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# Analysis of the Beta-Endorphin Structure-Related Activity on Human Monocyte Chemotaxis: Importance of the N- and C-Terminal

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SACERDOTE, P. AND A. E. PANERAI. *Analysis of the beta-endorphin structure-related activity on human monocyte chemotaxis: Importance of the N- and C-terminal.* PEPTIDES **10**(3) 565–569, 1989.—We evaluated the chemotactic activity of beta-endorphin and beta-endorphin-related peptides on human monocytes. We tested beta-endorphin(1–31) and fragments (1–16), (1–17), (1–27) in which the N-terminal of the opioid is preserved, N-acetyl-beta-endorphin(1–31) and fragments (6–31) and (28–31) in which the C-terminal is preserved, and fragment (2–17) that lacks both the N- and C-terminal. The fragments in which the N- and C-terminal were preserved [with the exception of fragment (28–31)] showed a chemotactic effect, while the lack of both terminals deprived the peptides of any activity. Moreover, only the N-terminal-mediated effects were naloxone reversible, while the C-terminal effects were not. These results indicate that while the intact N-terminal is necessary for opioid like effects, both N- and C-terminal can mediate effects on the immune system, thus offering evidence for a nonopioid receptor-mediated effect of opioid peptides on the immune system.

Beta-endorphin Monocytes Chemotaxis Opioid receptors Immune system
Beta-endorphin N-terminal fragments Beta-endorphin C-terminal fragments

EVIDENCE has accumulated in recent years that neuropeptides are common mediators of the central nervous system (CNS) and the immune system (IS) (6). One of the neuropeptides that influence a number of immune functions both in rodents and human is beta-endorphin (BE). BE depresses some T- and B-lymphocyte functions in vitro, such as active T cell rosette formation, lymphocyte proliferation, antibody production, and may augment other lymphoid functions (14). For example, the opioid peptide can enhance natural killer cell function and induce monocyte chemotaxis (11,16). However it soon appeared that not all the effects on the immune system are classically reversible by naloxone and several binding studies indicated the presence on lymphocytes and other components of the IS of specific "non-opioid" receptors (3,5). It is well known that in opioid peptides an intact N-terminal is necessary in order to manifest opioid binding and opioid effects. Moreover, any modication of the N-terminal, e.g., acetylation or deletion of one or more amino acids, results in a loss of classical opioid effects, i.e., analgesia. On the contrary, the opioid effects on IS are qualitatively, although not quantitatively, maintained when even several amino acids are deleted from the C-terminal (1).

An effect of BE on chemotaxis of human monocytes has been known for some time, but the portion of the BE molecule responsible for this effect has not been identified (11,16).

In the present study we evaluated the chemotactic activity of several N- and C-terminal BE fragments on the human monocyte chemotaxis, in order to better characterize the opioid receptors present on monocytes.

## METHOD

The peptides we employed in our studies and their sequences are reported in Table 1.

Human- and camel-beta-endorphin, alpha-, gamma-, delta-endorphin, N-acetyl-beta-endorphin, Des-Tyr-beta-endorphin, and fragment (28–31) were purchased from Peninsula (Belmont, CA) or Sigma (St. Louis, MO) and h-beta-endorphin(6–31) was a generous gift from the late C. H. Li (San Francisco, CA).

### *In Vitro Chemotaxis of Human Monocytes*

Human peripheral blood was obtained from healthy volunteers. Mononuclear cells were separated by sedimentation over Ficoll-Paque (Pharmacia) (13). The time elasping between collection of

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TABLE 1

| Name | Amino Acid Sequence |
|---|---|
| Beta-endorphin | YGGFMTSZKSZTPLVTLFKBAIIKBAYKKGZ |
| Delta-endorphin | YGGFMTSZKSZTPLVTLFKBAIIKBAY |
| Gamma-endorphin | YGGFMTSZKSZTPLVTL |
| Alpha-endorphin | YGGFMTSZKSZTPLVT |
| N-Acetyl-beta-endorphin | Ac-YGGFMTSZKSZTPLVTLFKBAIIKBAYKKGZ |
| Des-Tyr-gamma-endorphin | GGFMTSZKSZTPLVTL |
| h-Beta-endorphin(6–31) | TSZKSZTPLVTLFKBAIIKBAYKKGZ |
| h-Beta-endorphin(28–31) | KKGZ |

blood sample and separation never exceeded four hours. In preliminary studies, we observed, in fact, that six hours is the time since sampling during which chemotactic activity to formylpeptide is 100% preserved. The cells were washed and resuspended in Dulbecco's modified Eagle medium to which 1% BSA 20 nM Hepes were added, and diluted to a final concentration of one million monocytes/ml. The chemotaxis assay was performed using a 48-well microchemotaxis chamber (Neuroprobe, Bethesda, MD). Fifty thousand cells/well were placed in the upper compartment and chemoattractant substances at different concentration in the lower one. A 5 μm pore polycarbonate filter separates the upper and lower chamber, in order to allow the cells to migrate actively through the pores, minimizing random movements. After 90 minutes incubation at 37°C, the migrated cells adherent to the distal part of the filter were fixed and stained (12,13). These migrating cells were quantitated microscopically by counting three fields in triplicate using an optical image analyzer (IBAS, Zeiss). Data are expressed as chemotactic index (C.I.), that is the ratio between migration toward test attractants and buffer alone. The number of migrating cells in the buffer alone controls generally ranged from 20–50 cells/field. As positive control, the migration to the well-known chemotactic peptide f-Met-Leu-Phe (fMLP) was assessed. A chemotactic index of 5–6 was observed at $10^{-8}$ M fMLP. When required, the mu selective opioid receptor antagonist naloxone (SIFAC, Como, Italy) was added at the fixed concentration of $10^{-11}$ M to lower wells together with the peptide under investigation.

In a further experiment, an N-terminal BE sequence [beta-endorphin(1–17)] and a C-terminal BE sequence [beta-endorphin 6–31)] were associated at graded doses (from $10^{-14}$ to $10^{-8}$), and

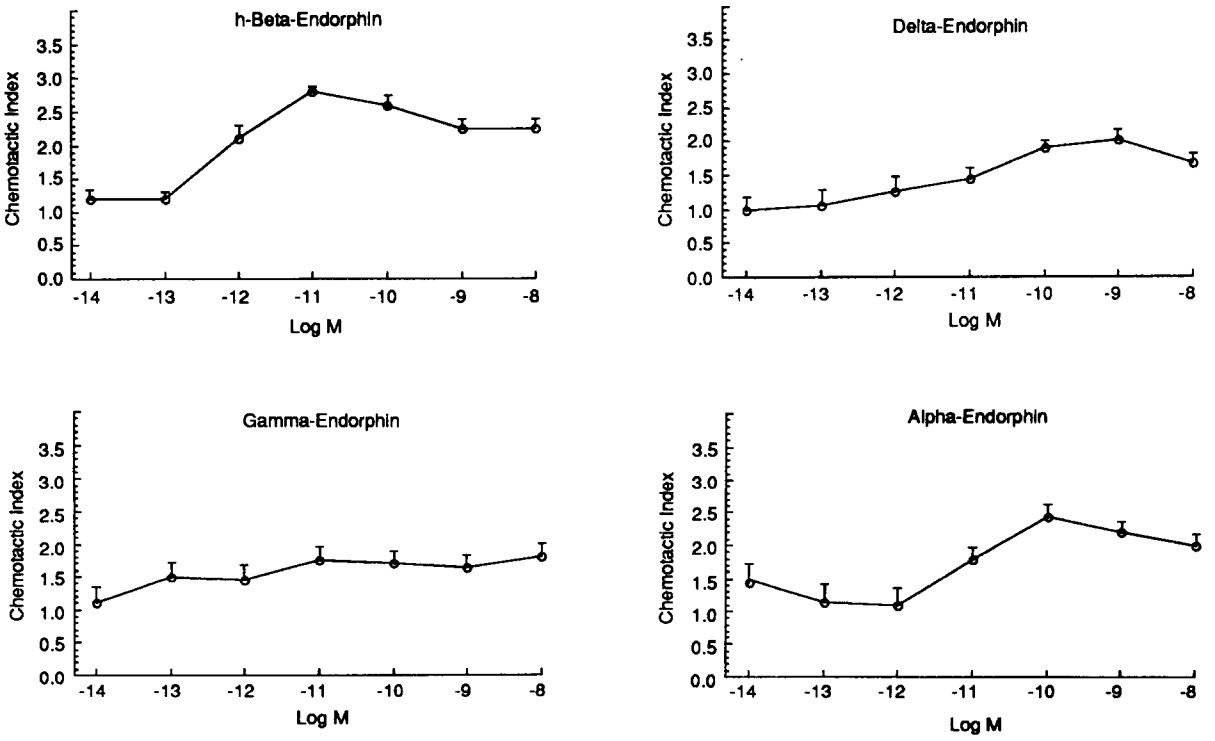


FIG. 1. Human monocyte chemotactic activity to the intact N-terminal beta-endorphin-related sequences. Values are means ± SD obtained from five experiments.

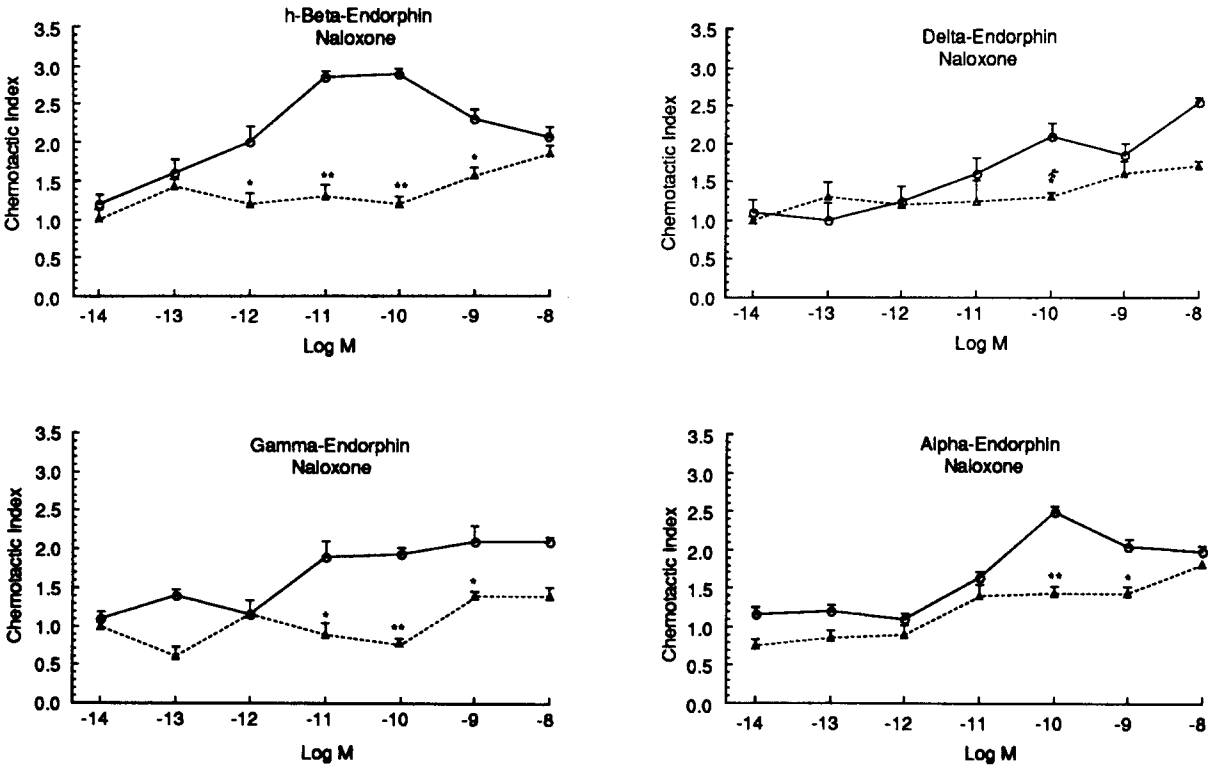


FIG. 2. Effect of a fixed naloxone concentration ($10^{-11}$ M) on N-terminal beta-endorphin-induced chemotaxis. (○) peptides only; (△) peptides + naloxone.

the chemotactic activity evaluated.

Statistical evaluation of results was performed by the two-way analysis of variance.

## RESULTS

As expected, all the peptides with the intact N-terminal elicited the chemotactic effect on human monocytes shown in Fig. 1. The whole BE molecule is the most active, its chemotactic activity is already evident at $10^{-12}$ and reaches the peak activity (C.I. = 3.5) at $10^{-10}/10^{-9}$ M, in agreement with what has been observed for other chemotactic neuropeptides (12,13). The shorter N-terminal fragments are active at similar concentrations, but the peak activity is lower. The chemotaxis induced by N-terminal fragments was reversed by the opioid antagonist naloxone, as shown in Fig. 2, at a dose ($10^{-11}$) devoid of any chemotactic effect [$F(1,55) = 166.2$, $p<0.005$ for BE; $F(1,55) = 49.9$, $p<0.005$ for delta-endorphin; $F(1,55) = 148.9$, $p<0.005$ for gamma-endorphin; $F(1,55) = 41$, $p<0.005$ for alpha-endorphin. It appears from the figure that the inhibition by naloxone is full at concentrations of the peptides in the range $10^{-14}/10^{-10}$, while it is less evident at higher concentration of the peptides. Figure 3 shows that the C-terminal fragments N-acetyl-beta-endorphin and h-beta-endorphin(6–31) are chemoattractant with a potency identical to the N-terminal fragments shorter than BE(1–31), but that on the contrary their effect is not inhibited by naloxone. The four amino acid C-terminal fragment (28–31) is not effective, as well as fragment (2–17) that lacks both the N- and C-terminals; these results are reported in Fig. 4.

When N- and C-terminal sequences were added together in the lower chamber, no higher response was observed, but we observed a potentiating effect. In fact a chemotactic response is elicited at a concentration as low as $10^{-14}$ M, at which the two peptides alone are inactive (Fig. 5).

## DISCUSSION

The results presented are consistent with previous data showing that BE can interact with the IS at two different sites, depending whether N- or C-terminal sequences of the molecule bind the receptor (6).

Both the N- and C-terminal peptides have chemotactic activity, but only the chemotaxis induced by the N-terminal fragment is inhibited by naloxone, indicating a ligand-receptor interaction similar to the one observed in the other systems. It is remarkable that naloxone at $10^{-11}$ can block the chemotactic activity of higher concentration of BE. The relative affinity for the mu site of BE and naloxone in tissues other than the immune system has been extensively studied with controversial results, but at least in some brain areas, i.e., striatum, naloxone possesses higher affinity for the mu receptor than BE (4). In addition, the medium utilized in the chemotaxis experiments contains high concentration of $Na^+$, and it is well established that $Na^+$ favors the binding of opiate antagonists (7).

The fragment BE(2–17), which lacks both the N- and C-terminals, is completely inactive. Indeed, the whole BE sequence is more active than fragments in which only the N- or C-terminals are preserved. On the basis of the data presented, it is impossible to give a full explanation for this greater potency. The experiments we conducted using both the N- and C-terminal peptides together seem to rule out the existence of different populations of cells bearing either the opioid or "nonopioid" receptor, since no

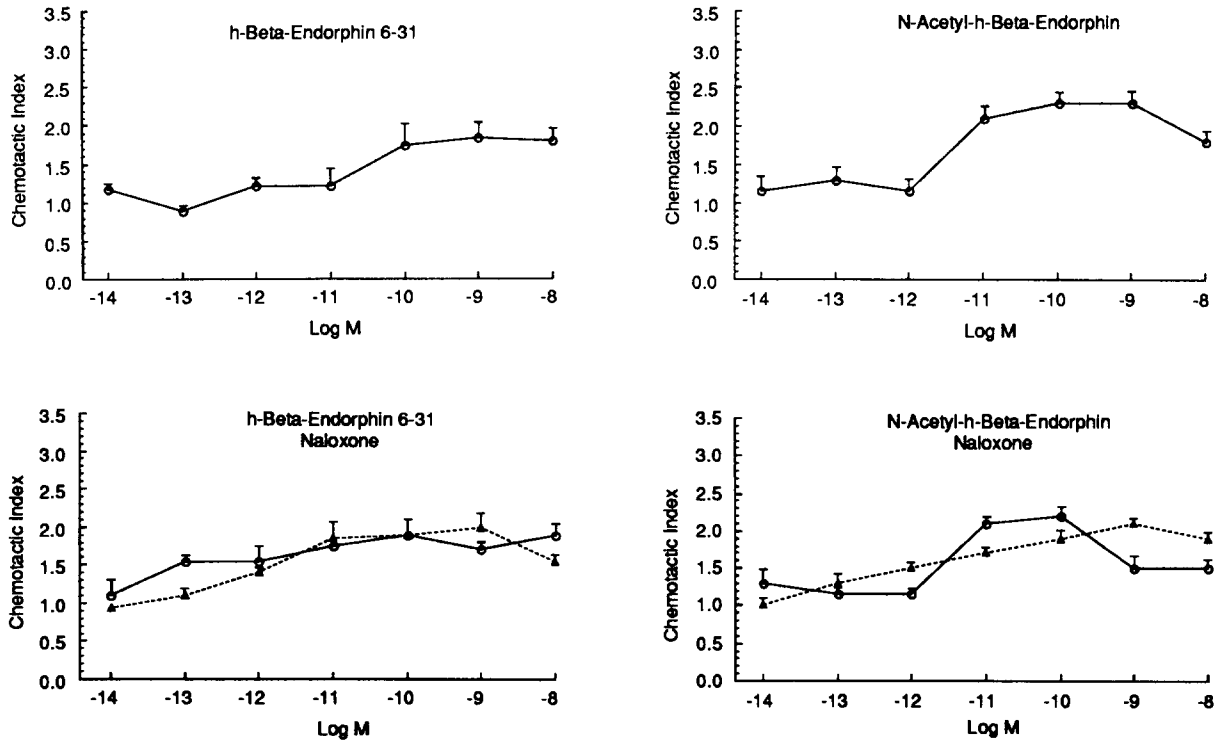


FIG. 3. Chemotactic activity to the C-terminal beta-endorphin-related sequences in the absence of (○), and the presence of $10^{-11}$ M naloxone (△).

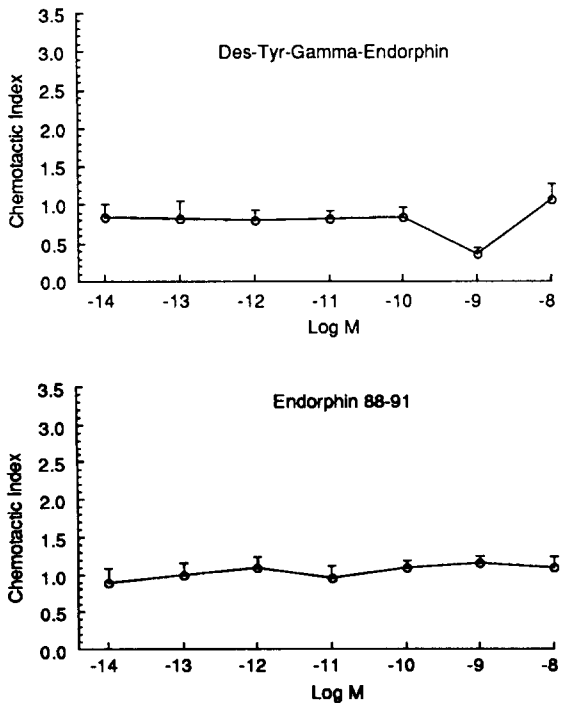


FIG. 4. Human monocyte chemotactic activity to beta-endorphin fragment (2–17) and (28–31).

additive biological effect was observed. If these fragments had intrinsic chemotactic action on separate receptors located on different cells, an additive biological response had to be observed. It is feasible that the complete molecule, consisting of 31 amino acids, possesses a conformation given by the secondary structure, which yields higher affinity for the N-receptor. This conformation is probably lost in the shorter fragments. The same seems to be true for the C-terminal fragments; in fact, the N-acetyl-beta-endorphin is more active than fragment (6–31), and the last C-terminal four amino acids are not sufficient for the C-terminal effect. An interesting aspect which remains to be elucidated is a possible interaction between the opioid receptor and the nonopioid receptor in a receptor complex. A hypothetical facilitation between

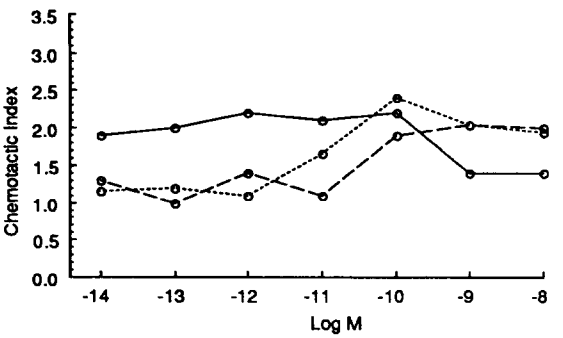


FIG. 5. Chemotactic activity of beta-endorphin(1–17) (——), beta-endorphin(6–31) (---), and combination of the two fragments (— —).

the two receptors could be suggested by the potentiation observed when N- and C-terminal peptides are tested together. It is well known that subliminal concentrations of two stimuli can show a marked synergism in the activation of cells of the IS, i.e., proliferation, and that a maximum stimulatory response is always reached independently from the number of stimuli applied (10). We consistently observe the same peak C.I. when N- and C-sequences are applied alone or together, but the response is already present at $10^{-14}$ M.

An important characteristic of BE and BE-related peptide-induced chemotaxis is the fact that monocytes can respond to less than $10^{-11}$ M concentration of those agents. These concentrations, in fact, are approximately the estimated physiological plasma levels of BE, and these levels can further increase both in physiological and pathological conditions, such as delivery and stress (2, 8, 9).

One of the most interesting observations is the chemotactic effect to N-acetyl-beta-endorphin. It is well known, in fact, that this peptide is the major form of circulating BE, does not bind to the opioid receptor, and is devoid of any opioid-like effect, e.g., analgesia (1). This observation suggests that the acetylation might transform the native pituitary BE into another important peptide that modulates the immune response. Furthermore a series of recent studies has suggested that lymphocytes can synthetize BE and some other hormones, such as ACTH and TSH (6, 14, 15). These findings reveal a new important source of BE, and it could be of great interest to analyze the molecular forms of the lymphocyte-derived BE.

The in vivo role of this peptide and its fragments is unclear at this time, but the chemotactic response of monocytes, taken together with the many described immunological effects of BE, suggests that it can actively alter the host immune system. Finally, our data suggest that although BE and related peptides are common mediators of CNS and IS, they behave differently in the two systems.

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