

Opioid-mediated Suppression of Interferon- γ Production by Cultured Peripheral Blood Mononuclear Cells

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Abstract

Mounting evidence suggests that opiate addiction and stress are associated with impaired cell-mediated immunity. We tested the hypothesis that morphine and the endogenous opioid β -endorphin (β -END), a pituitary peptide released in increased concentrations during stress, can suppress the production of the key macrophage-activating lymphokine interferon- γ (IFN- γ) by cultured human peripheral blood mononuclear cells (PBMNC). Using a radioimmunoassay to measure IFN- γ , we found that exposure of PBMNC to biologically relevant concentrations of both opioids significantly inhibited IFN- γ generation by cells stimulated with concanavalin A and varicella zoster virus. Studies of the mechanism of suppression revealed (a) a classical opioid receptor is involved (suppression was antagonized by naloxone and was specific for the NH₂ terminus of β -END), (b) monocytes are the primary target cell for opioids (monocyte-depleted lymphocyte preparations showed little suppression), and (c) reactive oxygen intermediates (ROI) and prostaglandin E₂ are important mediators (scavengers of ROI and indomethacin eliminated the suppression). Based on these findings we suggest that opioid-triggered release of inhibitory monocyte metabolites may play a role in the immunodeficiency associated with narcotic addiction and stress.

Introduction

During the past several years, the immunodulatory effects of opiates and endogenous opioid peptides have received considerable attention (1-4). Evidence is accumulating from both in vivo and in vitro sources that points to important interactions between opiates and T lymphocytes (5-12) and mononuclear phagocytes (13-15). Of highly related interest have been studies with β -endorphin (β -END)¹ (15-19). From these latter

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1. *Abbreviations used in this paper:* ACTH, corticotropin; β -END, β -endorphin; con A, concanavalin A; GHBSS, Hank's balanced salt solution containing 0.1% gelatin; IFN- γ , interferon- γ ; NAC- β -END, N-acetylated β -endorphin; PGE₂, prostaglandin E₂; PBMNC, peripheral blood mononuclear cells; POMC, proopiomelanocortin; ROI, reactive oxygen intermediates; SOD, superoxide dismutase; VZV, varicella zoster virus.

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studies has evolved the concept that β -END as well as other neuropeptides function as communication signals between the nervous system and the immune system (3, 20, 21). Since β -END is released in increased quantities from the anterior pituitary during stress, the possibility exists that this peptide hormone is partly responsible for the suppressed cell-mediated immunity that accompanies stress (22-25).

Effective cell-mediated immunity has been known for some time to be dependent upon activation of macrophages by T lymphocytes, and interferon- γ (IFN- γ) now has been identified as the principal T cell lymphokine responsible for enhancement of macrophage microbicidal (26, 27) and tumoricidal (28, 29) activity. In addition to its macrophage-activating properties, IFN- γ , which is produced by activated T lymphocytes and natural killer cells (30), has multiple other functions in host defense (31-33).

Recently, Johnson et al. (34) have demonstrated that corticotropin (ACTH), a proopiomelanocortin (POMC)-derived peptide that is co-secreted with β -END, markedly suppresses INF- γ production in mouse spleen cell cultures. In the present study, we investigated the effects of β -END and morphine on IFN- γ production by cultured human peripheral blood mononuclear cells (PBMNC). We found that both of these opioids profoundly inhibit IFN- γ generation in this cell system. The mechanism of suppression appears to involve a classical opioid receptor in which monocytes serve as the primary target cell with depressed lymphocyte activation and INF- γ production occurring as a consequence of the release of inhibitory monocyte metabolites.

Methods

Cell preparations

PBMNC. Heparinized venous blood was obtained from healthy volunteers with a mean age of 36 yr (range 24-43 yr) who were not taking any medications. PBMNC were isolated by adding dextran (3 ml dextran to 10 ml blood) and layering on lymphocyte separation medium (Litton Bionetics, Inc., Kensington, MD). After centrifugation, the mononuclear cell layer was washed twice and resuspended in Hank's balanced salt solution containing 0.1% gelatin (GHBSS) to yield a final concentration of 2×10^6 PBMNC/ml GHBSS. Cytocentrifuge preparations were examined, and monocytes and lymphocytes were enumerated. In addition to morphologic features, monocytes were identified by staining for nonspecific esterase (35). Using these techniques, PBMNC suspensions were found to contain from 15 to 38% monocytes and from 62 to 85% lymphocytes.

Purified mononuclear cell preparations. Monocytes were isolated from heparinized venous blood using microexudate-coated tissue culture flasks (36, 37); final cell suspensions contained > 95% monocytes. Monocyte-depleted lymphocyte preparations were recovered from the nonadherent cells by sequential passage on microexudate-coated tissue culture flasks for 45 min followed by incubation on uncoated polystyrene tissue culture flasks (Corning Glass Works, Corning, NY) for 45 min. The lymphocyte preparations contained a mean of 97% lympho-

cytes (range 95–98% lymphocytes) and 3% monocytes (range 2–5% monocytes).

Cell viability. All cell preparations contained > 95% viable cells as assessed by trypan blue exclusion criteria. Exposure of cells to opioids during cell culture, both in the presence and absence of stimuli of INF- γ production, did not affect cell viability when compared with cultured control cells.

Cell culture medium. For all assays, PBMNC and purified cell preparations were incubated in a culture medium consisting of RPMI 1640 (Sigma Chemical Co., St. Louis, MO) with 20% defined bovine serum (HyClone Laboratories, Sterile Systems, Inc., Logan, UT) containing 100 U penicillin/ml, 100 μ g streptomycin/ml (Gibco, Grand Island, NY), and 2 mM L-glutamine (KC Biological, Inc., Lenexa, KA).

Opioids and related materials

Morphine sulphate and naloxone were kindly provided by the National Institute on Drug Abuse. Human sources of β -END₁₋₃₁, β -END₁₋₂₇, N-acetylated β -END₁₋₃₁ (NAC- β -END), [D-Ala²]- β -END₁₋₃₁, [D-Ala²]-Met-enkephalinamide, and ACTH₁₋₃₉ were obtained from Peninsula Laboratories, Inc., (Belmont, CA).

IFN- γ production and measurements

For each experiment, 100 μ l volumes of cell suspension (2×10^5 cells in GHBSS) were pipetted in replicates of five or six into 96-well flat-bottom microtiter plates (Flow Laboratories, Inc., McLean, VA), centrifuged, and resuspended in 100 μ l culture medium alone (control) or culture medium containing opioids or ACTH at indicated final concentrations. In experiments with naloxone, cells were preincubated with this antagonist for 30 min at indicated concentrations before adding opioids. After the stated period of exposure to opioids and ACTH, 10 μ l culture medium alone (unstimulated cells), culture medium containing an optimal-stimulating 10 μ g/ml dose of concanavalin A (con A; Sigma Chemical Co.), or a 1:50 dilution of varicella zoster virus (VZV; Whitaker M. A. Bioproducts, Walkersville, MD) (stimulated cells) was added, and plates were then incubated for 72 h (con A experiments) or 6 d (VZV experiments) at 37°C in a 5% CO₂ incubator. After centrifugation, supernatant fluids were collected.

To measure the secretion of IFN- γ into the supernatant fluids, radioimmunoassay (38) kits (Centocor Corp., Malvern, PA) were used with minor modifications of a previously described method (39). Briefly, 200 μ l of supernatant fluid was mixed with a polystyrene bead onto which a murine monoclonal antibody to human IFN- γ was bound. After 2 h incubation at room temperature, beads were washed four times with distilled water, and then a 200- μ l suspension of ¹²⁵I-anti-IFN- γ was added to the beads. After 2 h, the beads were again, washed four times with distilled water, and the amount of ¹²⁵I (counts per minute) bound to the beads was measured by a detector system (Crystal Multi Detector Gamma System; United Technologies, Packard Instrument Co., Inc., Downers Grove, IL) fit with a computer program that converts counts per minute to Units of IFN- γ . The concentration of IFN- γ in culture supernatant fluids was determined by linear regression analysis using the IFN- γ control and standards provided with the kit. Intra- and interassay variability with this method is low; standard deviations were generally < 10% of the mean for intra- and interassay comparisons.

Cell supernatants

To determine whether a suppressive factor was released into the culture supernatants of opioid-exposed cells, purified monocyte and lymphocyte preparations were constituted in wells of microtiter plates in the absence or presence of β -END, as described above. For this experiment, the number of monocytes and lymphocytes added to the microtiter wells was adjusted to approximate the number of each cell type found in mixed PBMNC preparations, i.e., 5×10^4 monocytes and 1.5×10^5 lymphocytes per well. After 72 h incubation at 37°C, plates were centrifuged and supernatants were harvested. Supernatant (100 μ l) was

then added to wells containing fresh purified lymphocyte preparations and con A.

Scavengers of reactive oxygen intermediates (ROI)

To test the role of oxygen metabolites in opioid-mediated suppression of IFN- γ production, PBMNC were suspended in culture medium containing the indicated final concentrations of the superoxide scavenger, superoxide dismutase (SOD; bovine erythrocyte, Sigma Chemical Co.) or catalase (bovine liver, Sigma Chemical Co.) to remove hydrogen peroxide. Heat-inactivated enzymes were prepared by autoclaving for 30 min at 20 atm pressure and were included as controls. Opioids and con A were then added sequentially as outlined in the protocol for IFN- γ production.

Studies of prostaglandin metabolism

To block prostaglandin metabolism, the cyclooxygenase inhibitor indomethacin (Sigma Chemical Co.) was added to PBMNC cultures, at a final concentration of 0.5 μ M for 30 min before adding opioids and con A sequentially and determining IFN- γ production. In one experiment, volunteers received indomethacin (Lederle Laboratories, Div. American Cyanamid Co., Wayne, NJ) 25 mg orally every 8 h for a total of eight doses before donating blood.

The effect of prostaglandin E₂ (PGE₂) on IFN- γ production was tested by adding PGE₂ (Sigma Chemical Co.) at the indicated final concentrations to PBMNC cultures before adding con A, and determining IFN- γ production after 72 h incubation.

To determine whether β -END mediated its effect through stimulation of prostaglandin metabolism, PBMNC (2×10^5 cells) were exposed to culture medium alone or medium containing β -END at 10^{-12} M for 3 h. Supernatants were harvested and stored at -70°C. PGE₂ was assayed using a competitive binding [¹²⁵I]PGE₂ radioimmunoassay (New England Nuclear, Boston, MA) with minor modifications. Briefly, pooled cell supernatants (0.5 ml) were acidified and extracted as recommended with C₁₈ followed by silica columns (Bond-Elut; Analytichem International, Inc., Harbor City, CA). Extracted samples were diluted 1:2,000 with assay buffer provided, and values of PGE₂ were interpolated from a standard dose-response curve.

Lymphocyte proliferative activity

Proliferative responses were measured in quadruplicate using 96-well microtiter plates with each well containing 2×10^5 PBMNC and culture medium alone or culture medium containing con A (10 μ g/ml). Cells were preincubated with opioids before adding con A as outlined in the procedure for determination of IFN- γ production. After 72 h incubation at 37°C, 0.5 μ Ci [³H]thymidine (New England Nuclear) was added to each well. After 6 h incubation, cells were washed four times with cold phosphate-buffered saline, and pooled cell fraction was added to 3 ml of scintillation fluid (Packard Instrument Co., Inc., United Technologies). Counts were performed on a scintillation counter (LS 5801; Beckman Instruments, Inc., Fullerton, CA).

Statistical analysis

Where appropriate, data were expressed as means \pm SE. In each experiment, *n* refers to cell samples from separate individual donors. For multiple group comparisons, the significance of differences between the means of different groups was determined by one-way analysis of variance; comparisons between individual groups were calculated by the method of Bonferoni, as reported by Wallenstein et al. (40). For comparisons of means of two groups, statistical significance was assessed by Student's *t* test. Statistical significance was defined as *P* < 0.05.

Results

Opioid suppression of con A-stimulated cells. The effect of opioids on con A-stimulated IFN- γ production was evaluated initially using concentrations of morphine and β -END that

were considered to fall within pharmacologically (10^{-10} – 10^{-6} M) and physiologically (10^{-14} – 10^{-10} M) relevant ranges, respectively (Fig. 1). While con A-stimulated control cells yielded close to 100 U IFN- γ , PBMC that were exposed to opioids at these concentrations generated < 50 U IFN- γ , a difference that was statistically significant for morphine at all concentrations and for β -END at 10^{-12} – 10^{-10} M. When cells were exposed to morphine and β -END at 10^{-18} – 10^{-12} M, significant inhibition of IFN- γ production was observed only at 10^{-12} M (data not shown), and at this concentration morphine and β -END had similar inhibitory potencies of 51 and 49%, respectively.

In the preceding experiments, PBMC were incubated with morphine and β -END for 3 h before adding con A. Using 10^{-8} M morphine and 10^{-12} M β -END, we found that maximal suppression of IFN- γ was achieved within 3 h, i.e., preincubation of PBMC with opioids for 1 h before adding con A resulted in only 25% suppression of IFN- γ release, whereas preincubation for 24 h did not afford any greater suppression than that observed when cells were exposed to opioids for only 3 h. Also, removal of the opioids by washing after the 3-h preincubation eliminated their suppressive effect, although removal after 24 h preincubation resulted in ~ 20% suppression (data not shown). Thus, for all subsequent experiments, PBMC were exposed to opioids for 3 h before adding con A, and the opioids were left in the cell cultures throughout the 72-h incubation.

Unstimulated control cells produced small amounts of IFN- γ (Fig. 1). In subsequent experiments, the value for unstimulated control cells, which averaged ~ 2 U IFN- γ , was subtracted from those obtained with stimulated cells. Incubation of PBMC with morphine (10^{-10} – 10^{-6} M) and β -END (10^{-14} – 10^{-10} M) alone for 72 h did not yield IFN- γ levels that were any higher than those observed with unstimulated control cells (data not shown).

Since other investigators have demonstrated that the POMC-derived peptide ACTH is capable of inhibiting IFN- γ production (34), we next compared the suppressive activity of β -END to that of ACTH. PBMC exposed to β -END and ACTH for 3 h at 10^{-12} M concentrations before stimulation with $10 \mu\text{g}$ con A for 72 h secreted only 24 ± 15 U and 18 ± 4 U IFN- γ , respectively, as compared with 115 ± 5 U IFN- γ generated by control cells ($P < 0.05$, $n = 3$). Thus, at a concentration of 10^{-12} M, the suppressive activity of these two anterior pituitary peptides appears to be relatively similar. At concentrations ranging from 10^{-18} to 10^{-14} M, neither peptide gave significant inhibition (data not shown).

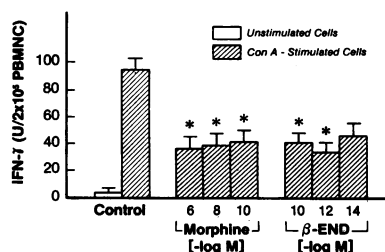


Figure 1. Effect of morphine and β -END on IFN- γ production by con A-stimulated PBMC. Cells were preincubated for 3 h in culture medium alone (control) or opioids, at the indicated concentrations, before adding

buffer (Unstimulated Cells) or con A (Con A-Stimulated Cells). After 72 h, IFN- γ release into culture supernatants was measured. Values are means \pm SE of experiments with four donors (* $P < 0.05$ compared with control cells).

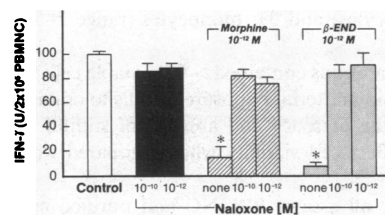


Figure 2. Effect of naloxone on opioid-mediated suppression of IFN- γ production. PBMC were preincubated with naloxone or buffer (none) for 30 min before adding culture medium alone,

morphine, or β -END at the indicated concentrations. After 3 h incubation, con A was added to the cells, and IFN- γ release was measured at 72 h. Control cells were preincubated in culture medium alone and stimulated with con A in the absence of naloxone and opioids. Values are means \pm SE of experiments with three donors (* $P < 0.05$ compared with control cells).

Involvement of a classical opioid receptor in suppression.

To determine whether opioids mediate their suppressive effect on PBMC IFN- γ production via a classical opioid receptor mechanism, we first studied the influence of the opioid antagonist naloxone in this system. Whereas naloxone itself did not significantly inhibit IFN- γ production, it completely abrogated the suppressive activities of morphine and β -END at equimolar concentrations (Fig. 2).

Responses to endorphins that involve classical opioid receptors are mediated through the NH₂ terminus of these peptides, and *N*-acetylation of β -END markedly decreases its affinity for the opioid receptor rendering it inactive (41, 42). When we compared the suppressive activity of NAC- β -END to β -END at concentrations from 10^{-14} to 10^{-10} M, significant suppression of PBMC IFN- γ generation was observed only with β -END (Fig. 3). The effect of altering the carboxy terminus of β -END was assessed by studying the suppressive activity of β -END₁₋₂₇, a manipulation that did not change its inhibitory potency (Fig. 3). Taken together with the experiments demonstrating antagonism by naloxone, these findings indicate that a classical opioid receptor is involved in opioid-mediated suppression of PBMC IFN- γ production.

To control for proteolytic degradation of β -END and to assess whether the *N*-terminal five amino acid sequence of β -END was also inhibitory, the suppressive activities of the

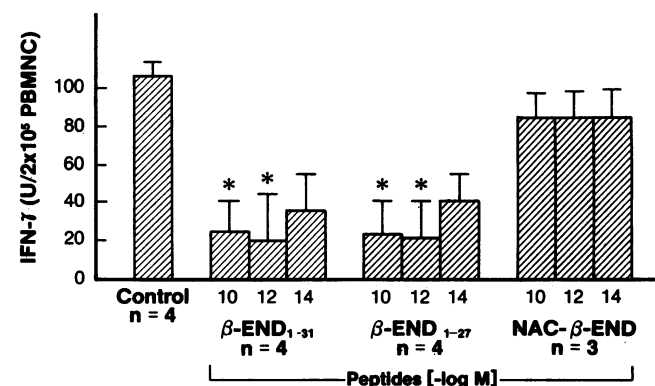


Figure 3. Influence of alterations of β -END on suppressive activity. PBMC were preincubated for 3 h with culture medium alone (control), β -END₁₋₃₁, β -END₁₋₂₇, and NAC- β -END₁₋₃₁, at the indicated concentrations, before stimulation with con A for 72 h. Values are means \pm SE for the given number of donors (* $P < 0.05$ compared with control cells).

Table I. Suppressive Activities of Peptides Resistant to Enzymatic Degradation

| Peptide concentration | IFN- γ Generation by cells exposed to* | | |
|-----------------------|---|-------------------------------------|---|
| | β -END | [D-Ala ²]- β -END | [D-Ala ²]-met-enkephalinamide |
| <i>M</i> | | | |
| 10 ⁻¹² | 48±8.5 [‡] | 45±8.5 [‡] | 64±9.1 |
| 10 ⁻¹⁴ | 62±11 | 53±12 | 46±8.7 [‡] |
| 10 ⁻¹⁶ | 62±12 | 60±12 | 56±13 |
| 10 ⁻¹⁸ | 67±17 | 68±17 | 60±17 |

* PBMNC were incubated for 3 h in culture medium containing peptides, at the indicated final concentrations, before adding con A and measuring IFN- γ (U/2×10⁵ PBMNC) release after 72 h culture.

[‡] *P* < 0.05 compared with the value with control PBMNC (95±6.4 U IFN- γ) incubated in culture medium with con A in the absence of peptides. All values are means±SE (*n* = 5).

hydrolysis-resistant analogues [D-Ala²]- β -END and [D-Ala²]-Met-enkephalinamide (43) were compared with that of β -END (Table I). Similar inhibitory potencies were observed with [D-Ala²]- β -END and β -END, which suggests that the intact β -END molecule is capable of mediating IFN- γ suppression. A different dose-response relationship was seen with [D-Ala²]-Met-enkephalinamide when compared with β -END. Whereas β -END and [D-Ala²]- β -END were both significantly inhibitory at 10⁻¹² M, [D-Ala²]-Met-enkephalinamide consistently gave the greatest suppression of IFN- γ release at a concentration of 10⁻¹⁴ M with all five donors tested. This finding suggests the possibility that in this cell system the opioid receptor for β -END may not be identical to the met-enkephalin receptor.

Role of the monocyte in suppression. Receptors for opioids have been described for both lymphocytes (10–12, 17–19, 44, 45) and monocytes (13). Since monocytes have been shown to play a primary role in modulating various lymphocyte activities, we investigated their contribution to opioid-mediated suppression of PBMNC IFN- γ production. Monocytes were first removed from PBMNC preparations by adherence techniques yielding cell populations that contained > 95% lymphocytes. When these relatively pure lymphocyte preparations were exposed to β -END before adding con A, the suppression of IFN- γ release was less pronounced than that observed with simultaneously studied PBMNC preparations that contained an average of 28% monocytes (Fig. 4). This finding suggested that monocytes play a role in the suppression of PBMNC IFN- γ release. Since equivalent numbers of cells (2 × 10⁵ lymphocytes or 2 × 10⁵ PBMNC per well) were used in this experiment, the greater IFN- γ production by control-purified lymphocytes as compared with control PBMNC may reflect the removal of monocytes that are normally somewhat inhibitory, or it could simply be due to the presence of a greater number of lymphocytes in the purified lymphocyte preparations.

To further investigate the involvement of monocytes in opioid-mediated suppression of IFN- γ production, relatively pure populations of monocytes and of lymphocytes were incubated in the absence and presence of β -END for 72 h. The supernatants were then harvested from these cells and added to purified lymphocyte preparations. After 72 h incubation with con A, IFN- γ release from these lymphocyte preparations was

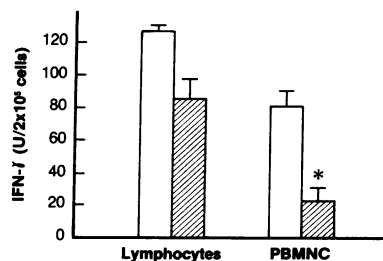


Figure 4. Effect of β -END on IFN- γ production by monocyte-depleted lymphocyte preparations. Monocytes were removed by adherence techniques from PBMNC suspensions, which contained a mean of 28% monocytes (range 22–30%

monocytes), yielding lymphocyte preparations with a mean of only 3% monocytes (range 2–5% monocytes). After 3 h preincubation in culture medium alone ([□] control cells) or 10⁻¹² M β -END ([▨] β -END-exposed cells), cells were stimulated with con A for 72 h. Values are means±SE of experiments with four donors (* *P* < 0.05 compared with control cells).

then measured. Whereas supernatant fluids obtained from the β -END-exposed monocytes significantly inhibited IFN- γ generation by lymphocytes, those from β -END-exposed lymphocyte preparations did not (Table II). These findings indicated that (a) the monocyte is the primary target cell involved in opioid-mediated suppression of IFN- γ production, and (b) the interaction between opioids and monocytes leads to the release of a factor(s) that is capable of suppressing IFN- γ production by lymphocytes.

Since previous investigators have shown that monocyte-derived ROI (46), prostaglandin metabolites (47, 48), or both of these factors (49) are capable of inhibiting other lymphocyte activities, we studied their involvement in opioid-mediated suppression of IFN- γ production. When morphine and β -END-exposed PBMNC were incubated in the presence of SOD or catalase, opioid-mediated suppression of IFN- γ production was abolished (Fig. 5). Neither SOD nor catalase alone significantly altered IFN- γ production by control PBMNC (data not shown). Similarly, the addition of 0.5 μ M indomethacin to the cell cultures completely blocked morphine and β -END-mediated suppression, whereas indomethacin alone

Table II. Effect of Supernatants from Purified Cell Cultures on IFN- γ Production

| Supernatant source* | β -END added to supernatant | IFN- γ generation (U/1.5 × 10 ⁵ lymphocytes) [‡] |
|---------------------|-----------------------------------|---|
| Cell preparation | Exposed to | |
| Monocytes | Culture medium | 109±16 |
| Monocytes | β -END | 51±10 |
| Monocytes | Culture medium | 97±6 |
| Lymphocytes | Culture medium | 128±4 |
| Lymphocytes | β -END | 144±18 |

* Suspensions of purified monocytes (5 × 10⁴ cells) or lymphocytes (1.5 × 10⁵ cells) were cultured for 72 h in culture medium alone or in culture medium containing β -END (10⁻¹² M); supernatants were then harvested and added to suspensions of freshly isolated lymphocytes (1.5 × 10⁵ cells).

[‡] IFN- γ release after 72 h culture in the presence of supernatants and con A. Values are means±SE (*n* = 3).

^{||} β -END (10⁻¹² M) was added to supernatants harvested from monocytes that had been cultured for 72 h in culture medium alone.

^{||} *P* < 0.05 compared with each of the other IFN- γ values.

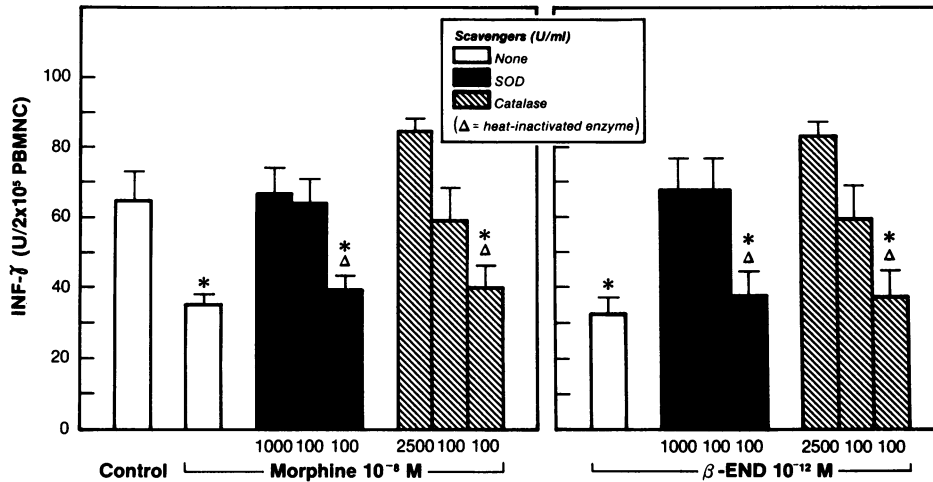


Figure 5. Effect of scavengers of ROI on opioid-mediated suppression of IFN- γ production. PBMC were preincubated for 3 h in culture medium alone (control) or opioids in the absence or presence of SOD, catalase, and heat-inactivated enzymes at the indicated concentrations before stimulation with con A for 72 h. Values are means \pm SE of experiments with five donors (* $P < 0.05$ compared with control cells).

had little effect on IFN- γ production by control PBMC (Fig. 6). Moreover, administration of indomethacin to volunteers for 2–3 d before isolating their PBMC, resulted in inhibition of the suppressive activities of morphine and β -END with these cells in vitro (Fig. 7).

Next, the suppressive capacity of one of the prostaglandin metabolites considered likely to be involved in this system, namely PGE₂, was studied. When PGE₂ was added to PBMC, significant suppression of con A-stimulated IFN- γ production was observed at a concentration of 10⁻⁸ M (Table III). Finally, to test whether β -END is capable of mediating the release of PGE₂ from PBMC, cells were exposed to 10⁻¹² M β -END for 3 h (the period of time that had been determined to be necessary for optimal IFN- γ suppression), and PGE₂ was measured in the culture supernatants. Supernatants from β -END-exposed cells contained $(1.3 \pm 0.3) \times 10^{-8}$ M PGE₂ as compared with $(3.5 \pm 1.4) \times 10^{-9}$ M PGE₂ in supernatants from control cells incubated in culture medium alone for 3 h ($P < 0.05$, $n = 5$).

In summation, the results of these experiments suggest that opioids trigger the release of ROI intermediates and PGE₂ from monocytes and that each of these metabolites in turn is

involved in the suppression of IFN- γ production by cultured PBMC.

Effect of opioids on lymphocyte proliferation. Since the generation of IFN- γ by con A-stimulated PBMC reflects a state of T lymphocyte activation, we next investigated the effect of opioids on lymphocyte-proliferative activity. When PBMC were exposed to morphine and β -END for 3 h before adding con A, marked inhibition of lymphocyte proliferation was observed after 72 h of cell culture (Table IV). Thus, opioid-mediated suppression of IFN- γ production appears to occur as a consequence of ineffective T cell activation.

Opioid suppression of VZV-stimulated cells. To evaluate whether opioids would also suppress IFN- γ generation by cells upon stimulation with a specific antigen, we incubated PBMC with VZV. Preliminary experiments indicated that control PBMC did not secrete appreciable quantities of IFN- γ in response to VZV after only 72 h of culture, but by 6 d IFN- γ release was readily detected. When PBMC were preincubated with opioids for periods from 3 to 48 h before adding VZV, there was no detectable suppression of IFN- γ secretion (data not shown). However, when the preincubation period was extended to 72 h before adding VZV, significant

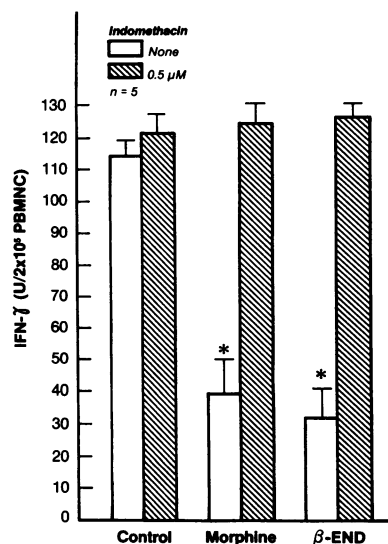


Figure 6. Effect of indomethacin on opioid-mediated suppression of IFN- γ production. PBMC were preincubated for 60 min in the absence (none) or presence of indomethacin (0.5 μ M) before adding culture medium alone (control), 10⁻⁸ M morphine, or 10⁻¹² M β -END. After 3 h, con A was added and cells were cultured for 72 h. Values are means \pm SE of experiments with five donors (* $P < 0.05$ compared with control cells).

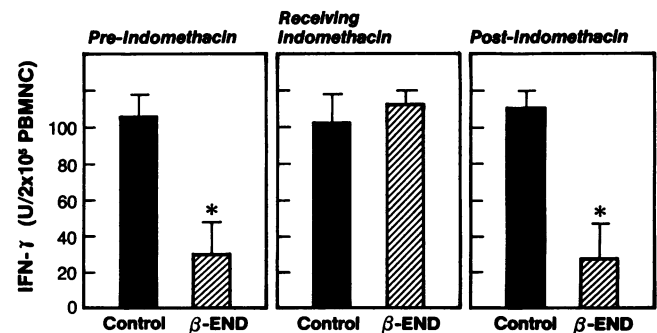


Figure 7. Effect of oral administration of indomethacin on β -END-mediated suppression of IFN- γ production. PBMC were isolated from three donors 7 d before, 7 d after, and within 4 h of receiving a course of indomethacin (25 mg every 8 h for a total of eight doses). Cells were preincubated for 3 h in culture medium alone (control) or 10⁻¹² M β -END before stimulation with con A for 72 h. Values are means \pm SE (* $P < 0.05$ compared with control cells).

Table III. Effect of PGE₂ on PBMNC IFN- γ Production

| PGE ₂ concentration* | IFN- γ generation (U/2 \times 10 ⁵ PBMNC) [‡] |
|---------------------------------|---|
| <i>M</i> | |
| Control | 91 \pm 6 |
| 10 ⁻¹² | 92 \pm 9 |
| 10 ⁻¹⁰ | 76 \pm 4 |
| 10 ⁻⁹ | 79 \pm 6 |
| 10 ⁻⁸ | 58 \pm 7 [§] |
| 10 ⁻⁷ | 54 \pm 8 [§] |

* PBMNC were incubated for 3 h in culture medium in the absence (control) or presence of PGE₂, at the indicated final concentrations, before adding con A.

[‡] IFN- γ release after 72 h culture. Values are means \pm SE (*n* = 6).

[§] *P* < 0.05 compared with control.

inhibition of IFN- γ production was observed with both morphine and β -END (Fig. 8). These findings indicate that opioids are capable of suppressing PBMNC IFN- γ production in response to a specific antigen (VZV) but that a relatively prolonged interval of opioid exposure is required before the suppressive effect becomes manifest.

Discussion

In this study, we have demonstrated that morphine and β -END, at remarkably low concentrations, are capable of suppressing IFN- γ production by PBMNC stimulated by both a nonspecific T cell mitogen (con A) and a specific antigen (VZV). Our data suggest that monocytes are the primary target cell for opioids in this system and that morphine and β -END operate via a classical opioid receptor mechanism. Other investigators have shown that blood monocytes possess classical opiate receptors (13), and activation of these receptors has been implicated in the inhibition of the production of another lymphokine, T lymphocyte chemotactic factor (18). Additional studies will be necessary to determine whether β -END binds to the same class of receptors on monocytes as does morphine, i.e., mu receptors, or if a separate class of receptors is involved, such as epsilon receptors, which have been described in other tissues (50, 51). Other investigators have

Table IV. Effect of Opioids on Lymphocyte Proliferative Activity*

| Stimulus | Opioid | Lymphocyte proliferation (cpm \times 10 ³) | IFN- γ generation (U/2 \times 10 ⁵ PBMNC) |
|----------|------------------------------------|---|--|
| None | None | 3.9 \pm 0.2 | 0.4 \pm 0.09 |
| Con A | None (control) | 23.7 \pm 5.3 | 107.6 \pm 14.6 |
| Con A | Morphine (10 ⁻⁸ M) | 7.4 \pm 3.5 [‡] | Not tested |
| Con A | β -END (10 ⁻¹² M) | 3.0 \pm 0.4 [‡] | 27.6 \pm 6.6 [‡] |

* 2 \times 10⁵ control and opioid-exposed PBMNC were incubated in the absence or presence of con A for 72 h before assaying for proliferative activity and measuring IFN- γ release. Values are means \pm SE (*n* = 3).

[‡] *P* < 0.05 compared with con A-stimulated control cells.

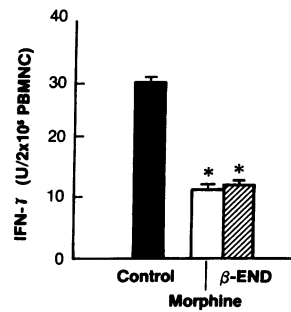


Figure 8. Effect of opioids on IFN- γ production by VZV-stimulated PBMNC. Cells were preincubated for 72 h in culture medium alone (control), 10⁻⁸ M morphine, or 10⁻¹² M β -END before adding VZV. After 6 d, IFN- γ release into culture supernatants was measured. Values are means \pm SE of experiments with three donors (* *P* < 0.05 compared with control cells).

shown that β -END is rapidly internalized by human polymorphonuclear leukocytes (52). Whether receptor-mediated internalization of β -END by monocytes also occurs and the importance of such a step in the sequence of events leading to suppression of IFN- γ production are at the present time unknown.

β -END has been shown to stimulate human peripheral blood lymphocyte natural killer cytolytic activity (53–56), and recent studies indicate that β -END augments interferon production by these cells (57). IFN- γ production by activated T lymphocytes is correlated with blastogenesis (58), and this response is regulated by the monokine interleukin 1 and the T cell growth factor interleukin 2 (59–61). The opioid-mediated suppression of PBMNC IFN- γ generation that we observed in the present study appeared to be associated with depressed T lymphocyte-proliferative activity, and it is possible that reduced production of or responsiveness to one or more of these interleukins is a more proximate cause of suppressed IFN- γ secretion.

In keeping with the results of other investigators (47, 48, 62), our findings with indomethacin suggest that a product of monocyte prostaglandin metabolism plays an important role in suppression of T lymphocyte IFN- γ generation. In the present study, β -END was found to trigger the release of PGE₂ from PBMNC. In contrast to the limited capacity of lymphocytes to produce this arachadonic acid metabolite, monocytes are known to be a rich source of PGE₂ (63). PGE₂ is recognized to be a potent inhibitor of many monocyte and lymphocyte functions (63, 64), including IFN- γ production (65), a finding corroborated in the present study. In a previous study, Goodwin et al. (66) found that physical stress was associated with depressed mitogen-induced lymphocyte proliferation which appeared to be linked to monocyte-derived PGE₂. It is interesting to speculate that a stress-responsive peptide, such as β -END, may have triggered these monocytes to release PGE₂. Since PGE₂ appears to exert most of its physiologic effects through changes in intracellular cyclicAMP (63), it is possible that cyclic nucleosides play an important role as second messengers in β -END-mediated suppression of IFN- γ production. The fact that scavengers of ROI also blocked opioid-mediated suppression of IFN- γ production is consistent with the view that these metabolites may contribute to prostaglandin metabolism (49).

Although the biological significance of our findings is at present unknown, the most relevant paradigms approximated by this *in vitro* model may be those of opiate addiction and chronic stress since relatively prolonged periods of exposure to morphine and β -END were required to obtain maximal suppression of IFN- γ production. If this is the case, the defective

cell-mediated immunity associated with these disorders could in part be explained by monocyte-derived PGE₂ occurring as a consequence of opioid stimulation of these cells. Given this scenario, the observation that oral administration of indomethacin blocked the suppressive activities of opioids may have clinical implications. Also, if opiate addiction and stress are shown to be cofactors in the pathogenesis of opportunistic infections in diseases such as the acquired immunodeficiency syndrome (67), therapeutic strategies ultimately may be developed to counteract the immunosuppressive activities of the opioids involved under these conditions. Finally, recent studies indicate that splenic macrophages and lymphocytes express the POMC gene (68–70), and it is possible that the β -END that is produced by these cells may play an important role in the down-regulation of lymphocyte IFN- γ generation.

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