Effect of β-Endorphin on Production of Antibodies and IL-4 under Conditions of Opioid Receptor Blockade

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β-Endorphin *in vivo* produced different effects on antibody production depending on the administered dose. Blockade of opioid receptors with naloxone (but not naltrindole) abolished the effects of β-endorphin. The peptide activated IL-4 production *in vitro* via activation of δ-opioid receptors.

Key Words: *β-endorphin; opioid receptors; antibody production; interleukin-4*

Opioid peptide β-endorphin (BE) plays an important role in the interaction between the nervous and immune system and is characterized by a wide range of immunoregulatory activities. The peptide suppresses antibody production in the spleen and inhibits proliferative response of splenocytes *in vivo* [6,8]. In *in vitro* system the peptide stimulates lymphocyte proliferation, antibody production, and IL-4 synthesis [1,5]. The role of various opiate receptors in immunoregulatory effects of BE remains poorly understood.

Our aim was to evaluate the role of μ- and δ-opioid receptors in the realization of immunoregulatory effects of BE, in particular, in modulation of immune response and IL-4 production.

MATERIALS AND METHODS

In vivo experiments were carried out on outbred male mice weighing 20-22 g. BE (Sigma) was singly intraperitoneally injected in a volume of 0.2 ml (dose range 0.0005-100,0000 μg/kg). Naloxone (Narcan) in a dose of 0.2 mg/kg and naltrindol (ICN) in a dose of 0.1 mg/kg were injected subcutaneously 20 min before BE. Control mice received 0.9% NaCl according to the same scheme. All mice were simultaneously sensitized with sheep erythrocytes (10^8 cells in 0.02 ml subcutaneously into the plantar surface of the right hindpaw) 1 h after BE injection. The resolving dose of the antigen (10^9 cells in 0.02 ml) was injected on day 4; 0.02 ml NaCl was injected into the left (control) hindpaw. The animals were sacrificed on day 5 under ether narcosis. Taking into account the fact that local immunization induced changes only in the regional (right) lymph node [2], the cellularity of the regional lymph node, the number of antibody-producing cells (APC), and the intensity of delayed-type hypersensitivity (DTH) reaction were evaluated.

Peripheral blood leukocytes from healthy male volunteers were cultured in 96-well round-bottom plates for 72 h in the presence of phytohemagglutinin P (Sigma, 2.5 μg/ml). Each culture contained 2×10^5 cells in 0.2 ml medium 199 supplemented with 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml gentamicin, and 10% fetal calf serum (ICN). ^{3}H-Methylthymidine (2 μCi) was added 18 h before the end of culturing. Radioactivity of samples was measured on a Guardian scintillation counter (Wallac). BE was used in a concentration of 10^{-7} M, naloxone and naltrindole were added in concentrations of 10^{-6}, 10^{-8}, and 10^{-10} M. IL-4 concentration in the supernatants of 48-h cultures stimulated with phytohemagglutinin was measured using ProCon kits. Previous experi-

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ments showed that BE had no effect on spontaneous proliferative activity and cytokine production [1,7].

The data were processed statistically using one-way dispersion analysis (dose-effect relationships) and *t* test (for intergroup differences).

RESULTS

The *in vivo* effects of BE directly depended on the administered dose of the peptide. Analysis of the dose-effect relationship revealed a significant effect of BE on relative (F=3.6; p<0.003) and absolute (F=3.95; p<0.001) parameters of antibody production (Table 1). The peptide suppressed the humoral immune response in a dose of 100 μg/kg and stimulated it in a dose of 0.0005 μg/kg. BE had no significant effect on lymph node cellularity and intensity of DTH reaction. Thus, BE *in vivo* can both stimulate and suppress the formation of antibody-producing cells. The stimulating effect of the low dose is more pronounced that the suppressing effect of the high dose.

Blockade of opiate receptors with naloxone abolished both the suppressing effect of the high dose and stimulating effect of the low dose of BE on the relative and absolute number of APC (Table 2). At the same time, injection of BE against the background of δ-receptor blockade with naltrindole did not abolish the stimulatory effect of low dose of BE (0.0005 μg/kg) and increased the number of APC in response to administration of high dose 100 μg/kg, which suppressed the intensity of antibody pro-

TABLE 1. Effect of BE on Number of APC, Cellularity, and Intensity of DTH Reaction (M±m)

BE dose, μg/kg	n	Lymph node	Nucleated cells, ×10⁶		Index of DTH reaction, %
			lg APC per 10⁶ nucleated cells	lg APC per organ	
Control	9	4.62±0.89	2.43±0.10 (271.28)	3.04±0.01 (1088.94)	17.22±2.41
100	9	4.33±0.71	1.98±0.22* (95.28)	2.57±0.26* (370.54)	19.84±3.68
10	8	5.25±0.70	2.06±0.26 (115.56)	2.76±0.25 (569.35)	23.92±3.46
1	9	4.87±1.06	2.46±0.18 (286.09)	3.05±0.16 (1128.59)	20.25±4.25
0.1	8	7.55±1.35	2.25±0.18 (117.27)	3.08±0.13 (1198.91)	18.82±6.17
0.01	9	5.33±1.13	2.68±0.09 (474.99)	3.34±0.13 (2192.95)	26.72±3.88
0.001	8	5.90±1.73	2.62±0.73 (421.49)	3.21±0.13 (1607.54)	21.27±4.88
0.0005	9	5.82±0.81	2.87±0.08* (743.72)	3.61±0.07* (4031.74)	28.72±4.10

Note. Here and in Table 2: index of DTH reaction=$(R_{ex}-R_c)/R_c \times 100\%$, where R_{ex} and R_c are the weight of experimental and control paw, respectively. Geometric means (antilogarithm) of APC number are shown in parentheses. *p<0.05 compared to the control according to Fished *t* test.

TABLE 2. Effect of BE on Nymber of APC, Cellularity, and Intensity of DTH Reaction under Conditions of Opioid Receptor Blockade (M±m)

Experimental conditions	n	Lymph node	Nucleated cells, ×10⁶		Index of DTH reaction, %
			lg APC per 10⁶ nucleated cells	lg APC per organ	
Control	18	5.34±0.62	2.35±0.10 (223.55)	3.02±0.11 (1043.31)	22.43±3.19
BE, 100 μg/kg	18	4.71±0.51	1.99±0.14* (98.80)	2.36±0.15* (423.03)	22.16±2.73
BE, 0.0005 μg/kg	17	5.98±0.66	2.67±0.07* (465.21)	3.40±0.08* (2519.33)	27.95±2.73
BE, 100 μg/kg+naloxone	11	5.64±0.97	2.21±0.14 (161.61)	2.87±0.18 (747.62)	23.79±4.27
BE, 0.0005 μg/kg+naloxone	12	5.95±0.66	2.16±0.12 (143.12)	2.90±0.14 (798.77)	28.57±6.77
BE, 100 μg/kg+naltrindole	11	7.18±41.24	2.50±0.07 (314.71)	3.29±0.05* (1945.42)	23.32±4.17
BE, 0.0005 μg/kg+naltrindole	11	9.07±1.24*	2.52±0.09 (327.67)	3.43±0.12* (2673.98)	25.67±3.12
Naloxone	12	6.40±0.73	2.23±0.09 (170.40)	3.01±0.11 (1013.98)	16.08±2.02
Naltrindole	8	6.98±0.55	2.04±0.24 (110.87)	2.87±0.21 (755.38)	18.36±1.92

Note. *p<0.05 compared to the control according to unpaired Student *t* test.

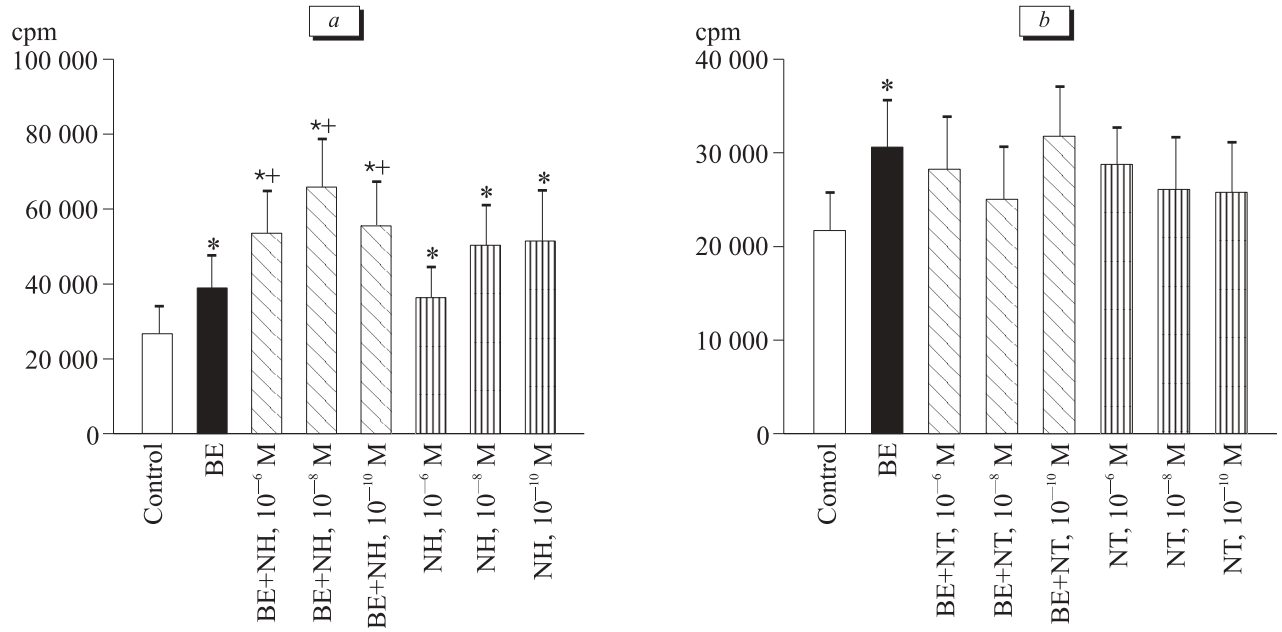

Fig. 1. Effect of BE on proliferative response of lymphocytes in the presence of phytohemagglutinin (n=10) under conditions of opioid receptor blockade with naloxone hydrochloride (NH, *a*) and naltrindole hydrochloride (NT, *b*). Here and on Fig. 2: *p<0.05 compared to the control according to paired Student t test; ^{+}p<0.05 compared to BE.

duction. Moreover, injection of 0.0005 μg/kg BE against the background of naltrindole blockade significantly increased lymph node cellularity compared to the control. The intensity of immune inflammation was not affected by combination of BE with opioid receptor agonists. Thus, naloxone irrespective of the administered dose leveled the effects of BE. Since naloxone is a μ-opioid receptor agonist, these *in vivo* findings attest to interaction of BE with μ-opioid receptor and transmission of the stimulating signals through this type of receptors. The interaction of BE with δ-receptors was observed only after administration of high doses of the agonist, which attests to the possibility of realization of immunosuppressive effects through these receptors [3].

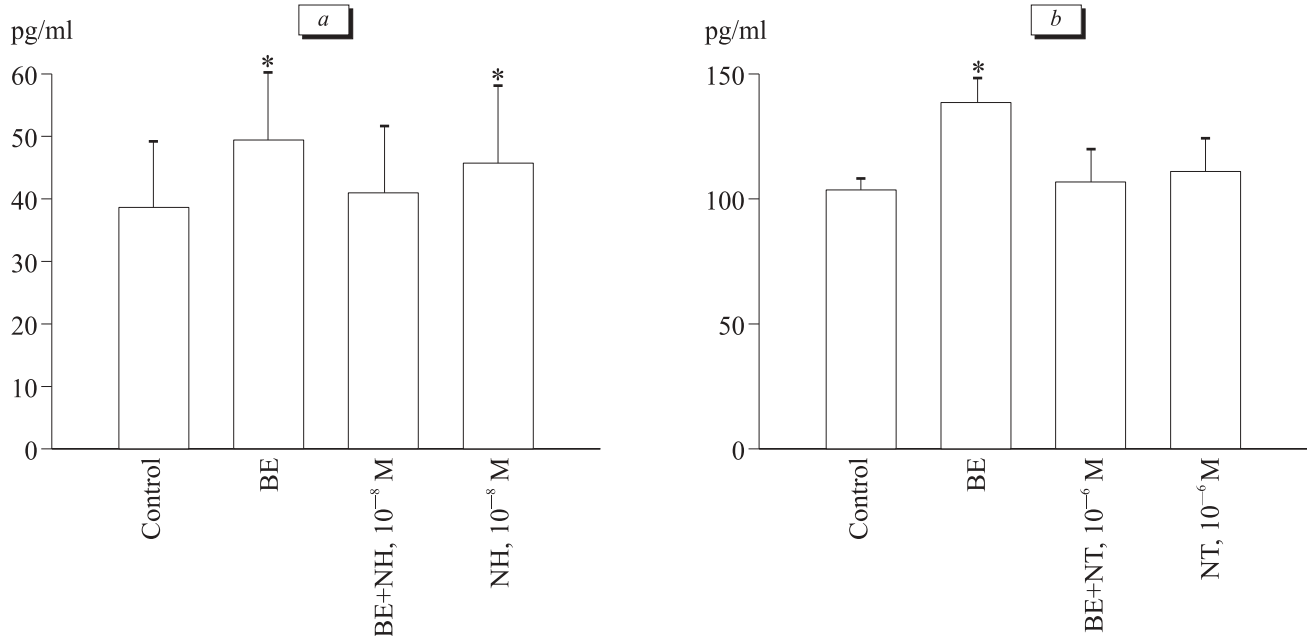

Fig. 2. Effect of BE on phytohemagglutinin-induced IL-4 production (n=6) under conditions of opioid receptor blockade with naloxone hydrochloride (NH, *a*) and naltrindole hydrochloride (NT, *b*).

In *in vitro* system BE potentiated the proliferative response of lymphocytes. Against the background of opioid receptor blockade with naloxone lymphocyte proliferation increased compared to both the control and effect of BE alone (Fig. 1, *a*). Unexpectedly, naloxone exhibited an independent stimulatory effect. After combined administration of BE and naltrindole in various concentrations the test parameters did not significantly differ from the control, which probably suggested that the blockade of δ-opioid receptors abolished the stimulating effects of BE. Incubation of cultured cells with naltrindole alone had no effect on their proliferation. The effect of BE on the level of IL-4 was similar (Fig. 2). The increase in BE-induced production of IL-4 was abolished by naltrindole, but not naloxone, which produced an independent IL-4 stimulating effect. Thus, the data obtained in *in vitro* system showed that BE stimulated functional activity of leukocytes and IL-4 production via interaction with δ-opioid receptors.

The suppressive effect of BE on antibody production after intraperitoneal administration of the antigen *in vivo* was described previously [6,9]. The capacity of BE to activate immune reactions *in vivo* was also reported [4,5]. Opposite effects of BE *in vivo* can be explained by morphine-like action of BE in high doses [9]. The stimulating effects of low doses of BE on the number of APC agrees with its capacity to stimulate lymphocyte proliferation and production of IL-4, a cytokine playing an essential role in the formation of humoral immune response. Different roles of μ- and δ-opioid receptors in the regulation of immune reactions *in vivo* and *in vitro* can be explained by different pathways of realization of BE effects, first of all, by its capacity to act via naloxone-sensitive and naloxone-unsensitive mechanisms [7] and by the existence of BE-binding sites on immune cells, which are not analogous to BE-binding sites in CNS.

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