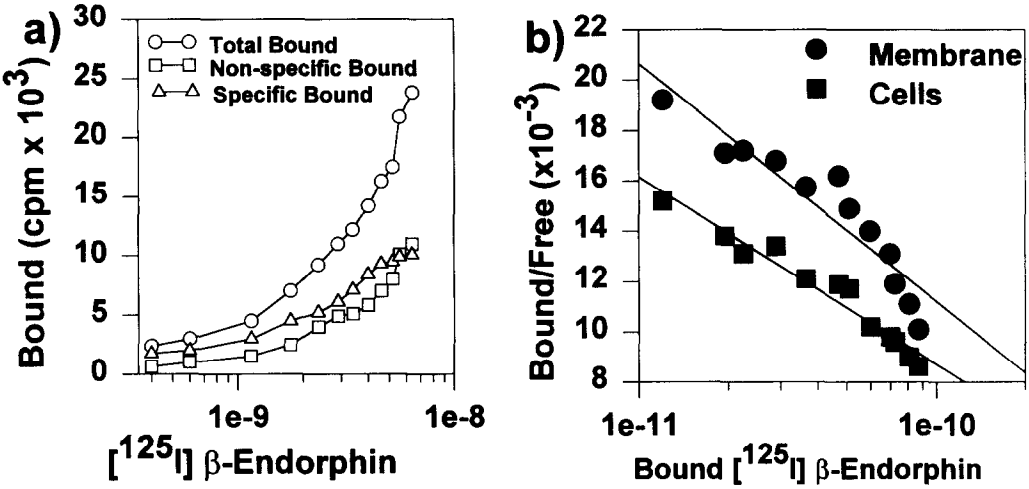

Fig. 3

Binding isotherm for intact Mφ's **(a)** and Scatchard plots **(b)** of [^{125}I] β-End binding to intact Mφ's and membranes. Cells (1 x 10^6cells·tube^{-1}) and membranes (1.5 mg protein) were prepared as described in Methods and incubated for 90 min at 4°C with increasing concentrations of [^{125}I] β-End (0.4 x 10^{-9} - 6.4 x 10^{9} M). In these representative experiments, the binding isotherms revealed a single saturable site for both intact cells and membranes using the nonlinear curve-fitting program *Ligand* (41). For these experiments, the K_d for intact Mφ's was 10.1 x 10^{-9}M with a binding capacity of 9,120 sites·cell^{-1}, while the K_d for Mφ membranes was 9.4 x 10^{-9}M with a binding capacity of 11,197 sites·cell^{-1}.

Competition studies comparing intact cells and membranes using unlabeled β-End can be found in Figure 4. Inhibition curves comparing intact Mφ's and membranes revealed no significant differences in the ability of unlabeled β-End to compete with [^{125}I] β-End (Figure 4). Significant inhibition occured at doses exceeding 1 x 10^{-8}M unlabeled β-End and only one site was predicted from the data.

Due to the fact that data from saturation and competition studies were similar for both intact cells and membranes, and lower non-specific binding was seen in intact cell preparations, competition of [^{125}I] β-End with opiate ligands and β-End (1-31) analogs were done using only intact Mφ's. Figure 5 reveals competition curves for the opiate antagonist naloxone, the δ opiate agonist 2,5 DPDP-enkephalin, the κ opiate agonist U69,593, and the μ opiate agonist DAMGO. The results indicate that none of the opiate ligands tested competed effectively with [^{125}I] β-End for the

site on Mφ's.

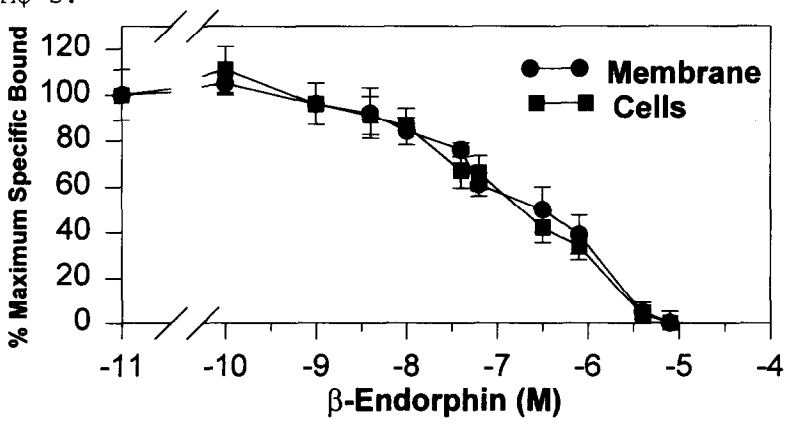

Fig. 4

A comparison of inhibition of [^{125}I] β-End binding to intact Mφ's and membrane preparations by unlabeled β-End (1×10^{-10} - 9×10^{-5} M). Cells (1×10^{6}) or membranes (1.5 mg·mL $^{-1}$) were incubated for 90 min at 4°C with 8 nM of [^{125}I] β-End. The data represent the mean ± sem of 7 separate experiments.

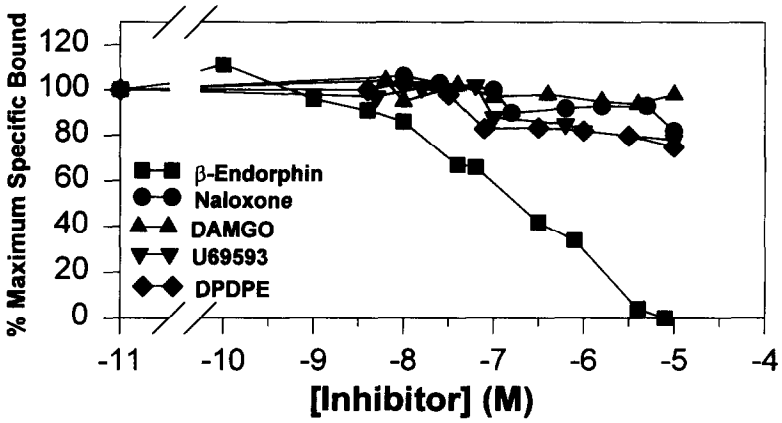

Fig. 5

Competition of [^{125}I] β-End binding to intact murine Mφ's by the opiate receptor ligands naloxone, DAMGO, U69,593, and 2,5 DPDP-enkephalin. Cells were incubated for 90 min at 4°C with [^{125}I] β-End (8 nM) and increasing concentrations (10^{-10} - 10^{-5}M) of unlabeled ligands. The data represent the means of 4 separate experiments. Standard error bars were omitted for clarity.

In order to determine the region of the β-End molecule required for binding to this site, β-End (1-31) analogs and fragments were used in competition studies (Fig. 6). β-End fragments (6-31) and (1-5,16-31) were equipotent to β-End (1-31), suggesting that the C terminal portion of the molecule is important in receptor recognition.

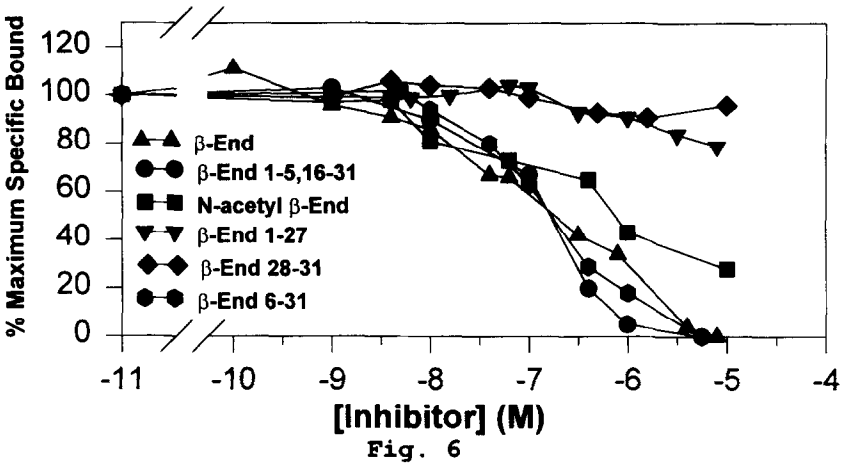

Fig. 6

Competition of [^{125}I] β-End binding to intact murine Mφ's by unlabeled β-End, fragments of β-End, and n-acetylated-β-End. Cells were incubated for 90 min at 4°C with [^{125}I] β-End (8 nM) and increasing concentration (10^{-10} - 10^{-5}M) of ligand. The data represent the means of 3 separate experiments. Standard error bars were omitted for clarity.

N-acetylated β-End was approximately 10-fold less potent than β-End (1-31). Both β-End (1-27) and (28-31) showed minimal ability to compete with β-End (1-31). Therefore, it appears that the C terminus is absolutely required for binding at this site. Moreover, it appears that extension towards the N terminus enhances binding due to the fact that β-End (6-31) and (1-5,16-31) were equipotent to β-End (1-31). Surprisingly, β-End (28-31) could not compete. The N terminus appears not to recognize this site because β-End (1-27) could not compete with β-End (1-31). Likewise, naloxone, which interferes with binding of N-terminal portions of β-End (1-31) at opiate sites in the central nervous system, also failed to effectively compete with β-End (1-31) (Fig. 5). It is interesting to note though, that N-acetylated β-End was 10-fold less potent, suggesting that an intact N-terminus may be of some importance in receptor recognition.

Discussion

Converging lines of evidence regarding the functional effects of β-End on immune function, the existence of β-End receptors on immunocytes, and the production of β-End by immune cells provides support for a physiological role of β-End in immunoregulation. Since Mφ's are potent immune effectors and are central to the regulation of immune function, and in view of our previous reports on the expression of a naloxone-resistant β-End receptor on the pro-monocytic cell line U-937(31), we sought to identify and characterize this site on fully differentiated tissue Mφ's.

Results from the present investigation revealed that thioglycollate-elicited murine peritoneal Mφ's express a naloxone-

insensitive receptor for β-End. This is the first report, to our knowledge, of the existence of this site on freshly obtained fully differentiated tissue Mφ's. This receptor appears to interact with the C-terminal region of the peptide and binding is not antagonized by classical mu, delta, or kappa opiate ligands. The affinity of this receptor for β-End 1-31 appears to be similar to the one which we have previously described on the human pro-monocytic cell line U-937 (31) in that the K_d's of the two sites are comparable (K_d = 9.75 x 10^{-9}M in this study vs 12 x $^{-9}$10 M on U-937 cells). The affinity of this site is intermediate when compared to other naloxone-resistant sites. It is lower than that found on cultured murine splenocytes (4 nM)(30), transformed human lymphocytes (3 nM)(26), freshly obtained and cultured human lymphocytes (4-8 nM)(32), Con A stimulated murine splenocytes (1 nM) (34), human glioblastoma cells (2 nM) (28), and the high affinity site on the terminal complex of human complement (1-6nM)(29,42). In contrast, it is higher than that found on rat hepatocytes (52 nM)(43), murine EL-4 thymoma cells (65 nM)(27), unstimulated murine splenocytes (100nM) (34), the low affinity site on the terminal complex of human complement (30-80 nM)(29,42) and a heparin-inducible β-End binding site in human plasma (60nM)(44).

Interestingly, Gelfand and colleagues have recently reported that a naloxone-resistant receptor for β-End exists on culture-derived murine bone marrow Mφ's (33). In their study, bone marrow cells were obtained from DBA/2J mice and cultured for 1 wk in the presence of supernatant containing murine Mφ colony stimulating factor (M-CSF). This procedure resulted in production of cells positive for mature Mφ surface markers (Mac $1^+, 2^+, 3^+$ and $F4/80^+$). Utilizing competition studies, they described a naloxone-insensitive β-End receptor with a K_d of approximately 20nM, compared to 10nM in the present investigation. Results from both studies are similar in that N-acetylated β-End was a potent inhibitor of β-End binding, whereas β-End 1-27 was ineffective. In contrast to Gelfand's data (33), we found that the carboxy terminal residues (β-end 28-31) of β-End were unable to compete with β-End 1-31. They also demonstrated that prostaglandin E_2, an agent with potent immunosuppressive effects on Mφ's (35), induced an upregulation of these sites mediated by cAMP. Therefore, this site is similar, but not identical, to the one we've characterized on peritoneal Mφ's. The subtle differences between the two sites may be due to several factors including differences in Mφ phenotype, maturational environment (culture maturation vs *in vivo* maturation), or the approach to radioreceptor binding experiments (i.e competition experiment-derived K_d vs saturation experiment-derived K_d). Demonstration of the existence of this site on fully differentiated peritoneal Mφ's is important because most functional studies have utilized peritoneal Mφ's in the study of β-End modulation.

One recent study has shown that the dose-dependent enhancement of murine peritoneal Mφ phagocytosis by β-End could not be inhibited by pre-treatment of Mφ's with 5 x 10^{-6}M naloxone (24). However in the vast majority of *in vitro* functional studies,

naloxone is able to reverse, or significantly reduce, the effects induced by incubation of Mφ's with β-End. For example, the β-End-induced enhancement of suboptimally activated (IFN-γ and LPS) murine peritoneal Mφ anti-tumor cytotoxicity could be significantly (80%) reduced when Mφ's were pre-treated with naloxone (10^{-5}M)(9). Moreover, enhancement of murine bone marrow Mφ differentiation M-CSF and LPS could be completely abrogated by naloxone treatment (14). Other studies have shown that superoxide production from human PBMC (11) or P388 cells (Mφ cell line)(13) can be stimulated dose-dependently by β-End and completely inhibited by naloxone. Likewise, the β-End mediated increase in human Mo chemotaxis can be blocked by naloxone (6).

Several possible explanations exist to account for the discrepancies between β-End binding studies on Mφ's and the effects of β-End on various *in vitro* Mφ functions. First, there may be another receptor for β-End with a much higher affinity (10^{-11}M or higher) than the site described herein that is sensitive to naloxone inhibition. This site may not be detectable on Mφ's using classical radioligand binding techniques. Second, naloxone may be exerting its effects in functional studies, not by binding to the same receptor as β-End, but to another site. Evidence to support this contention comes from studies that have documented ^{3}H-naloxone binding to immune cells (45,46,47). In addition, very high concentrations of naloxone (e.g. 10^{-5}M) are often used to reverse the effects of β-End. Such concentrations may have non-specific effects in addition to those mediated by a selective naloxone binding site. Interestingly, Ovadia et al. (1989) demonstrated that ^{3}H-naloxone binding to rat splenocytes could not be displaced by 5 x 10^{-5}M β-End (47).

Peptidases may also be active under the conditions of functional but not binding assays. Ectopeptidases (i.e. CD13), known to be present on Mo (48) and Mφ membranes (49), act to cleave β-End in functional assays. The effects of ectopeptidases are minimized in binding studies due to the addition of protease inhibitors and the use of cold temperatures to protect receptor integrity. Ectopeptidase cleavage of β-End may result in the generation and binding of smaller peptides (i.e. β-End fragments lacking the C-terminal tetrapeptide) to specific naloxone-sensitive receptors. Lastly, fetal bovine serum which is commonly used in functional studies may contain protease activity and binding proteins for β-End (44,50). This may generate different peptide signals from β-End or lower the effective concentration of β-End. Along these lines, it is interesting to note that naloxone failed to block the enhancing effect of β-End in a phagocytosis assay done in the absence of fetal bovine serum (24). Whereas the effects of β-End on other Mφ *in vitro* functions, performed in the presence of fetal bovine serum, have shown either no effect (51,52), or an effect that can be antagonized by naloxone (8,9,13,14). It is likely that one, or a combination, of the above factors are responsible for discrepancies seen between our radioreceptor ligand binding experiments and *in vitro* functional studies regarding the effects of β-End on Mφ's.

While it appears that β-End binds to and alters various Mϕ functions, the source of β-End peptide is unknown. It is unlikely that β-End secretion from the pituitary plays a direct role in tissue Mϕ modulation through the relatively low affinity (10^{-8}M) receptor described in this paper. Interestingly, there have been numerous reports, in both humans and animals, documenting the production of β-End immunoreactivity in cells of the immune system (53,54,55). However, these studies have not demonstrated that this is authentic β-End 1-31. These findings bring up the possibility that local production of β-End by immune or other inflammatory cells (including Mϕ's) can regulate Mϕ function in an autocrine or paracrine fashion. At present, the physiological relevance of such regulation is unknown.

In summary, we have described a naloxone-insensitive receptor for β-End that is present *in vivo* on inflammatory Mϕ's. It is similar to the receptor we have previously described on the pro-monocytic cell line U937. This site has an affinity of 9.75×10^{-9}M as determined by saturation experiments and Scatchard analysis. In addition, there are approximately 10,000 sites per cell, regardless of whether membrane preparations or intact cells were utilized in binding studies. Further studies are needed in order to determine second messenger(s), receptor regulation, and physiological function.

Acknowledgements

This work was supported by DA-04196 (B. M. Sharp) and by an Institutional Training Grant, T32-DA-07239, in support of J. A. Woods.

References

1. M.S. HARBUZ AND S.L. LIGHTMAN, J. Endocrinol. 134 327-339 (1992).
2. E.G. FISCHER. Immunopharm. Immunotox. 10(3) 265-326 (1988).
3. R.A. NORTH. NIDA Research Monographs, (Series 71) R.M. Brown, D.H. Cloet, and D.P. Friedman(Eds) U.S. Dept. Health and Human Services (1986).
4. H. AKIL, E. YOUNG, AND S.J. WATSON, Peptides 2 289-292 (1981).
5. N.E.S. SIBINGA AND V. GOLDSTEIN, Ann. Rev. Immunol. 6 219-249 (1988).
6. M.R. RUFF, S.M. WAHL, S. MERGENHAGEN, AND C.B. PERT, Neuropeptides 5 363-366 (1985).
7. D.E. VAN EPPS, AND L. SALAND, J. Immunol. 132 3046-3053 (1984).
8. A.M. CASELLAS, H. GUARDIOLA, AND F.L. RENAUD, Neuropeptides 18 35-40 (1991).
9. K. HAGI, K. UNO, K. INABA, S. MURAMATSU, J. Neuroimmunol. 50 71-76 (1994).

10. B. MANFREDI, P. SARCEDOTE, M. BIANCHI, L. LOCATELLI, J. VELJIC-RADULOVIC, AND A.E. PANERAI, J. Neuroimmunol. 44 43-48 (1993).
11. B.M. SHARP, W.F. KEANE, J.H. SUH, G. GEKKER, D. TSUKAYAMA, AND P.K. PETERSON, Endocrinology 117 793-795 (1985).
12. B.M. SHARP, D.T. TSUKAYAMA, G. GEKKER, W.F. KEANE, AND P.K. PETERSON, J. Pharmacol. Expt. Ther. 242 579-582 (1987).
13. J.M. TOSK, J.R. GRIM, K.M. KINBACK, E.J. SALE, L.P. BOZZETTI, AND D. WILL, Int. J. Immunopharm. 15 615-620 (1993).
14. K. HAGI, K. INABA, H. SAKUTA, AND S. MURAMATSU, Blood 86 1316-1321 (1995).
15. P.K. PETERSON, B.M. SHARP, G. GEKKER, C. BRUMMITT, AND W.F. KEANE, J. Clin. Invest. 80 824-831 (1987).
16. R.N. MANDLER, W.E. BIDDISON, R. MANDLER, S.A. SERRATE, J. Immunol. 136 934-939(1986).
17. H.M. JOHNSON, E.M. SMITH, B.A. TORRES, J.E. BLALOCK, Proc. Nat. Acad. Sci. 79 4171-4174(1982).
18. W. GILMORE AND L.P. WEINER, Immunopharmacology 17 19-30 (1989).
19. P. VAN DEN BERGH, J. ROZING, AND L. NAGELKERKEN, Immunology 72 537-543 (1991).
20. N.A. SHAHABI, M.Z. BURTNESS, AND B.M. SHARP, Biochem. Biophys. Res. Comm. 175 936-942 (1991).
21. H.W. MCCAIN, I.B. LAMSTER, J.M. BOZZONE, AND J.T. GRBIC, Life Sci. 31 1619-1624 (1982).
22. S.L. BROWN, AND D.E. VAN EPPS, Cellular Immunol. 103 19-26 (1986).
23. P. VAN DEN BERGH, J. ROZING, AND L. NAGELKERKEN, Cell. Immunol. 149 180-192 (1993).
24. M. ICHINOSE, M. ASAI, AND M. SAWADA, J. Immunol. 42 311-316 (1995).
25. L.M. HEMMICK AND J.M. BIDLACK, Life Sci. 41(16) 1971-1978 (1987).
26. E. HAZUM, K. CHANG, AND P. CUATRECASAS, Science 205 1033-1035 (1979).
27. L. SCHWEIGERER, W. SCHMIDT, H. TESCHEMACHER, AND S. WILHELM, Neuropeptides 6 445-452 (1985).
28. M. WESTPHAL AND C.H. LI, Proc. Nat. Acad. Sci. 81 2921-2923 (1984).
29. L. SCHWEIGERER, H. TESCHEMACHER, S. BHAKDI, AND M. LEDERLE, J. Biol. Chem. 258 12287-12292 (1983).
30. N.A. SHAHABI, K.M. LINNER, AND B.M. SHARP, Endocrinology 126 1442-1448 (1990).
31. N.A. SHAHABI, P.K. PETERSON, AND B.M. SHARP, Endocrinology 126 3006-3015 (1990).
32. P. BORBONI, G. DI COLA, G. SESTI, M.A. MARINI, P. DEL PORTO, M. SAVERIA, G. MONTANI, R. LAURO, R. DE PIRRO, Biochem. Biophys. Res. Comm. 163 642-648(1989).
33. R.A. GELFAND, H.T. WEPSIC, L.N. PARKER, AND M.R. JADUS, Immunol. Lett. 45 143-148 (1995).
34. L. JIA, H. HARA, T. OKOCHI, AND S. NEGORO, Int. J. Immunopharm. 14 809-819 (1992).
35. W.J. JOHNSON, S.D. SOMERS, AND D.O. ADAMS, In: Contemporary Topics in Immunobiology. Macrophage Activation, D.O. Adams and M.G. Hanna (Eds)13 127-146, Plenum, New York (1984).

36. T.A. HAMILTON AND D.O. ADAMS, In: Tumor Immunology, W. Den Otter and E.J. Ruitenberg (Eds)89-107, Elsevier, Amsterdam (1987).
37. D.S. JESSOP, S.L. LIGHTMAN, AND H.S. CHOWDREY, J. Neuroimmunol. 49 197-203 (1994).
38. D.B. BYLUND, AND M.L. TOEWS, Am. J. Physiol. 265 L421-L429 (1993).
39. K. KUMAGAI, K. ITOH, S. HINUMA, AND M. TADA, J. Immunol. Meth. 29 17-25 (1979).
40. M.M. BRADFORD, Anal. Biochem. 72 248-251 (1976).
41. G.A. MCPHERSON, J. Pharmacol. Meth. 14 213 (1985).
42. L. SCHWEIGERER, S. BHAKDI, AND H. TESCHEMACHER, Nature 296 572-574 (1982).
43. J.R. DAVE, N. RUBINSTEIN, AND R.L. ESKAY, Endocrinology 117 1389-1396 (1985).
44. A. HILDEBRAND, L. SCHWEIGERER, AND H. TESCHEMACHER, J. Biol. Chem. 263(5) 2436-2441 (1988).
45. N.E. FALKE, E.G. FISCHER, AND R. MARTIN, Cell. Biol. Int. Rep. 11 10041-1047 (1985).
46. J.J. MADDEN, R.M. DONAHOE, J. ZWEMER-COLLINS, D.A.SHAFER, AND A. FALEK, Biochem. Pharmacol. 23 4103-4109 (1987).
47. H. OVADIA, P. NITSAN AND O. ABRAMSKY, J. Neuroimmunol. 21 93-102 (1989).
48. R.A. ASHMUN AND A. T. LOOK, Blood 75(2)462-469 (1990).
49. P.T.W. VAN HAL, J.P.M. HOPSTAKEN-BROOS, J.M. WIJKHUIJS, A.A. TEVELDE, C.G. FIGDOR, AND H.C. HOOGSTEDEN, J. Immunol. 149 1395-1401 (1992).
50. H. SATO, Y. SUGIYAMA, Y. SAWADA, T. IGA, AND M HANANO, Life Sciences 37 1309-1318 (1985).
51. W.C. KOFF AND M.A. DUNEGAN, J.Immunol. 135 350-354 (1985).
52. R. PECK, Ann. N.Y. Acad. Sci. 496 264-270 (1987).
53. S.J. LOLAIT, J.A. CLEMENTS, A.J. MARKWICK, C. CHENG, M. MCNALLY, A.I. SMITH, AND J.W. FUNDER, J. Clin. Invest. 77 1776-1779 (1986).
54. E.M. SMITH, D.HARBOUR-MCMENAMIN, AND J.E. BLALOCK, J. Immunol. 135 779s-782s (1985).
55. M. THROSBY, Z. YANG, D. LEE, W. HUANG, D.L. COPOLOV, AND A.T. LIM, Endocrinology 133 2889-2896 (1993).