Role of β-Endorphin in the Regulation of Proinflammatory Cytokine Production by Peripheral Blood Monocytes *in Vitro*

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β-Endorphin activated interleukin-1β production in the culture of unfractionated cells with lipopolysaccharide, but had no effect on the synthesis of tumor necrosis factor-α and interleukin-6. This peptide produced a slight stimulatory effect on spontaneous production of interleukin-1β by cultured leukocytes. Opioid receptor blockade with naloxone and naltrindole did not abolish the stimulatory effect of β-endorphin on interleukin-1β production. β-Endorphin did not modulate the synthesis of interleukin-1β, tumor necrosis factor-α, and interleukin-6 in a purified fraction of monocytes.

Key Words: *β-endorphin; interleukin; tumor necrosis factor; naloxone; naltrindole*

Opioid peptide β-endorphin (BE), hypophyseal hormone of the proopiomelanocortin group, exhibits a wide range of immunoregulatory activity [9]. BE produces immunosuppressive and immunostimulatory effects. The directionality of BP-induced changes depends on various factors, including the type of immune response (cellular or humoral), Th1/Th2 balance, and composition of the cell fraction *in vitro*.

BE modulates function of lymphocytes, natural killer cells, and macrophages. BE modifies polarization of T helper cells, activates synthesis of interleukin-4 (IL-4), interferon-γ, and IL-2 [13], and stimulates nitric oxide production [10]. Little is known about the role of BE in regulation of secretory function of monocytes, including the production of proinflammatory cytokines IL-1β, tumor necrosis factor-α (TNF-α), and IL-6 [10]. These cytokines play a key role in activation and development of the immune response. The mechanisms for the effect of this peptide mediated by the interaction with μ-opioid receptors (OR) and δ-OR are poorly understood. Published data show that these receptors are expressed on various populations of immune cells [12].

Here we evaluated the role of μ-OR and δ-OR in the BE-mediated regulation of production of IL-1β, TNF-α, and IL-6 in the suspension of unfractionated cells and purified fraction of monocytes.

MATERIALS AND METHODS

Leukocytes were isolated from the peripheral venous blood of healthy donors (men, 22-30 years). Heparinized venous blood was maintained at 37°C for 2 h, leukocyte-containing top layer of the plasma was taken, centrifuged at 400*g* for 20 min, and suspended in RPMI-1640 (ICN) containing 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml gentamicin, and 10% fetal bovine serum (ICN). The cells (1×10^6) were cultured in 24-well plates (Costar) with 1 ml complete nutrient medium in a humid atmosphere (5% CO_2) at 37°C for 24 h. Culturing was performed in the presence of 2.5 μg/ml phytohemagglutinin (PHA, Sigma) and 0.1 μg/ml lipopolysaccharide (LPS, *Escherichia coli* O26:B6, Sigma). The cultures were simultaneously treated with BE (Sigma), PHA, and LPS in concentrations of 10^{-7}-10^{-11} M. Naloxone hydrochloride and nal-

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trexone hydrochloride (ICN) were used in a concentration of 10^{-6} M. The supernatant of cultured cells was frozen and stored at -20°C.

The monocyte fraction was isolated mechanically. The mononuclear cell fraction was isolated in a Ficoll-Verografin density gradient (ρ=1.077) at 400*g* for 40 min. The cell suspension was washed 2 times, suspended in RPMI-1640 medium, and maintained at 4°C for 1 h to prevent the isolation-induced activation. The cooled suspension of mononuclear cells was placed in a sterile glass Petri dish and incubated in a thermostat at 37°C for 1 h. Nonadherent cells were removed. Adherent cells were isolated with a scraper, suspended in RPMI-1640 medium, washed 2 times, and maintained at 4°C for 1 h. Monocytes (5×10^5 cells/ml) were cultured under conditions similar to culturing of the leukocyte fraction.

The concentrations of IL-1β, TNF-α, and IL-6 in supernatants of cultured cells was measured using Tsitokin kits according to manufacturer's instructions.

Statistical treatment of data involved paired two-factor analysis of variance, Fisher's least significant difference test (evaluation of the dose-response relationship), and paired Student's *t* test (evaluation of the effect of combined treatment with BE and opioid receptor antagonists).

RESULTS

LPS significantly increased the production of IL-1β, TNF-α, and IL-6 only in cultures with the monocyte fraction, but was ineffective in leukocyte culture.

BE (10^{-7}-10^{-11} M) activated LPS-induced production of IL-1β in the suspension of unfractionated cells. However, BE had no effect on the synthesis of IL-6 and TNF-α (Table 1). BE in concentrations of 10^{-7}-10^{-9} M produced a minor stimulatory effect on spontaneous production of IL-1β. BE did not modulate cytokine production in the presence of PHA in the suboptimal dose. BE had no effect on spontaneous and LPS-induced synthesis of IL-1β, TNF-α, and IL-6 in the monocyte fraction (Table 2). OR blockade with nonselective OR antagonist naloxone (δ-OR and μ-OR) and selective δ-OR

TABLE 1. Effect of BE on Cytokine Production in the Suspension of Unfractionated Cells (*n*=8, *M*±*m*)

Cytokine	Experimental treatment	Control	BE concentration, M		
			10^{-7}	10^{-9}	10^{-11}
IL-1β, pg/ml	Without inductor	193.01±39.16	271.66±77.96*	266.57±49.18*	238.22±50.19
	PHA, 2.5 μg/ml	228.59±48.19	220.56±51.70	294.13±92.04	246.510±68.065
	LPS, 0.1 μg/ml	190.87±54.43	305.76±49.50***	300.95±76.95***	279.41±62.40**
TNF-α, pg/ml	Without inductor	253.72±52.60	286.79±61.43	277.46±67.08	290.90±62.32
	PHA, 2.5 μg/ml	315.69±56.76	354.26±61.82	328.50±57.27	344.16±64.08
	LPS, 0.1 μg/ml	269.59±53.90	297.93±66.45	295.42±68.59	295.49±60.45
IL-6, pg/ml	Without inductor	1115.41±30.54	1084.09±50.23	1101.83±56.55	1109.70±46.66
	PHA, 2.5 μg/ml	1144.11±24.99	1156.29±32.12	1133.00±31.49	1119.24±39.01
	LPS, 0.1 μg/ml	1094.73±36.40	1082.85±28.72	1051.07±36.16	1115.41±33.88

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

TABLE 2. Effect of BE on Cytokine Production in the Purified Fraction of Monocytes (*n*=8, *M*±*m*)

Cytokine	Experimental treatment	Control	BE concentration, M		
			10^{-7}	10^{-9}	10^{-11}
IL-1β, pg/ml	Without inductor	153.22±33.48	121.65±19.83	249.92±84.88	118.71±28.97
	LPS, 0.1 μg/ml	230.26±42.25	202.84±36.47	223.97±71.16	235.21±53.29
TNF-α, pg/ml	Without inductor	137.52±51.33	153.81±58.24	148.31±57.56	133.20±55.31
	LPS, 0.1 μg/ml	163.21±60.48	178.81±69.78	174.97±68.63	178.73±70.21
IL-6, pg/ml	Without inductor	1204.61±354.15	1174.58±356.34	1281.19±357.40	1243.46±332.03
	LPS, 0.1 μg/ml	1435.55±379.02	1442.89±340.04	1346.26±342.73	1414.19±367.26

antagonist naltrindole did not abolish the stimulatory effect of BE in a concentration of 10^{-7} M on IL-1β production (F=21.71, p=0.002; Fig. 1). Naltrindole had an independent stimulatory effect on IL-1β production (F=21.2, p=0.002). The measurement of TNF-α concentration under the influence of opioids revealed statistically significant effects of BE (F=6.07, p=0.039), naloxone (F=4.64, p=0.068), and naltrindole (F=6.55, p=0.034). Comparison of the mean values showed that combined addition of BE and OR antagonists into cultures decreased TNF-α level. BE and OR antagonists had no effect on IL-6 concentration.

Our results show that BE produce an immunomodulatory effect on cultured cells from healthy donors, which is related to stimulation of IL-1β production in the presence of antagonists and decrease in TNF-α level. Evaluation of the dose-response relationship showed that BE in a concentration of 10^{-7} M is most potent in modulating cytokine production (similarly to the effect of BE on lymphocyte proliferation) [1,2]. All effects of BE were most significant in unfractionated cultures, which suggests that realization of the effects of opioid peptides requires cooperation of cell populations.

Another important fact suggests activation of cells via the CD14/TLR4/MD2 receptor complex [7] expressed primarily on monocytes. Receptors for LPS are also expressed on granulocytes (*e.g.*, neutrophils) [4,11]. In our study these cells were present in the mixed cell fraction. Neutrophils play a role in the secretion of proinflammatory cytokines [5,6]. On the other hand, expression of mRNA for IL-1β, TNF-α, and IL-6 in the population of unfractionated cells was not observed after activation of neutrophils with LPS. Under these conditions, neutrophils contain only mRNA for antiinflammatory factors (IL-1Ra, IL-6Ra, TNF-SR1, TNF-SRII, *etc*.) [14]. Complete activation of neutrophils with LPS occurs only in the presence of monocytes. Removal of these cells decreases neutrophil survival and contributes to monocyte apoptosis [12].

BE does not modulate cytokine production in PHA-containing cultures, which is probably related to the fact that lymphocytes serve as a primary target for PHA. BE-binding sites, including μ-OR and δ-OR, are expressed on lymphocytes only upon

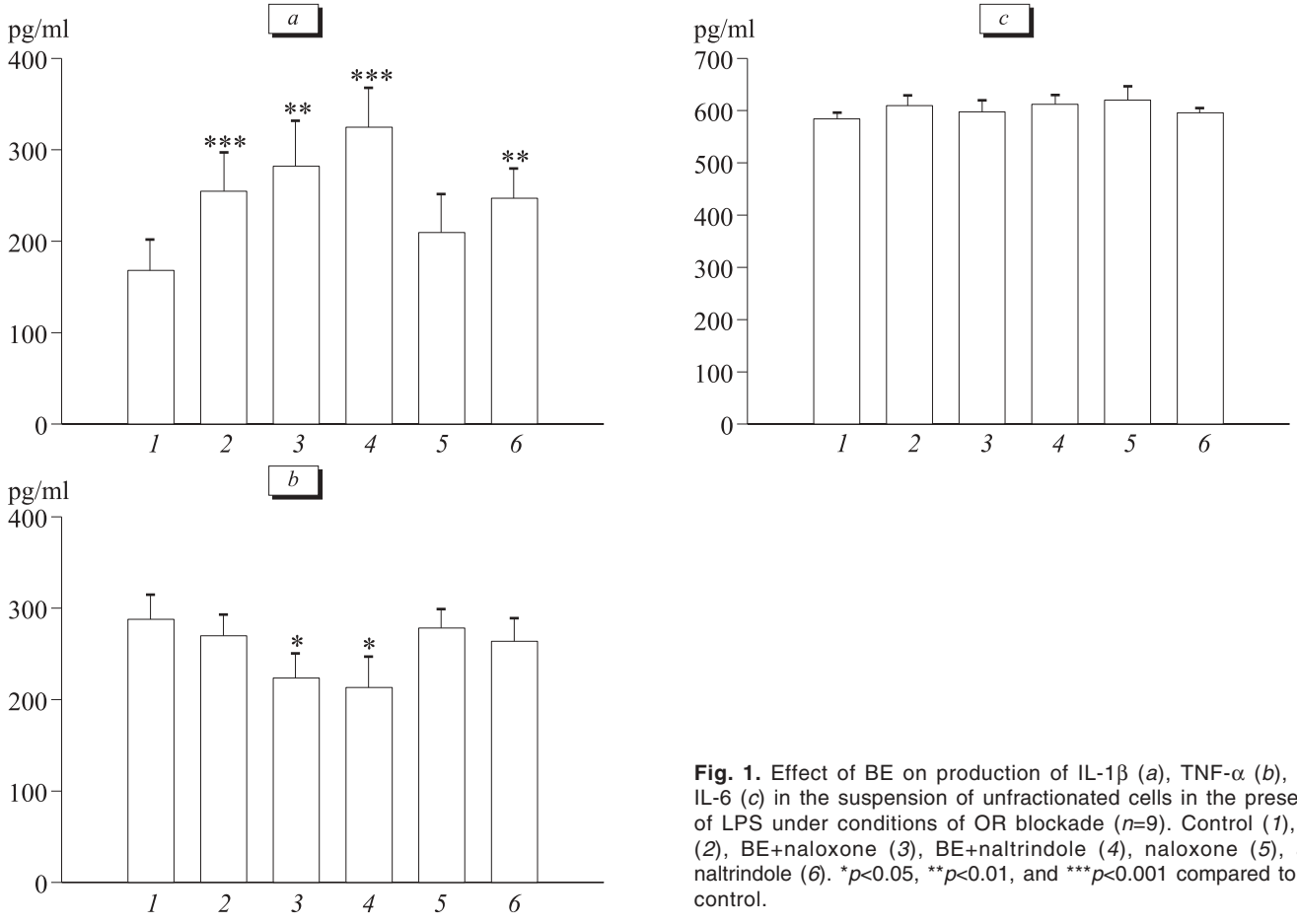

Fig. 1. Effect of BE on production of IL-1β (*a*), TNF-α (*b*), and IL-6 (*c*) in the suspension of unfractionated cells in the presence of LPS under conditions of OR blockade (n=9). Control (*1*), BE (*2*), BE+naloxone (*3*), BE+naltrindole (*4*), naloxone (*5*), and naltrindole (*6*). *p<0.05, **p<0.01, and ***p<0.001 compared to the control.

direct activation with PHA or concanavalin A at suboptimal doses [12]. Indirect activation of monocytes under these conditions is insufficient for the initiation of OR expression.

OR blockade does not abolish the effect of BE, which suggests the existence of additional non-opioid binding sites on immune cells. High-affinity naloxone-insensitive receptor for BE is expressed on T lymphocytes, macrophages, adrenals, and nerve cells [3]. The effect of combined treatment with agonists and antagonists can be also realized via the interaction between opioids and various domains of the peptide chain within one receptor [8]. Previous studies showed that the effect of BE and naloxone on lymphocyte proliferation is similar to the inhibition of TNF-α production [2]. Naloxone has an agonistic effect on IL-4 production [1,13]. Therefore, BE plays an important role in the regulation of function of monocytes and macrophages.

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