Short paper

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The phosphorylation of the CD3 γ chain of T lymphocytes is modulated by β-endorphin

The neuropeptide β-endorphin can modulate the response of T and B cells to mitogenic or antigenic stimulation. In the present report we describe a novel mechanism by which β-endorphin can interfere with T cell activation. It is shown here that β-endorphin can modulate the phorbol ester-induced phosphorylation of the γ chain of the CD3 complex. The effect of β-endorphin is dose dependent and appears to be mediated via interaction of β-endorphin with an opiate receptor on lymphocytes. Evidence is presented that the modulatory effect of β-endorphin is specific for the phosphorylation of the CD3 γ chain. β-Endorphin does not affect the phosphorylation of total cell protein, nor does it have any effect on the phosphorylation of the CD4 determinant on T cells. The possible consequence of a change in CD3 γ chain phosphorylation is discussed.

1 Introduction

Protein phosphorylation is one of the most common means of regulating cellular processes. In T lymphocytes the calcium- and phospholipid-dependent PKC plays a pivotal role in the induction of T lymphocyte growth and differentiation. Activation of T lymphocytes with mitogen or antigen induces the phosphorylation of PKC substrates in these cells such as CD4, CD8, LFA-1 and CD3, the signal-transducing complex associated with the TcR for antigen [1, 2]. Inhibition of PKC ativity by a specific antagonist abrogates the response of T lymphocytes to mitogenic stimulation [3]. Furthermore, phorbol ester-induced activation of PKC can, in combination with a calcium ionophore, induce T cell growth [4].

Peptide hormones and growth factors such as arginine-vasopressin, adrenocorticotropic hormone (ACTH), platelet-activating factor (PAF) and platelet-derived growth factor (PDGF) are known to modulate cell functioning via interaction with PKC activity [5–8]. We report here that the peptide hormone β-endorphin modulates the phosphorylation of a PKC substrate in T lymphocytes. β-Endorphin can either enhance or inhibit the phorbol ester-induced phosphorylation of the CD3 γ chain, depending on the concentration of the peptide. The modulation of CD3 γ chain phosphorylation is specific and does not reflect a general effect of β-endorphin on protein phosphorylation. Phorbol ester-induced phosphorylation of total cell protein or of the PKC substrate CD4 is not modulated by β-endorphin. The fact that β-endorphin modulates CD3 γ chain phosphorylation is indicative for the involvement of a kinase signaling pathway in the modulation of T cell activation by β-endorphin.

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2 Materials and methods

2.1 Cell isolation

Human peripheral blood T lymphocytes were isolated by density gradient centrifugation on Ficoll Isopaque (Pharmacia, Uppsala, Sweden) and rosetting with SRBC treated with 2-aminoethylisothiouronium bromide (AET) as described [9].

2.2 ^{32}P labeling of the cells

Isolated T cells were incubated for 30 min in phosphate-free Eagle's medium supplemented with 2% heat-inactivated dialyzed FCS and labeled with ^{32}P for 2 h in medium with 100 μCi = 3.7 MBq/ml of ^{32}P-orthophosphate (Amersham Int., Amersham, GB). Labeled cells (2×10^7 per sample) were incubated with β-endorphin in the concentrations indicated for 15 min and then stimulated with 2×10^{-8} M 4β-PBu_2 (Sigma, Taufkirchen, FRG) for 15 min. The reaction was stopped by washing with ice-cold PBS, followed by lysis of the cells.

2.3 Cell lysis and immunoprecipitation

Cells were lysed in lysis buffer containing 1% NP40, 150 mM NaCl, 1 mM PMSF, 5 mM EDTA, 10 mM triethanolamine, 20 mg/ml trypsin inhibitor (Sigma) and 1% BSA, pH 7.8 [10]. After preclearing the lysates with pansorbin, the CD3 γ chain was precipitated, as described by Cantrell et al. [11], with mAb RIV 9 [12], and the CD4 determinant was immunoprecipitated with mAb RIV 6 [13]. The immunoprecipitates were extensively washed in lysis buffer without BSA and analyzed by SDS-PAGE on a 12% gel run under reducing conditions, followed by autoradiography. Autoradiograms were analyzed with the use of a 2-D video analysis system.

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0014-2980/90/0404-0943$02.50/0

3 Results and discussion

Incubation of ^{32}P-labeled human peripheral blood T lymphocytes with the phorbol ester 4β-PBu_2 induces the phosphorylation of the γ chain of the CD3 complex. T cells preincubated with β-endorphin and then treated with PBu_2 exhibit a different phosphorylation of the CD3 γ chain as compared to cells only treated with PBu_2 (Fig. 1B). β-Endorphin does not affect the phosphorylation of the CD3 γ chain in the absence of PBu_2 (data not shown). The dose-response curve for the effect of β-endorphin on the PBu_2-induced phosphorylation of the CD3 γ chain is bimodal. The phosphorylation of the CD3 γ chain is inhibited at 10^{-13}–10^{-12} M β-endorphin. The maximal inhibitory effect of β-endorphin was 56% ± 12%. In contrast, preincubation of human T cells with a higher concentration of the peptide (10^{-11} M) enhances the phosphorylation of the CD3 γ chain with 39 ± 7% (Fig. 2). A comparable bimodal dose-response curve could also be observed for the modulatory effect of β-endorphin on the proliferative response of T cells after stimulation with the T cell mitogen Con A [14].

The CD4 determinant is another substrate of PKC on the membrane of T lymphocytes. It is interesting that β-endorphin has no effect on the phosphorylation of the CD4 determinant (Fig. 1A), suggesting that the effect of β-endorphin is specific for CD3 γ chain phosphorylation. This hypothesis is supported by the fact that the phosphorylation pattern of total cell protein (Fig. 1C) nor ^{32}P incorporation in trichloroacetic acid precipitates of total cell protein is changed detectably by β-endorphin preincubation (results not shown).

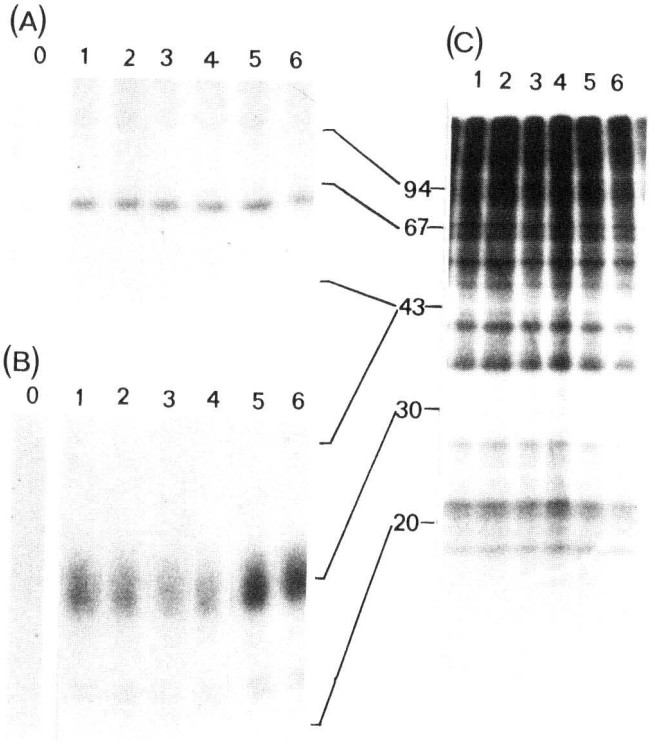

Figure 1. Effect of different concentrations of β-endorphin on the PBu_2-induced phosphorylation of T cell determinants. ^{32}P-labeled T cells were incubated with β-endorphin (lanes 0, 1, 2: control without β-endorphin, lanes 3, 4: 10^{-10} M β-endorphin, lanes 5, 6: 10^{-11} M β-endorphin) for 15 min and then stimulated with 2×10^{-8} M PBu_2 for 15 min (except lane 0: control without PBu_2). (A) CD4 immunoprecipitate, (B) CD3 immunoprecipitate, (C) total cell protein. Molecular mass markers as indicated (kDa). Representative experiment out of five with comparable results.

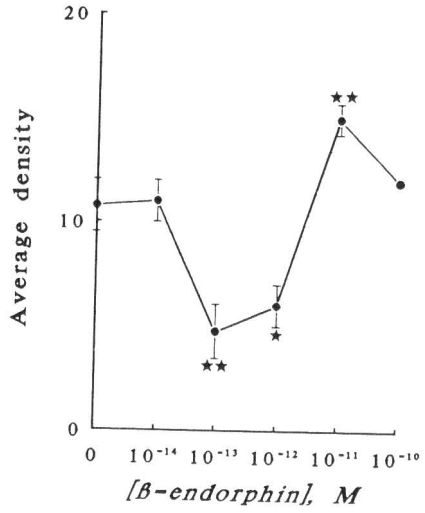

Figure 2. Effect of different concentrations of β-endorphin on the PBu_2-induced phosphorylation of the CD3 γ chain as determined by analysis of the autoradiograms with use of a 2-D video imaging analysis system. Mean ± SD of five different experiments. * $p < 0.01$; ** $p < 0.005$.

β-Endorphin is an endogenous opioid peptide that can exert its effect via binding to an opiate receptor with the N-terminus of the peptide. There is pharmacological evidence, obtained from functional assays, for the presence of opiate binding sites on lymphocytes (reviewed in [15]). However, it has been demonstrated that β-endorphin can also influence immune responses such as antibody synthesis and T cell proliferation via a non-opiate receptor-mediated mechanism [14–17]. Binding of β-endorphin to opiate receptors can be prevented by acetylation of the N terminus of the peptide [18]. To investigate whether opiate receptors are involved in the modulatory effect of β-endorphin on CD3 γ chain phosphorylation, T cells were incubated with the N-terminal acetylated form of β-

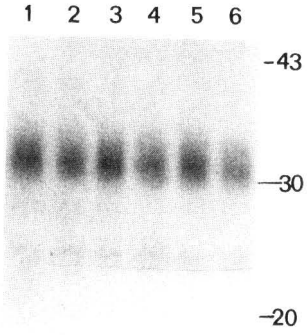

Figure 3. Effect of N-acetyl-β-endorphin on PBu_2-induced CD3 γ chain phosphorylation. Cells were incubated with N-acetyl-β-endorphin and stimulated with PBu_2 as described in Sect. 2.2 (lanes 1, 2: control without N-acetyl-β-endorphin, lanes 3, 4: 10^{-13} M N-acetyl-β-endorphin, lanes 5, 6: 10^{-11} M N-acetyl-β-endorphin). Representative experiment out of three.

endorphin prior to activation with PBu_2. N-acetyl-β-endorphin does not have any effect on the PBu_2-induced phosphorylation of the CD3 γ chain (Fig. 3). These results suggest that the effect of β-endorphin on CD3 γ chain phosphorylation is mediated via interaction with an opiate receptor on lymphocytes.

As mentioned above, the effect of β-endorphin seems to be specific for CD3 γ chain phosphorylation. These data indicate that the modulatory effect of β-endorphin is not mediated via a direct effect on either PKC or ATPase activity. Modulation of PKC or ATPase activity would also affect the phosphorylation of other proteins. Recent work by Alexander et al. [19] has shown that a membrane-associated phosphatase in T cells can rapidly dephosphorylate the CD3 γ chain. Moreover, it has been suggested that other kinases are also involved in the phosphorylation of the CD3 γ chain [20, 21]. It may well be possible that the effect of β-endorphin on CD3 γ chain phosphorylation is mediated via differential effects on kinase and phosphatase activity.

4 Concluding remarks

The functional consequences of an interference by β-endorphin with the phosphorylation of the CD3 γ chain are as yet unknown. Phosphorylation of T cell determinants has been described as an obligatory step in the regulation of their endocytosis [22–25]. Mutant CD4 molecules that lack a PKC phosphorylation site do not undergo endocytosis in response to PKC stimulation, whereas wild-type CD4 molecules are rapidly internalized after PKC stimulation [24]. In addition, the phosphorylated form of the CD3 γ chain has been found predominantly in the cytosolic fraction of T cells [23]. Phosphorylation and subsequent internalization of the TcR/CD3 complex may serve a negative feedback mechanism: receptor internalization being one of the mechanisms by which the cell can prevent stimulation by antigen [25–27].

It may well be possible that modulation of the phosphorylation of the CD3 γ chain results in interference with the internalization of the TcR/CD3 complex and, thus, with the capacity of T cells to respond to (repeated) antigenic stimulation. We have preliminary evidence that β-endorphin can indeed interfere with the PBu_2-induced down-regulation of the expression of CD3 on the surface of T lymphocytes (unpublished results).

In conclusion our data show a novel mechanism by which the neuropeptide β-endorphin can modulate T cell function. We suggest that the neuropeptide exerts its action via interfering with the phosphorylation of a specific PKC substrate in T cells, the CD3 γ chain.

Received November 30, 1989.

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