IMMUNOLOGY AND MICROBIOLOGY

β-Endorphin Effects on Antibody Production, Proliferation, and Secretion of Th1/Th2 Cytokines *In Vivo*

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Intraperitoneal injection of β-endorphin in doses of 1, 0.01, and 0.0005 μg/kg under conditions of systemic immunization increased the count of antibody-producing cells in the spleen and the titer of anti-erythrocyte antibodies in the plasma of experimental animals. Intraperitoneal β-endorphin stimulated proliferative activity of splenocytes in mice in the presence of both B- and T-cell mitogen, did not change the production of IFN-γ, reduced the level of IL-2, and stimulated the secretion of IL-4, the main Th2-polarizing factor.

Key Words: *β-endorphin; splenocytes; cytokines; IL-2; IL-4; IFN-γ*

The role of endogenous opioid peptides, *e.g.* β-endorphin, the most active and polyfunctional representative, in immunogenesis regulation has been studied for more than 30 decades. β-Endorphin is a component of the universal functional system (hypothalamic-pituitary-adrenal axis); together with other hormones, it is released into the peripheral blood during stress, injury, mental strain, and exercise [10]. In addition, the peptide is produced locally by cells of various organs and systems, including the immune system cells, in response to antigen or in the presence of high concentrations of proinflammatory cytokines, causing numerous immunoregulatory effects realized by the para- and autocrine mechanisms [13]. However, we still have no clear-cut picture of the opiatergic processes developing under conditions of unfolding immune reactions. Most published data on the immunoregulatory effects of β-endorphin have been obtained *in vitro*; therefore these results cannot be extrapolated to living organism because of the existence of multi-level regulatory systems and cell-cell cooperation *in vivo*, which are extremely difficult to simulate *in vitro*.

Few previous *in vivo* studies have revealed mainly the inhibitory effect of the peptide, for example, on antibody production in the spleen [3]. On the other hand, we have found that low doses of β-endorphin, comparable to those in stress, stimulate antibody production in the lymph nodes in response to local injection of the antigen [1]. The stimulating effects of β-endorphin on lymphocyte proliferation, antibody production, and IL-4 production *in vitro* were demonstrated [1,2,15].

Here we studied the effects of β-endorphin on antibody production in the spleen of mice immunized with thymus-dependent antigen and on proliferative activity of splenocytes and production of IL-2, IL-4, and IFN-γ *in vivo*.

MATERIALS AND METHODS

The experiment was carried out on outbred male mice (17-22 g). β-Endorphin (Skytek Laboratories) was injected intraperitoneally in single doses of 100, 1, 0.01, and 0.0005 μg/kg. Controls were injected with equivalent volumes of 0.9% NaCl. The choice of these doses

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was based on previous results [1]. The animals injected with β-endorphin were then divided into 2 groups. Group 1 animals were intraperitoneally immunized with sheep erythrocytes (SE) in a single dose of 10^8 cells/0.2 ml 0.9% NaCl 1 h after the peptide injection. On day 5, the animals were decapitated under ether narcosis and plasma titers of anti-erythrocyte antibodies were evaluated by direct hemagglutination test and the count of antibody producing cells (APC) in the spleen was evaluated by local hemolysis in agarose gel. Group 2 animals were sacrificed 1 h after the peptide injection, splenocytes were isolated and cultured in plastic round-bottom 96-well plates (Medpolymer) for 72 h. Each culture contained 5×10^5 cells/0.2 ml complete culture medium (prepared *ex tempore*) of the following composition: RPMI-1640 (Biolot) with 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Biolot). The mitogens were *E. coli* B55:O5 LPS (10 μg/ml; Sigma) and concanavalin A (ConA, 20 μg/ml; Sigma). ^{3}H-Methylthymidine (10 μl) was added to the wells 18 h before the end of culturing. Radioactivity of samples was measured on a Guardian scintillation counter (Wallac).

For evaluation of secretory activity of splenocytes, the supernatants of 24-h (IL-2) and 48-h (IL-4, IFN-γ) cultures were collected into Eppendorf tubes, frozen, and stored at -20°C. The cytokines were measured by solid phase EIA using Bender Medsystems kits by the method recommended by the manufacturer.

The data were statistically processed by unpaired one-way (for antibody production parameters) or two-way (for evaluation of splenocyte proliferative and secretory activities) analysis of variance and Fisher LSD post-hoc test. The data in the figures were presented as the mean and standard error of the mean ($M\pm m$).

RESULTS

Injection of β-endorphin in doses of 1, 0.01, and 0.0005 μg/kg to mice led to a significant increase in APC count in the spleen (F=4.38; p<0.005). The strongest effect was recorded in the groups of animals receiving the peptide in a dose of 0.01 μg/kg. Injection of β-endorphin in the high dose (100 μg/kg) caused no significant changes in APC counts in comparison with the control (Fig. 1). A similar dose-effect relationship was found for the effect of β-endorphin on the titer of antibodies to SE in mouse plasma (F=12.08; p<0.001). A significant elevation of plasma titers of anti-erythrocyte antibodies was observed in response to β-endorphin in doses of 1, 0.01, and 0.0005 μg/kg (Fig. 1). The peptide in a dose of 100 μg/kg caused virtually no changes in the titer of antibodies to SE.

Spontaneous proliferative activity of splenocytes virtually did not change under the effects of the studied concentrations of the peptide (Fig. 2), but significant stimulatory effects of β-endorphin on the level of radioactive label incorporation by splenocytes (F=4.4; p<0.003) were revealed under the background of mitogen stimulation. The proliferative response was clearly stimulated in the presence of LPS in splenocyte cultures treated with β-endorphin in doses of 1, 0.01, and 0.0005 μg/kg. Stimulation with ConA resulted in obvious manifestation of the effect of β-endorphin in all the studied doses.

Analysis of β-endorphin effect on the production of IL-2, IFN-γ, and IL-4 (the key cytokines for functional differentiation of T-helpers towards Th1 or Th2 type) has shown that despite comparable activation of proliferation in cultures with ConA and LPS, cytokine production was markedly stimulated in the presence of only ConA, while in the cultures with LPS it dif-

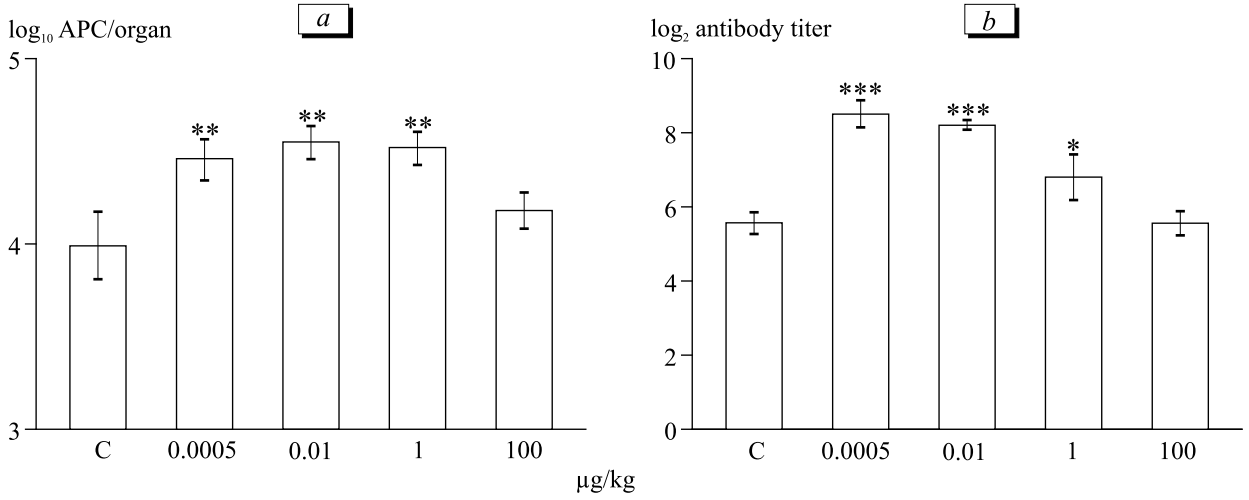

Fig. 1. Effect of β-endorphin on APC count (*a*; *n*=10) in the spleen and titer of antibodies (*b*; *n*=10) to SE in the peripheral blood of mice. Here and in Figs. 2 and 3: *p<0.05, **p<0.01, ***p<0.001 in comparison with the control (C; Fisher's LSD test).

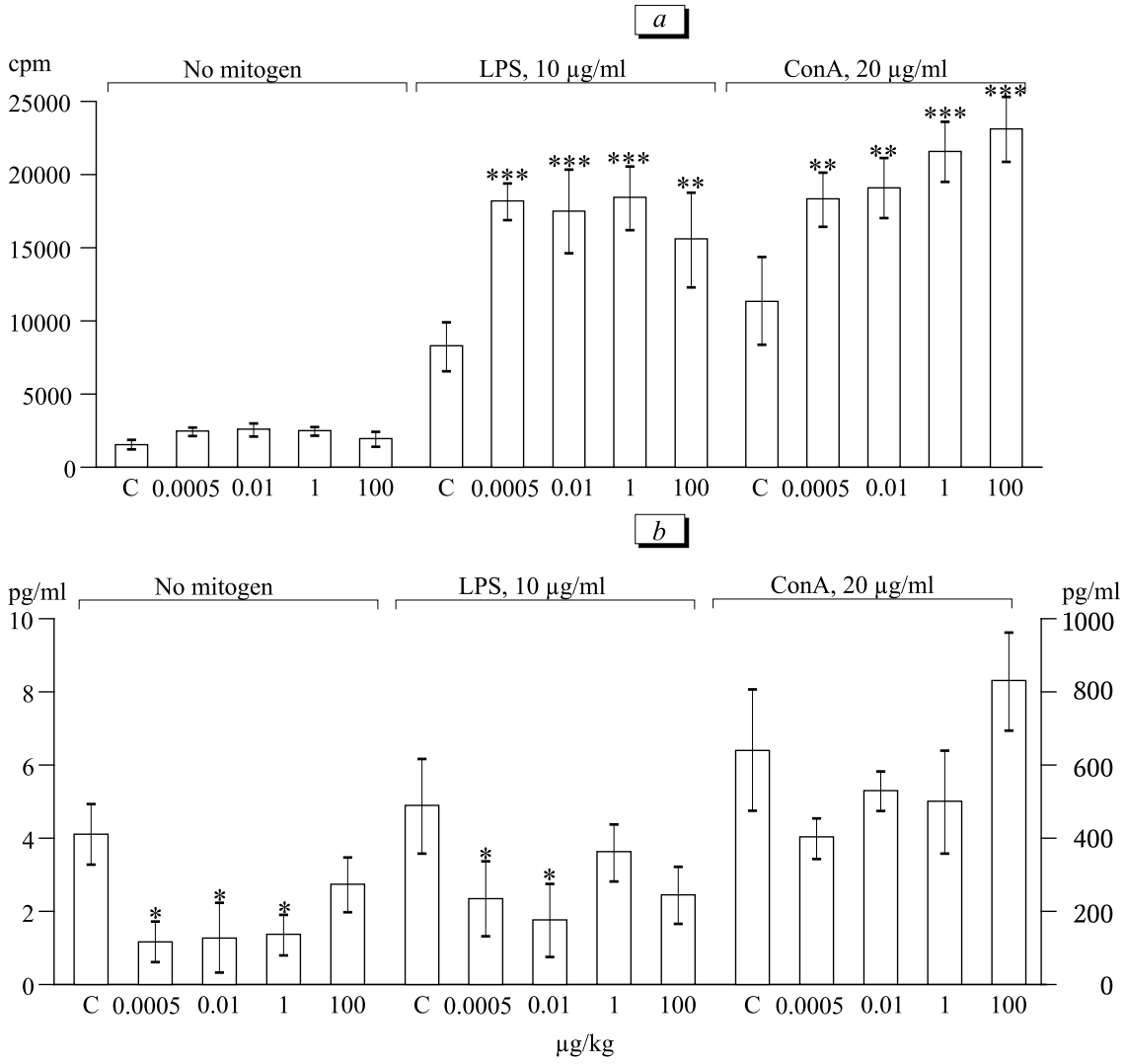

Fig. 2. Effect of β-endorphin on proliferative activity of mouse splenocytes (*a*; *n*=11) and production of IL-2 by these cells (*b*; *n*=6). Main (left) axis: spontaneous and LPS-induced production of IL-2; extra (right) axis: ConA-induced production.

fered negligibly from cultures without stimulation. This could be due to the fact that ConA was a polyclonal stimulant, while LPS in mice was a direct B-cell mitogen and its direct stimulatory effect was due to expression of toll-like molecules on the surface of B-lymphocytes [14].

Despite β-endorphin stimulation of splenocyte proliferative response, the production of IL-2 changed negligibly in ConA-stimulated cultures (Fig. 2), but decreased in spontaneous cultures and in the presence of LPS (F=3.55; p<0.013) in animals receiving β-endorphin in doses of 1, 0.01, 0.0005 μg/kg, respectively.

By contrast, the production of IL-4 increased in ConA-stimulated cultures after injection of β-endorphin in physiological doses of 0.01 and 0.0005 μg/kg (F=2.9; p<0.027, Fig. 3). In the presence of LPS, the effect of the peptide was observed only in its lowest dose of 0.0005 μg/kg (F=2.73; p<0.034).

The production of IFN-γ under the effect of β-endorphin changed just negligibly. An obvious inhibitory effect of the peptide in doses of 1, 0.01, and 0.0005 μg/kg in stimulated and not cultures was worthy of note.

Hence, intraperitoneal injection of β-endorphin under conditions of systemic immunization stimulates antibody production, proliferative activity of splenocytes, stimulates production of IL-4, and reduces secretion of IL-2 promoting polarization of T-helpers towards Th2 cells. An inhibitory effect of the peptide on APC count and antibody production has been described [3]. We observed various dose-dependent effects of the peptide on antibody production under conditions of local immunization. In a dose of 100 μg/kg β-endorphin inhibited APC formation, while in doses comparable to its doses in the peripheral blood of stressed animals (0.01-0.0005 μg/kg) it stimulated the process [1].

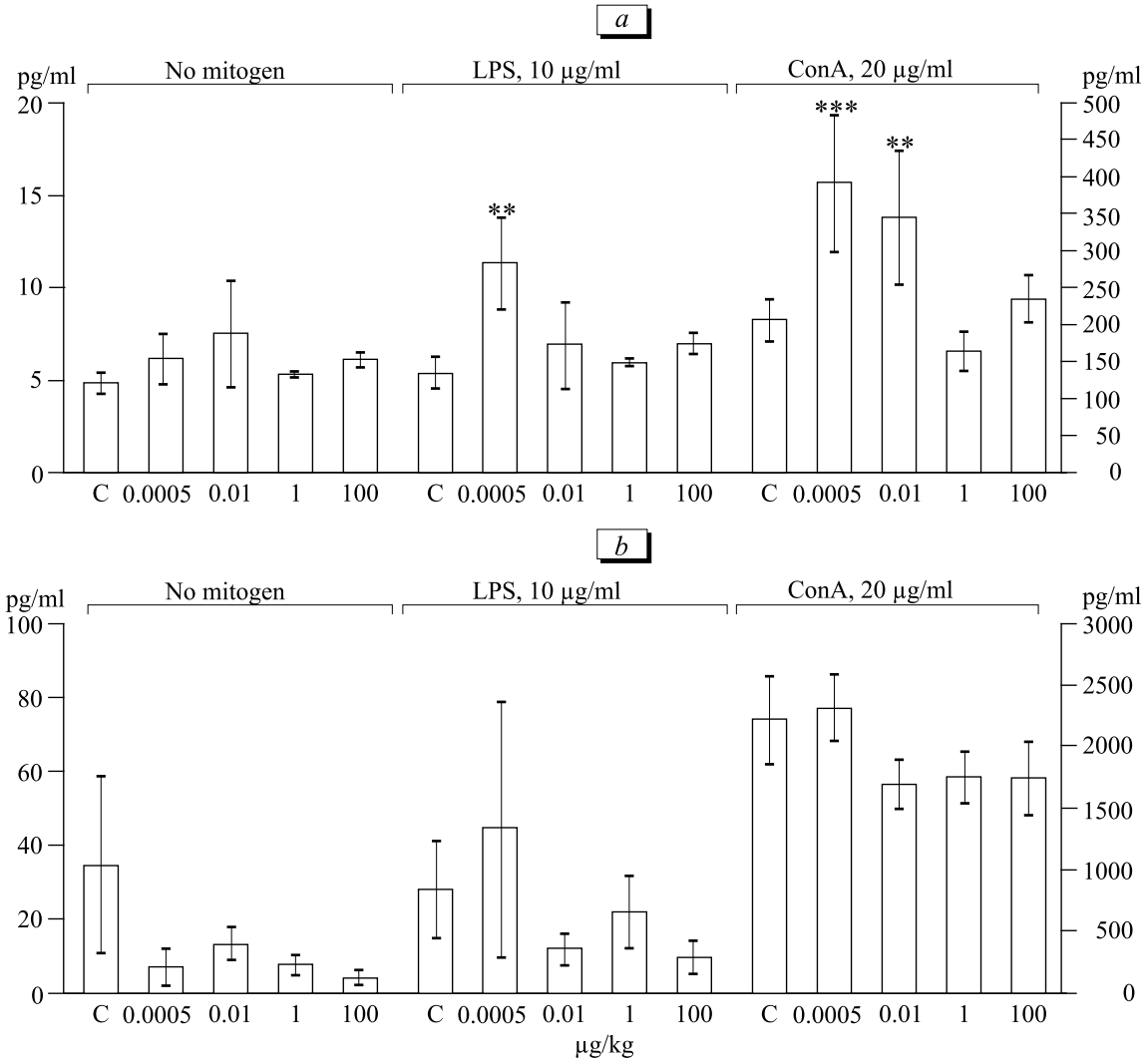

Fig. 3. Effect of β-endorphin on production of IL-4 (*a*; *n*=12) and IFN-γ (*b*; *n*=6) in mouse splenocyte culture. Main (left) axis: spontaneous and LPS-induced production; extra (right) axis: ConA-induced production.

Published data on β-endorphin effects on secretory and functional activities of T-lymphocytes *in vivo* are scanty and ambiguous. Different authors have demonstrated both the inhibitory [9] and stimulatory [6] effects of the peptide on proliferative activity of splenocytes. Splenocyte proliferation *in vivo* was stimulated by β-endorphin, but this effect was not paralleled by an increase in IL-2 production [8]. Reduction of IL-2 levels under the effect of β-endorphin has been demonstrated in β-endorphin-deficient mice. High levels of IL-2 mRNA have been observed in genetically modified animals in comparison with the controls [12].

Detection of IFN-γ and IL-4, two most significant Th1/Th2 polarizing factors, indicated a pronounced increase of IL-4 production in response to doses comparable to those found in the peripheral blood in stress. No data on β-endorphin modulation of IL-4 production *in vivo* are available up to the present time. *In vitro* β-endorphin stimulates the production of IL-4 by mouse $CD4^+$ lymphocytes [15] and by human peripheral blood leukocytes [2]. The absence of the peptide effects on spontaneous proliferative activity and production of IL-4 can be explained by low density of opioid peptide binding sites on nonstimulated lymphocytes [7].

In contrast to the production of IL-4, that of IFN-γ changed just negligibly exhibiting a clear-cut trend to inhibition. β-Endorphin elevated the levels of IFN-γ and IFN-γ mRNA in rat splenic NK cells [5]. Similar results were previously obtained on a mixed splenocyte culture [4]. On the other hand, the lectin-induced production of IFN-γ by mononuclears [11] and by $CD4^+$ cells (in the presence of monocytes) [2] reduced in the presence of β-endorphin. Presumably, the final destination of the peptide effect on the production of IFN-γ is determined mainly by the proportion and interactions of cells of congenital and adaptive immunity in culture.

Hence, β-endorphin is a positive regulator of humoral immune response *in vivo* promoting the switchover of the T-lymphocyte differentiation towards Th2, responsible for the formation of B-cellular response.

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