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Two opposing modes of action of β-endorphin on lymphocyte function

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SUMMARY

Five opioid peptides (α-, β-, and γ-endorphin, methionine- and leucine-enkephalin) were tested for their effect on the concanavalin A-induced proliferative response of splenocytes of adult male F344 rats. The continuous presence of these opioid peptides during culture of T cells did not affect proliferation. However, 30 min of preincubation with β-endorphin (β-end), but not with the other opioid peptides, resulted in a dose-dependent enhancement of proliferation of 50–100%. This potentiating effect of β-end on proliferation was preceded by an increase in the production of interleukin-2 (IL-2) and in the extent of IL-2 receptor expression. The stimulatory effect of β-end was not prevented by naloxone, indicating that classical opioid receptors were not involved. The continuous presence of β-end (or α-end) in cultures of cells that had been preincubated with β-end completely abolished the stimulatory effect, pointing towards the potential of β-end to regulate T-cell function via different mechanisms.

INTRODUCTION

Interleukins are considered to be the major regulatory mediators of cells of the immune system. In the past few years, however, endogenous opioid peptides (endorphins and enkephalins) have also been shown to interact with virtually every immune function.

The effects of opioid peptides on mitogen-induced T-cell proliferation have been intensively studied, as reviewed in Sibinga & Goldstein.[1] Several groups[2–4] have shown that opioid peptides enhance proliferative responses. However, the mechanism by which these peptides act remains rather conflicting. Naloxone-sensitive (opioid receptor mediated) and naloxone-insensitive mechanisms have been claimed. At the level of lymphokine secretion, interleukin-1 (IL-1) production by murine bone marrow macrophages,[5] IL-2 production by activated lymphocytes,[6] and interferon production by mononuclear cells[7,8] have been described to be enhanced by opioid peptides.

Besides these observations, a variety of other leucocyte activities has been studied, among which the enhancing effects of opioids on natural killer cell cytotoxicity[9,10] and chemotaxis[11,12] and inhibitory effects on *in vitro* antibody production[13] are best characterized.

Abbreviations: ACTH, adrenocorticotropic hormone; CTLL, cytotoxic T-lymphocyte line; β-end, β-endorphin; IL-2, interleukin-2; leu-enk, leucine-enkephalin; met-enk, methionine-enkephalin; POMC, pro-opiomelanocortin.

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In vivo, there are several sources for the production of opioid peptides. First, opioid peptides are synthesized by cleavage from the pro-opiomelanocortin (POMC) derived precursor β-lipotropin in the central nervous system. There they exert local effects, among which analgesia and behavioural effects are best established.[14] Alternatively, they are secreted from the pituitary into the circulation, after which these peptides may affect cells from non-neural tissues including lymphocytes. This may indicate a possible link between the neuroendocrine and the immune system.

Second, there are several reports concerning the production of opioid peptides in non-neural tissues. In this regard, various parts of the female and male reproductive tract[15] and adrenal medullary cells[16] have been shown to express POMC and synthesize related peptides, whereas pro-enkephalin mRNA has been detected in the rat heart.[17] In the past recent years, the immune system has also been recognized as a source of opioid peptide production. Smith & Blalock[18] have demonstrated the presence of the POMC-derived peptides, adrenocorticotropic hormone (ACTH) and β-endorphin (β-end) in cells of the immune system. Likewise, infection of murine splenocytes with Newcastle disease virus results in expression of the POMC gene.[19] Furthermore, Lolait *et al.*[20] have shown that murine splenic macrophages produce β-end of the same size as β-end derived from the central nervous system. As for the pituitary, the expression of POMC in peripheral blood mononuclear cells is stimulated by corticotropin releasing factor.[21] In the periphery this occurs via an IL-1-dependent mechanism.[22] With regard to these observations it is tempting to consider opioid peptides as paracrine hormones and to speculate on the role of these peptides in the regulation of an immune response.

Enkephalins and endorphins share the N-terminal amino acid sequence and differ in the number and composition of the C-terminal residues. Receptors to which opioid peptides bind by the N-terminal amino-acids are called opioid receptors. In the central nervous system four types of opioid receptors (mu, kappa, delta, epsilon) have been identified but have not yet been cloned. The presence of multiple opioid receptors on cells of the immune system was implicated by the observation that morphine and methionine-enkephalin (met-enk) both affected active rosetting of T lymphocytes.[23] More recently, these receptors have indeed been demonstrated on lymphocytes.[24,25] Besides opioid receptors, there are some reports concerning so called non-opioid receptors for β-end on transformed B lymphocytes[26] as well as on thymoma cells.[27] In a naloxone-irreversible manner, these receptors bind the C-terminal part of β-end.

The presence of multiple receptors for opioid peptides on lymphocytes may explain the inconsistency in the effects (reviewed in Refs 1 and 28) that these peptides are claimed to have on the immune system. To gain a better understanding of the actions of these peptides we compared five endogenous opioid peptides in their effect on immune function. As a model we used splenocytes of adult male rats and we focused on lymphocyte proliferation, IL-2 production, and IL-2 receptor expression.

In this paper we describe two different modes of action of the opioid peptide β-end in the modulation of immune function: an up-regulating effect via a non-opioid receptor mechanism and a down-regulating effect mediated via interaction with opioid receptors.

MATERIALS AND METHODS

Cell preparations

Experiments were performed with splenocytes of male F344 rats (8–15 weeks of age), derived from the breeding colony of the TNO-Institute for Applied Radiobiology and Immunology (Rijswijk, The Netherlands).

Rats were killed by inhalation of carbon dioxide and the spleens were removed asceptically. Single-cell suspensions were prepared as described previously.[29] Cells were pelleted by centrifugation (10 min, 350 *g*) and resuspended in 4 ml lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0·1 mM EDTA, pH 7·0) and kept on ice for 7 min to lyse the erythrocytes. Subsequently, 4 ml foetal calf serum (FCS) were layered under the suspension and cells were centrifuged (20 min, 50 *g*). The cell pellet was resuspended in standard culture medium (SCM) consisting of RPMI-1640 supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol, 20 mM HEPES, and 5% heat-inactivated FCS. Cell viability, as determined by trypan blue exclusion, was always greater than 90%.

Proliferation assays

Splenocytes (5×10^4/well) were cultured in triplicate in SCM in flat-bottomed 96-well microtitre plates (Nunc, Roskilde, Denmark) in a humidified CO_2 incubator. Cells were stimulated with 0·5 μg/ml concanavalin A (Con A; Pharmacia, Uppsala, Sweden). During the last 6 hr of culture, 0·25 μCi = 9·25 kBq [^{3}H]thymidine (2 Ci/mmol: RCC Amersham, Amersham, Bucks, U.K.) was added to measure proliferation. Results are expressed as mean proliferation ± SD.

Incubations with opioid peptides

The effect of the neurohormones α-end (Boehringer Mannheim GmdH, Penzberg, Germany), β-end, γ-end, met-enk, and leu-enk (Sigma Chemical Co., St Louis, MO) on the Con A-induced proliferative response was investigated in two ways. First, by adding these peptides directly to cultures in concentrations ranging from 10^{-6} to 10^{-16} M. Second, by preincubating the splenocyte suspension with the opioid peptides. To this end we prepared suspensions at 10^7 cells/ml and added the various peptides in concentrations between 10^{-8} and 10^{-14} M. After 30 min of incubation at 37° and 5% CO_2, the cells were washed twice with excess of culture medium to remove the peptides that remained unbound in suspension. Subsequently the cells were stimulated as described above.

Measurement of IL-2 production

Splenocytes were stimulated under identical conditions as described for the proliferation assays. At 24, 48, and 72 hr of culture, supernatants were harvested and IL-2 production was measured, using an IL-2-dependent mouse cytotoxic T-lymphocyte line (CTLL-2), as described by Gillis *et al.*[30] Results are expressed as U/ml; 1 U/ml is defined as the IL-2 concentration at which half-maximal proliferation of the CTLL was found.

Expression of the IL-2 receptor α-chain

Spleen cells ($3{\cdot}5 \times 10^5$/well) were cultured in 24-well plates (Nunc, Denmark) under identical conditions as described above. After 24, 48, and 72 hr of culture, cells were collected, washed, and stained with a saturating amount of the FITC-labelled monoclonal antibody OX39, directed against the p55-chain of the IL-2 receptor. OX39 was kindly provided by Dr A. F. Williams, Oxford, U.K. After incubation (30 min) on ice, cells were washed and analysed on a FACScan (Becton-Dickinson, Sunneyvale, CA).

Statistical analysis

Statistical significance was assessed by Student's *t*-test. Differences were considered statistically significant when $P < 0{\cdot}05$.

RESULTS

Selective enhancement of lymphocyte proliferation by β-end

The endogenous opioid peptides α-, β-, and γ-end, as well as met- and leu-enk, were investigated for their effect on the proliferation of rat splenocytes. Since the peptides by themselves were not mitogenic for rat splenocytes (data not shown), they were tested for their ability to influence T-cell proliferation in a culture of splenocytes in response to a suboptimal concentration of the T-cell mitogen Con A. Therefore, various concentrations of the peptides were added to the cultures at Day 0 and the proliferative response was determined at Day 3. The results shown in Table 1 demonstrate that addition of neither one of the peptides over a broad concentration resulted in changes in the proliferative response. Also, at Days 4 and 5 of culture the proliferative response was not affected. However, when presenting the opioid peptides by means of a preincubation procedure to the splenocytes, i.e. 30 min incubation at 37°, 5% CO_2, followed by a washing step, and subsequently stimulating with Con A, β-end induced an enhancement of the proliferative response (Fig. 1). The effect of β-end was most obvious on Day

Table 1. Continuous presence of opioid peptides in a culture of splenocytes stimulated with Con A does not affect the extent of proliferation

Neuropeptide added	Relative proliferative response (%)* Conc.: 10^{-12} M	10^{-10} M	10^{-8} M
α-end	106·8	105·8	102·9
β-end	101·1	92·4	105·0
γ-end	101·1	96·2	110·6
met-enk	93·0	100·5	102·9
leu-enk	99·0	91·6	102·7

* Splenocytes (5×10^4/well) were cultured with 0·5 μg/ml Con A in the continuous presence of various neuropeptides. On Day 3 of culture, the extent of proliferative response was determined by [^{3}H]thymidine incorporation. Cultures were performed in triplicate and SD values were always less than 10%. The means of the proliferative responses are expressed as a percentage of the response found with cells to which no neuropeptides were added (22,500 c.p.m.).

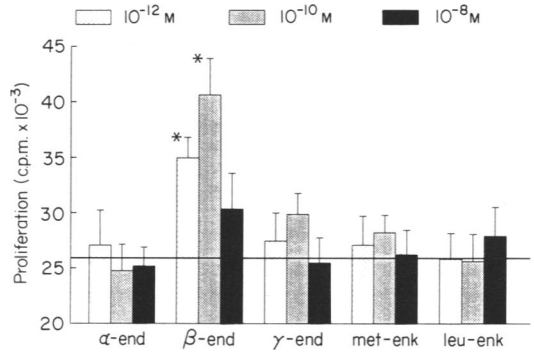

Figure 1. β-End selectively enhances T-lymphocyte proliferation. Cells were preincubated with various concentrations of opioid peptide and subsequently stimulated with 0·5 μg/ml Con A. Proliferative responses were determined at Day 3 by [^{3}H]thymidine incorporation, and represented as means ± SD. The proliferative response to Con A of cells preincubated with medium is represented by the horizontal line (26,000 ± 2218 c.p.m.). * $P < 0{\cdot}01$ compared with the value of control cultures, preincubated with medium.

3. On Day 4, the effect of β-end pretreatment was no longer detectable (data not shown). Apparently, β-end had affected the kinetics of the response. In contrast to β-end, preincubation with α- , or γ-end, or met- , or leu-enk did not affect the proliferative response.

Figure 2 shows in more detail the dose-dependency of the stimulatory effect of β-end. An increase in proliferation was already significant ($P < 0{\cdot}01$) after preincubation with 10^{-13} M. An even more significant increase ($P < 0{\cdot}005$), from 25,000 to 37,000 c.p.m., was found at concentrations of 10^{-12} to 10^{-9} M, at which the increase was maximal. At higher concentrations, the stimulating effect of β-end was lower. As will be pointed out

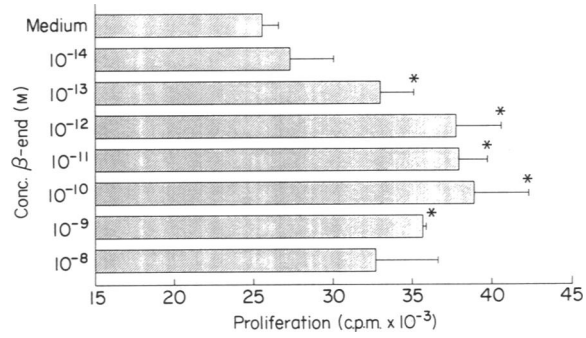

Figure 2. Dose-dependent enhancement of proliferation by β-end. Cells were preincubated with various concentrations of β-end, followed by stimulation with 0·5 μg/ml Con A. At Day 3 proliferation was determined by [^{3}H]thymidine incorporation. The results are represented as means ± SD. * $P < 0{\cdot}005$ compared with the value of the culture preincubated with medium.

later, this latter phenonemon may reflect a dualistic nature of β-end. The effect of β-end could not be explained by alterations (e.g. selective survival or enrichment of T cells) in the composition of the splenocyte population by the preincubation procedure (data not shown).

β-End enhances the production of IL-2 and the expression of the IL-2 receptor

Subsequently, we investigated the specificity of the β-end-induced enhancement of T-cell proliferation, by analysing its effect on two different activation events that are preceding to and crucial for T-cell growth, i.e. IL-2 production and IL-2 receptor expression.

As can be seen in Fig. 3a, preincubation with β-end leads to an enhancement of IL-2 production for supernants harvested after 24 h of culture. Enhancement of IL-2 production was obvious for the concentration range of 10^{-12} to 10^{-10} M β-end in a dose-dependent manner. This increase was not due to a lower level of IL-2 consumption, since the rise in IL-2 production closely paralleled the dose–response effect of β-end on the proliferative response. Additional support was derived from the kinetics of IL-2 production (Fig. 3b). As for the proliferative response, preincubation with β-end resulted in a shift in the kinetics. Already after 24 hr of culture the amount of IL-2 in supernatants of cells that were preincubated with 10^{-10} M β-end was twofold higher than in supernatants of control cultures. After 48 hr of culture, the amount of IL-2 in the preincubated cultures had increased further, and still twofold higher amounts of IL-2 were found when the cells had been preincubated with β-end. At this time-point the extent of proliferation by cells with or without β-end pretreatment was low (≈ 500 c.p.m.) and significant differences were not found. After 72 hr of culture, the amount of IL-2 in preincubated cultures returned to normal levels. Therefore, it is most likely that the strongly enhanced proliferation of preincubated cells at that time is due to the production of more IL-2 and hence IL-2 consumption.

The effect of β-end preincubation on the induction of the IL-2 receptor α-chain was evaluated using a FITC-labelled anti-IL-2 receptor monoclonal antibody. β-End by itself did not induce IL-2 receptor expression (data not shown). However, when preincubation with β-end was followed by stimulation

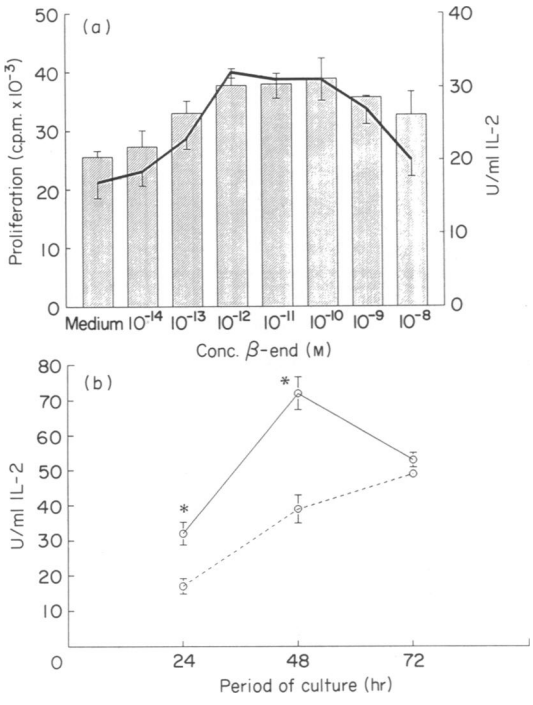

Figure 3. β-end augments IL-2 production. (a) Dose-dependent effect of β-end on IL-2 production. Spleen cells were preincubated with various concentrations of β-end and stimulated with 0·5 μg/ml Con A. Proliferation (bars) was determined at Day 3 by [^{3}H]thymidine incorporation. IL-2 levels (line) were determined in supernatants harvested after 24 hr of culture CTLL-2. All values are means ± SD. In the concentration range of 10^{-12}–10^{-9} M β-end, both the proliferative response and IL-2 production are significantly different from the medium control ($P < 0{\cdot}005$). (b) Kinetics of β-end-induced enhancement of IL-2 production. Cells were preincubated with 10^{-10} M β-end (solid line) or medium (dashed line) and stimulated with 0·5 μg/ml Con A. After 24, 48 and 72 hr of culture supernatants were taken and IL-2 was measured. All values are means ± SD. Asterisks indicate a significant increase ($P < 0{\cdot}005$) compared to untreated cells.

with Con A the extent of IL-2 receptor expression was higher as a consequence of preincubation with β-end (Fig. 4). This effect was only observed early in culture (24 and 48 hr), after 72 hr the percentages of IL-2 receptor positive cells were equal. Thus, β-end facilitated the enhanced expression of two different gene products: IL-2 and IL-2 receptor α-chain.

Enhancing effect of β-end on splenocyte proliferation is abolished by α- and β-end

In order to clarify the involvement of opioid receptors in the up-regulating effects we found, we applied the opioid receptor antagonist naloxone in a similar set of experiments. Splenocytes were preincubated (60 min) with naloxone (10^{-8} M) followed by preincubation with β-end, as described above, and by stimulation with Con A. Figure 5 shows that naloxone itself did not influence the proliferative response. As expected, preincubation with β-end enhanced proliferation. The application of naloxone did not prevent β-end from exerting its up-regulating effect on T-cell proliferation, thereby indicating that the effect of β-end is not mediated via interaction with opioid receptors, but rather via non-opioid receptors.

Next, we investigated whether we could modulate the effect of β-end with agonists for the opioid receptor. This was approached by preincubating the cells with β-end, followed by a washing step, stimulating with Con A and adding either α-, or β-end directly to the cultures (Table 2). Analogous to previous experiments, the continuous presence of α-, or β-end in Con A cultures of untreated splenocytes did not affect the extent of proliferation, while preincubation with β-end enhanced T-cell proliferation with 72% ($P < 0{\cdot}005$). Surprisingly, the continuous presence of α-end in a culture that had been preincubated with β-end abrogated the stimulatory signal. Also β-end was able to abrogate its own stimulatory signal by continuous presence of this peptide in preincubated cultures. As α-, and β-end share their N-terminal, i.e. opioid part, it was studied whether the down-regulating effect that we observed was mediated by opioid receptors. This was attempted by studying the effects of naloxone in a set of experiments in which cells were preincubated with medium or an optimal concentration of β-end, and subsequently stimulated with Con A in the presence of inhibitory concentrations of α-end or β-end (Table 3). The presence of naloxone in cultures that had not been preincubated with β-end did not affect the extent of proliferation (column 1). As expected, preincubation with β-end enhanced the proliferative response (columns 1 and 2, $P < 0{\cdot}005$), which was not influenced by the addition of naloxone. The addition of α-end to cultures of cells that had been preincubated with β-end, completely reversed the enhancing effect of β-end (column 3, $P < 0{\cdot}02$). However, in the presence of naloxone, α-end was no longer able to down-regulate the stimulatory signal of β-end. This indicated that the down-regulating signal of α-end is mediated via opioid receptors. In a similar fashion, β-end inhibited its own stimulatory activity (column 4, $P < 0{\cdot}02$). Also this inhibitory signal of β-end was completely prevented by naloxone.

DISCUSSION

In this study we investigated five naturally occurring opioid peptides for their effect on immune function. Only one of these peptides (β-end) was stimulatory under very stringent conditions. A preincubation step was a prerequisite for stimulation, whereas the continuous presence in culture appeared not to affect immune function.

A variety of T-cell functions was studied. We started by studying the effects on Con A-induced proliferation. In agreement with observations by Gilman *et al.*[2] we found that from the five peptides under investigation only β-end enhanced lymphocyte proliferation, in a naloxone-insensitive way. In addition to enhancement of proliferative response, β-end potentiated the production of IL-2 and the expression of the IL-2 receptor. The effects on IL-2 production were in contrast with the observations of Kusnecov *et al.*[4] Whereas this group reported enhancement of rat lymphocyte proliferation, this effect was not accompanied by increased IL-2 activity in supernatants of these cultures. Besides this conflicting observation, more reports on the effects of opioids on several leucocyte functions are inconsistent (reviewed in Refs 1 and 28). As will be discussed below, this may be explained from the presence of multiple receptors for opioid peptides on cells of the immune system.

β-End enhanced lymphocyte function only under very stringent conditions. Only a very short incubation time was necessary to initiate the stimulatory effect. Therefore, the

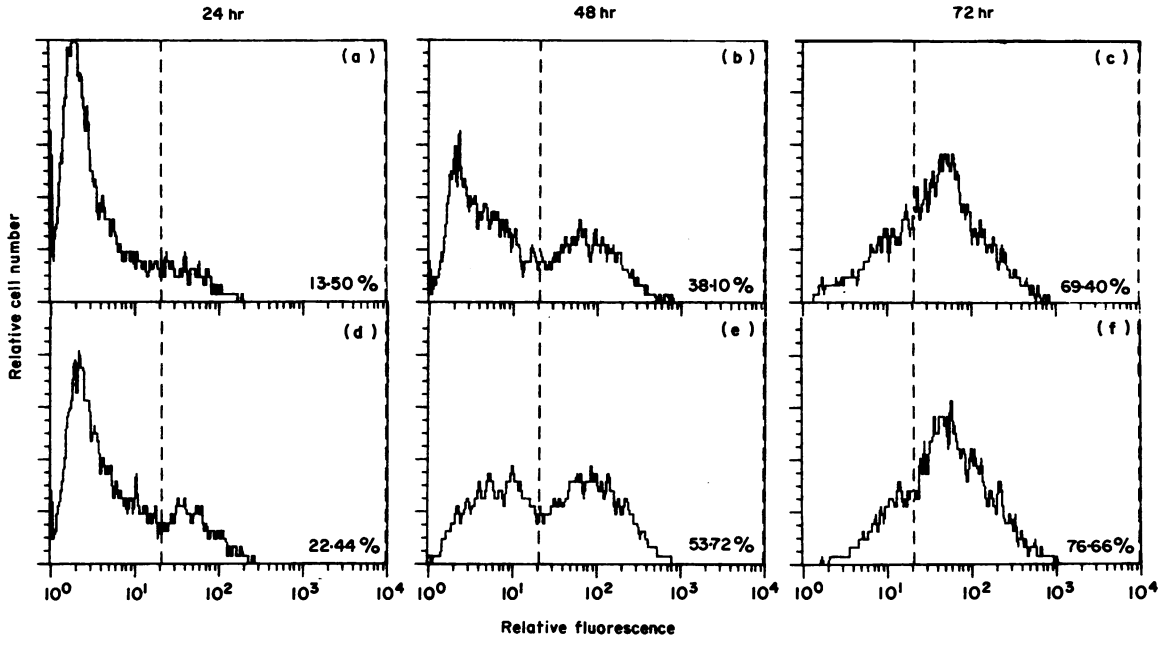

Figure 4. β-end facilitates enhanced IL-2 receptor expression. Cells were preincubated with medium (a, b, c) or 10^{-10} M β-end (d, e, f) and stimulated with 0·5 μg/ml Con A. IL-2 receptor expression was determined at 24, 48, and 72 hr by FACS analysis using the monoclonal antibody OX39. Percentages refer to the fraction of IL-2 receptor positive cells.

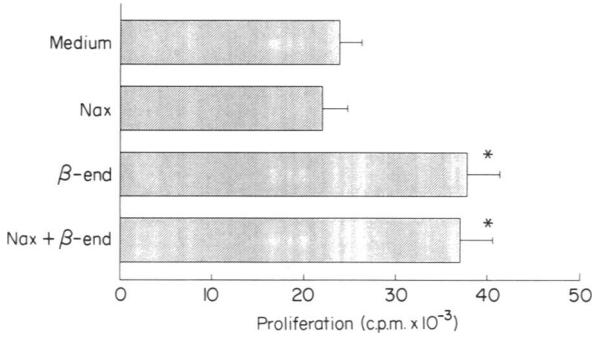

Figure 5. Naloxone (Nax) does not prevent the up-regulating effect of β-endorphin. Cells were treated with 10^{-8} M naloxone prior to preincubation with β-end. Subsequently, cells were stimulated with 0·5 μg/ml Con A. At Day 3 proliferative responses were determined by [^{3}H]thymidine incorporation. All values are means ± SD. * $P < 0{\cdot}005$ compared with the value of the cultures preincubated with medium.

Table 2. Opioid peptides abrogate the up-regulating effect of β-end

Addition of neuropeptide*		[^{3}H]thymidine incorporation† (c.p.m. ± SD)
Preincubation	Continuous presence	
—	—	24,311 ± 2009
—	α-end	22,560 ± 1423
—	β-end	25,098 ± 2591
β-end	—	41,902 ± 3266‡
β-end	α-end	28,981 ± 3011
β-end	β-end	26,740 ± 2290

* Preincubation and continuous presence refer to incubation procedures as described in Materials and Methods. Preincubation with β-end was done with 10^{-10} M, the continuous presene refers to 10^{-9} M of α- or β-end.

† Prior to stimulation with 0·5 μg/ml Con A, cells were treated as described above. Proliferative responses were determined by [^{3}H]thymidine incorporation at Day 3. The proliferative response in the absence of Con A was less than 500 c.p.m.

‡ $P < 0{\cdot}005$ compared with the value of untreated cultures.

receptor to which β-end bound within this short period is most likely of high affinity. This receptor was not one of the opioid receptors, because blocking experiments with non-toxic and saturating concentrations of naloxone, an opioid receptor antagonist, did not prevent the effect. In comparison with other opioid peptides β-end is unique in its C-terminal part. Therefore, it is likely that C-terminal amino acids of β-end are involved in the enhancing activity, mediated via one or more high-affinity non-opioid receptors. Preliminary data of experiments, in which moieties of β-end which lack the N-terminal amino acids are used, indeed favour this hypothesis (P. van den Bergh, manuscript in preparation).

In contrast to the up-regulating effect of preincubation with β-end, the addition of this peptide for the entire 3-day culture period without the preincubation procedure did neither increase nor decrease the extent of proliferation. Since one has to assume that also under these circumstances a similar mechanism of rapid, high-affinity binding of β-end has to occur, resulting in an up-regulation of proliferative response, there needs to be a

Table 3. β-end-induced stimulation is abrogated by interaction of α- and β-end with opioid receptors

	[^{3}H]Thymidine incorporation (c.p.m. ± SD)*			
Column†	1	2	3	4
Preincubation‡	Medium	β-end	β-end	β-end
Directly added to cultures‡			α-end	β-end
− Naloxone	38,064 ± 3082	51,727 ± 3923§	40,346 ± 2360¶	40,230 ± 2088¶
+ Naloxone	41,716 ± 3905	50,271 ± 3811§	52,727 ± 1496§	49,033 ± 378§

* Proliferative responses were determined by [^{3}H]thymidine incorporation at Day 3. Background values were less than 500 c.p.m.

† Column numbers to which is referred in the text.

‡ Prior to stimulation with 0·5 μg/ml Con A, spleen cells were either preincubated with medium (column 1) or preincubated with 10^{-10} M β-end (columns 2, 3, and 4). Subsequently, 10^{-10} M α-end (column 3) or 10^{-10} M β-end (column 4) was added. Cultures were performed in the presence or absence of 10^{-8} M naloxone.

§ $P < 0{\cdot}01$ compared with the value of cells preincubated with medium.

¶ $P < 0{\cdot}02$ compared with the value of cells preincubated with β-end.

negative signal down-regulating that effect, which causes the overall lack of changes in proliferative activity in the case of continuous presence of β-end. By preincubating the cells with β-end this down-regulating signal does not occur, and an enhancement of proliferative response is the result. However, when β-end is continuously present in culture it is no longer possible to discriminate between binding to the high-affinity receptor and other receptors. In this case β-end will deliver more than one signal, resulting in no overall change in extent of proliferation. In line with this idea is the fact that the stimulating effect of β-end was lower after preincubation with a higher concentration of β-end, i.e. 10^{-8} M (Fig. 2). Apparently, a preincubation with excess of β-end triggers the up-regulating mechanism as well as down-regulating events.

Subsequent experiments confirmed this hypothesis. The continuous presence of β-end in cultures that had previously been preincubated with β-end abrogated the stimulatory effect. Moreover, α-end was also able to abrogate the stimulatory signal of preincubation with β-end. As α-, and β-end share their opioid N-terminal region it appeared likely that the down-regulating effect was mediated via one or more opioid receptors. This was tested by adding naloxone as well as α-end to cultures that had been preincubated with β-end, followed by stimulation with Con A. Naloxone completely prevented the α-end-induced inhibition. Thus, the down-regulating signal was indeed delivered via interaction with receptors of the opioid type.

To observe the down-regulating capacity of opioid peptides it was required to preincubate cells with β-end and then add α- or β-end directly to the Con A-stimulated cultures. The continuous presence of α-end, without the preincubation step with β-end, did not inhibit Con A-induced proliferation. It is likely therefore that the negative signal by triggering opioid receptors specifically antagonizes the effect mediated after the preincubation step. Besides, it is possible that preincubation with β-end up-regulates the low number[24,25] of opioid receptors on cells of the immune system. This may result in a better availability and occupancy of these receptors, resulting in a detectable inhibition of proliferation. At the level of intracellular messengers it is noteworthy to mention the coupling of opioid receptors in neural tissues to G proteins and adenylate cyclase.[31] Possibly, a similar mechanism may occur in immune cells.

The experiments in this study have been performed with spleen cells consisting predominantly of T lymphocytes, B lymphocytes, and macrophages. As a model we used Con A-induced proliferation of T lymphocytes. Rather few experiments have yet been performed to investigate the effects of opioid peptides on purified T lymphocytes. Recently, it has been shown that β-end, met-enk, and leu-enk enhanced migration of human peripheral blood T lymphocytes, indicating the ability of opioid peptides to regulate the function of T lymphocytes directly.[12] However, preliminary results of experiments performed in our laboratory with purified T lymphocytes indicate that in our system β-end does not directly stimulate T cells. Since the Con A-induced proliferative response is dependent on the presence of antigen-presenting cells, β-end seems to interact with another cell population resulting in a more efficient accessory cell function for T lymphocytes (P. van den Bergh, manuscript in preparation). From this point of view, the observation that β-end regulates IL-1 production and release, which is required for IL-2 production, by murine bone marrow macrophages, is very interesting.[5]

Evidence that opioid peptides, like interleukins, are also products of cells from the immune system is accumulating.[18-20,22] These peptides might affect immune function in the way interleukins do. Following this line of thinking, β-end and other opioid peptides can be considered as regulatory entities between the various types of cells involved in an immune response. Rather than exerting drastic effects their contribution may lie in the area of fine-tuning of an immune response with very sensitive up- and down-regulating mechanisms. Although the immune cells produce opioid peptides in a low frequency and there are yet no reports demonstrating the biological activity of such immune-derived opioid peptides, to our view the relevance of opioid peptide-modulated lymphocyte function lies within the immune system. In this respect, opioid peptides may be both considered as immune-derived products with a local effect on

other cells of the immune system, as well as products from the central nervous system providing a neuroendocrine-immune link.

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