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Modulation of the Immune Response by POMC-Derived Peptides

I. Influence on Proliferation of Human Lymphocytes

COBI J. HEIJNEN,* JITSKE ZIJLSTRA,* ANNEMIEKE KAVELAARS,* GERDA CROISET,† AND RUDY E. BALLIEUX‡

Department of Immunology, University Hospital for Children and Youth "Het Wilhelmina Kinderziekenhuis," P.O. Box 18009, 3501 CA Utrecht, The Netherlands; †Rudolf Magnus Institute, Utrecht, The Netherlands; and ‡Department of Clinical Immunology, University Hospital, Utrecht, The Netherlands

The POMC-derived peptides β-endorphin and ACTH are capable of modulating an immune response in physiological concentrations. These neuropeptides can either enhance or inhibit the proliferative response of human peripheral blood lymphocytes after stimulation with the mitogen concanavalin A. The modulatory action of the peptides is not only dependent on the concentration but appears to be donor dependent. The response pattern observed is not determined by a selective affinity for certain amino acid sites on the molecules with "enhancing" or "inhibiting" activities, since fragments of β-endorphin and ACTH also produce a differential donor-dependent response pattern.

INTRODUCTION

The view that stress may influence immune function (Bartrop, Lazarus, & Luckhurst, 1977; Laudenslager, Ryan, Drugan, Hyson, & Marei, 1983; Shavit, Terman, Martin, Lewis, Liebeskind, & Gale, 1985; Lewis, 1985) has led to the investigation of possible modulatory effects on various immune functions of products released by the central nervous system (CNS) after a stressful stimulus.

Accumulating evidence indicates that the opioid peptides β-endorphin (βE) and ACTH, both of which are derived from the precursor molecule pro-opiomelanocortin (POMC), are important candidates in this respect. The presence on lymphocytes of specific receptors for ACTH and the nonopiate part of the βE molecule has been described, suggesting the possibility that these neuropeptides can influence directly cells of the immune system (Bost, Smith, Wear, & Blalock, 1987; Schweigerer, Schmidt, Teschemacher, & Gramsch, 1985; Hazum, Chang, & Cuatrecasas, 1979). The existence of opiate receptors on leukocytes is less clear. Mehrishi and Mills (1983) reported the presence of such a receptor, whereas Mendelsohn, Kerchner, Culwell, and Ades (1985) were unable to demonstrate the opiate receptor on lymphocytes.

The neuropeptide βE has been shown to enhance monocyte, lymphocyte, and neutrophil migration (Van Epps & Salan, 1984) and to increase NK cell function (Kay, Allen, Morley, 1984) as well as the production of interferon-γ (IFN-γ) (Brown & Van Epps, 1986). Alvarez-Mon, Kehrl, and Fauci (1985) have demonstrated that ACTH can enhance the mitogen-induced immunoglobulin production

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of human tonsillar B lymphocytes. However, the data regarding the influence of these neuropeptides on the proliferative response of lymphocytes are conflicting. For example, some authors claim that βE is capable of enhancing mitogenesis in cell cultures (Gilman, Schwarz, Milner, Bloom, & Feldman, 1982; Plotnikoff & Miller, 1983), whereas other authors report an inhibitory effect of this peptide (McCain, Lamster, Bozzone, & Grbic, 1982).

The possible modulation of immune reactivity by neuropeptides has been discussed thus far from the perspective of effects induced by cognitive stimuli involving the CNS. However, noncognitive stimuli such as viruses or endotoxins can induce lymphocytes to produce these "neuro"-peptides directly (Blalock, 1984; Harbour-McMenamin, Smith, & Blalock, 1985). The latter evidence opens up the possibility that these substances may serve as lymphokines in the regulation of the immune response. The recent observation that lymphocytes apparently process the POMC molecule in a stimulus-dependent way is of interest in this respect (Blalock, 1984). On the basis of these findings we investigated the specific immunomodulatory effects of various fragments of the neuropeptides ACTH and βE on the proliferative response of human peripheral blood lymphocytes.

Johnson, Smith, Torres, and Blalock (1982) have shown that βE1-31 slightly suppresses the primary antibody response of murine B cells *in vitro,* whereas α-endorphin (αE) which represents the first 16 amino acids of the molecule βE can effectively suppress the response. We have shown in the human system that βE enhances the primary antibody response (Heijnen & Ballieux, 1986), whereas αE inhibits the primary antibody response profoundly (Heijnen, Bevers, Kavelaars, & Ballieux, 1985).

The present study demonstrates that physiological concentrations of ACTH and βE as well as some of their fragments can modulate the proliferative response of human lymphocytes. The outcome of the proliferative response, however, is not only related to the neuropeptide used but appears also to be donor dependent.

MATERIALS AND METHODS

Human peripheral blood leukocytes (lymphocytes + 10–20% monocytes) were prepared by centrifugation of heparinized blood diluted once in minimal essential medium (MEM–Tris, GIBCO, Grand Island, NY) on Ficoll–Isopaque (Pharmacia, Sweden) density gradients (ρ = 1.077 g/cm^3) at 700*g* for 20 min. After the isolation procedure the cells were washed twice.

Incubation of PBL with the Neuropeptides

A quantity of 10×10^6 peripheral blood lymphocytes (PBL)/ml were preincubated in serum-free medium consisting of IMDM (Iscove's modified Dulbecco's medium, GIBCO) supplemented with 2% Ultrocer (Pharmacia, Sweden) as a serum replacement, for 30 min at 37°C. After the incubation period the cells were washed twice and incubated in the same medium for 2 h at 37°C in the presence of one of the neuropeptides: βE 1-31, 2-31, 1-17, 1-16, 6-16, 10-16; ACTH 1-39, 1-24, 4-9, 11-24 in concentrations ranging from 10^{-7} to 10^{-15} *M*. The neuropeptides mentioned above are a gift from Organon International B.V., Oss, The Netherlands. The synthetic neuropeptides were dissolved in IMDM and 2% Ultrocer

and used immediately after dissolvement. After the incubation period the cells were washed twice and resuspended in culture medium consisting of RPMI 1640 (GIBCO) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 m*M* glutamin, 5×10^{-5} *M* 2-mercaptoethanol, and 10% human AB serum.

Cell Cultures

Lymphocytes (1×10^5) were cultured for 4 days in round-bottom microtiter plates (Greiner) in 150 μl of culture medium supplemented with the various concentrations of concanavalin A.

DNA synthesis was measured after addition of 0.8 μl 2-^{14}C-labeled thymidin during the final 16 h of the culture. After the culture period the cells were harvested with a Cryoson cell harvester and counted in a liquid scintillation counter. The results of 21 donors are expressed as dpm $\times 10^{-3}$ (±SEM).

RESULTS

Influence of βE and ACTH on the Proliferative Response of PBL

Peripheral blood lymphocytes of normal donors were incubated with various concentrations of the neuropeptides βE or ACTH for 2 h at 37°C. After the incubation period the cells were washed and cultured for various days in the presence of the mitogen concanavalin A (Figs. 1a and 1b).

The results demonstrate that βE is capable of enhancing (25% of the cases) or inhibiting (55%) (20% of the donors show no effect) the proliferative response of human T cells in physiological concentrations (10^{-13} *M*) (Fig. 1a). ACTH (1-39) is inhibitory in 65% of the donors tested, whereas 23% show a positive effect (12% of the donors do not react) (Fig. 1b). The modulatory effect is not dependent on

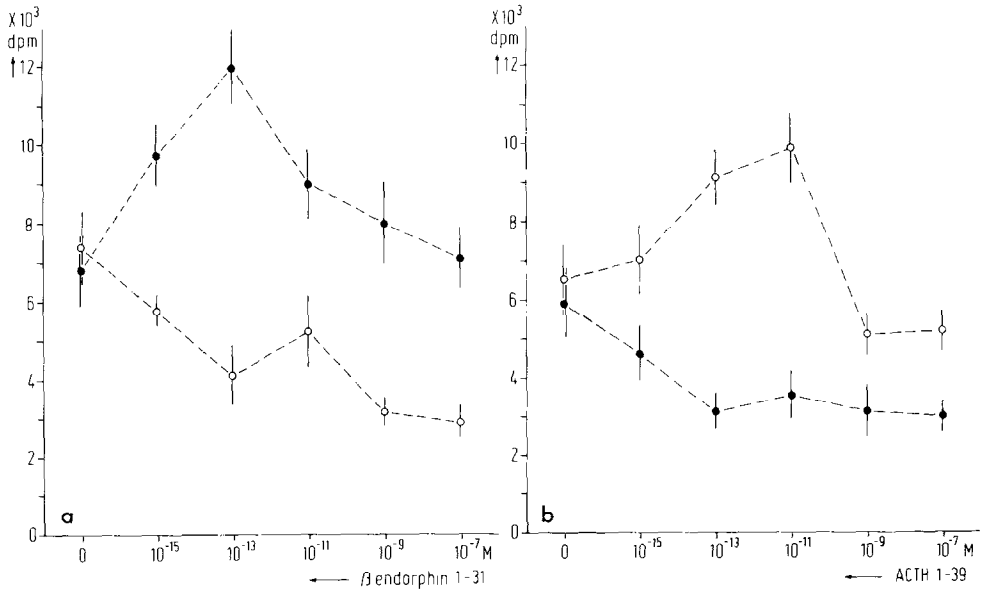

FIG. 1. Modulation of the proliferative response of Con A-stimulated human peripheral blood lymphocytes by βE 1-31 (a) and ACTH 1-39 (b).

the concentration of the mitogen, nor on the culture period, since the same pattern of modulation can be observed in any combination (data not shown). The concentration of the peptide used dictates primarily the effect observed (Fig. 1).

Modulatory Effect of Fragments of the Endorphin Molecule

In order to characterize the modulatory effect of various fragments of βE, we tested the activity of βE 2-31 (Fig. 2a), βE 1-17 and 1-16 (γE and αE, Fig. 2b) and βE 6-16 and 10-16 (Fig. 2C).

The results depicted in Fig. 2 show a comparison between the response patterns of two donors. When PBL of donor A are used, βE is capable of enhancing the response at the physiological dose of 10^{-13} *M*. When the N-terminal amino acid tyrosine is removed (2-31) the enhancement of the response is less pronounced. When we compare the response patterns of γE and αE we see a difference in the response especially at 10^{-15}–10^{-13} *M*. The fragments 6-16 and 10-16 also cause opposite effects on the proliferative response.

When we use the PBL of donor B we observe an overall inhibitory effect of all the peptide fragments used. It is clear from the results that the modulation of the response is not affected only by triggering of the opiate receptors, since the mod-

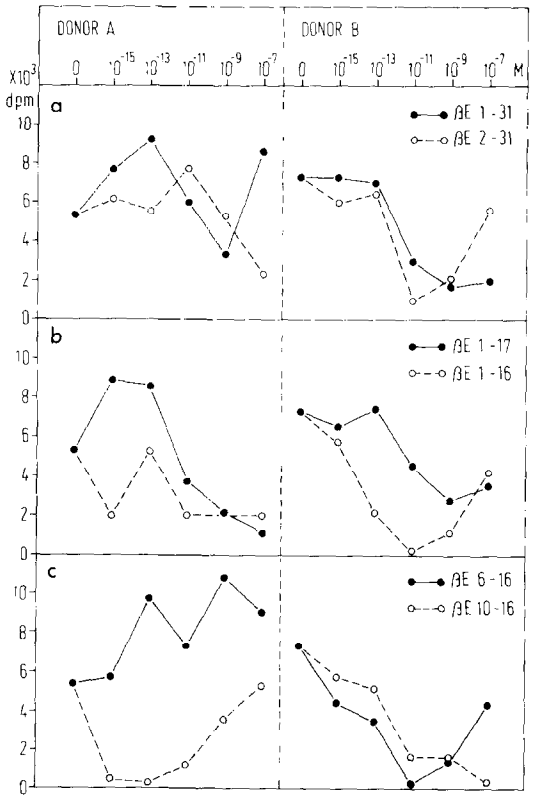

FIG. 2. Modulation of the proliferative response of Con A-stimulated human peripheral blood lymphocytes by βE 1-31 and 2-31 (a); βE 1-16 and 1-17 (b); and βE 10-16 and 6-16 (c).

ulatory activities of the peptide fragments are still retained in the absence of the N-terminal, the opioid specific part of the molecule (βE 2-31, 10-16, 6-16).

Modulation of the Proliferative Response by ACTH Fragments

The response pattern of two representative experiments using the PBL of two different donors shows that the ACTH fragment 11-24 follows the response pattern of ACTH 1-24 and ACTH 1-39, whereas ACTH 4-9 has only a marginal effect on the response (see Fig. 3). In 3 out of 20 experiments we demonstrated that ACTH 4-9 gave an enhancement of the response in the absence of a modulatory effect of either ACTH 1-24 or ACTH 1-39 (data not shown).

Reproducibility of the Modulatory Action of β-endorphin

It is a well-known phenomenon that the mitogenic response of the PBL of a given donor can widely vary over a period of several months. In order to test whether the peptide-induced response pattern was dependent on the reactivity of the donor to the mitogen or whether it was an inherent capacity of the PBL of the donor itself, we determined the modulatory activity of βE of two donors three times over a period of 9 months. The results depicted in Fig. 4 demonstrate clearly

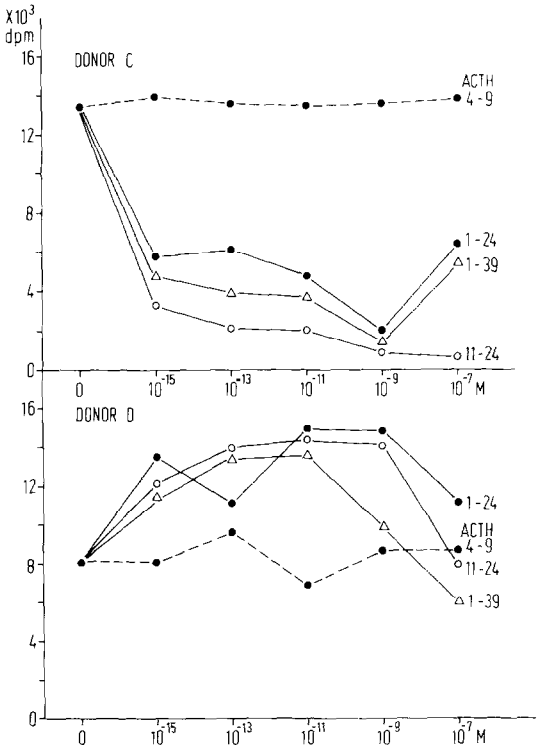

FIG. 3. Modulation of the proliferative response of Con A-stimulated human peripheral blood lymphocytes of two donors (donor C and D) by ACTH 4-9, 1-24, 1-39, and 11-24.

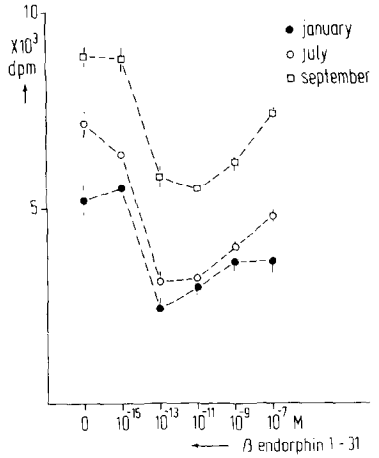

FIG. 4. Modulation by βE 1-31 of the proliferative response of Con A-stimulated human peripheral blood lymphocytes derived from one donor who was sampled three times over a period of 9 months. Values are given as dpm × 10^{-3} ± SEM.

that the peptide influences the proliferative response independent of the reactivity of the donors to the mitogen Con A.

DISCUSSION

We have shown that the neuropeptides ACTH and βE, when applied in physiological concentrations, are capable of modulating the proliferative response of human peripheral blood lymphocytes *in vitro*. The modulatory action of these peptides can be either positive or negative, depending on the concentration of the peptide as well as on the donor of the PBL. The peptide-mediated response pattern is an inherent capacity of the donor itself, as shown by the repeated testing in which βE1-31 produced the same response pattern, although the height of the mitogenic responses in the absence of the peptide varied in time. Data in the literature reporting the effect of βE1-31 on the proliferative response are conflicting on this point. Gilman et al. (1982) demonstrated that βE1-31 is capable of enhancing the proliferative response of rat splenic T cells after stimulation with the mitogen (Con A). In contrast, McCain et al. (1982) showed that this opioid peptide decreases the proliferation of human peripheral blood cells to the T-cell mitogen phytohemagglutinin (PHA). Plotnikoff and Miller (1983) reported that βE1-31 enhances human T-cell proliferation. The explanation for this discrepancy is unknown. One important point to note is that the concentrations of neuropeptides (10^{-13}–10^{-7} *M*) examined in the present study are much lower than those used in earlier work. In addition the assay systems used by the various authors differ with respect to the mitogenic stimulus used (Con A versus PHA) as well as the source of the lymphocytes (murine splenocytes versus human peripheral blood lymphocytes).

Brown and Van Epps (1986), who determined the influence of βE1-31 on IFN-γ production by lymphocytes, also reported that this peptide could either enhance or inhibit the response depending on the donor used, when applied in physiolog-

ical doses. It might be of interest to take into account the results of Claas and van Rood (1985) who reported that dtγE (βE2-17) may interact preferentially with certain HLA class I antigens (HLA-A 10, 11; HLA-B 13, 15, 22, and HLA-C6). HLA-antigens, together with the endorphin receptor on the lymphocyte, may form a "compound receptor" as has been proposed for insulin (Simonson & Olsson, 1983); the polymorphism of the HLA antigens may play a decisive role in the final modulatory capacity of the peptide.

In the present investigation of whether the type of immunomodulation is determined by a selective affinity of the lymphocyte for different amino acid sites of βE and ACTH, we can observe a profound difference in the proliferative response of donor A to βE1-16 and 1-17 (α- and γ-E, respectively) when used in a concentration of 10^{-15} *M*. This holds also true for the effects of βE6-16 and 10-16. The latter response patterns were only observed when βE1-31 generated an enhancement of the response. It is obvious from the results shown in Fig. 2 that when βE 1-31 is enhancing the response, it is very difficult to deduce a structure–activity relationship of the peptide. When βE1-31 inhibited the response an overall negative effect of the peptide fragments was observed. This inhibition is apparently not solely due to an interaction with an opioid receptor since removal of the N-terminal part of the molecule did not alter its modulatory capacity (see Fig. 2, donor B). Our results suggest that the negative effect of βE in this case is mediated most probably through the sequence 10-16.

From these results we can conclude that the type of modulation observed with βE1-31 is not caused by a selective affinity for certain amino acid sites on the molecules. The two donors reacted differentially to the various fragments, indicating that binding and response to the peptides are translated by the cells of the two donors in a different way.

The same conclusion holds true for the effect of ACTH. The active site of the ACTH molecule probably resides in amino acids 11-24. When ACTH 1-24 inhibits the response, ACTH 11-24 also inhibits and vice versa. The fragment ACTH 11-39, as well as ACTH 1-24 produced exactly the same response pattern (data not shown).

It is well-known phenomenon that the prohormone POMC is cleaved into various fragments by specific proteases present in the various sites of production (e.g., the pituitary) (Burbach, De Kloet, Schotman, & De Wied, 1981). The question of whether or not the lymphocyte is capable of processing the (exogenous) neuropeptides in a genetically determined way by a specific set of proteases that dictates the final outcome of the modulatory action may provide new insight into neuropeptide–immune interactions in both directions.

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