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REVIEW

Endogenous Opioid Peptides in Regulation of Innate Immunity Cell Functions

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Abstract—Endogenous opioid peptides comprise a group of bioregulatory factors involved in regulation of functional activity of various physiological systems of an organism. One of most important functions of endogenous opioids is their involvement in the interaction between cells of the nervous and immune systems. Summary data on the effects of opioid peptides on regulation of functions of innate immunity cells are presented.

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Opioid peptides comprise a large group of physiologically active bioregulatory factors exhibiting a broad spectrum of biological activity and interacting with opiate receptors (μ, δ, κ). Initially endogenous opioids were identified in the central nervous system, but later they were detected in many organs and tissues, including stimulated and not-stimulated cells of the immune system [1-3]. Opioid peptides regulate functions of the nervous system, processes of digestion, reproduction, lactation, stress-reaction, immunoregulation, etc. [4]; these peptides are also involved in development and pathogenesis of a number of neurological and autoimmune disorders. Now, in addition to three main well known families (endorphins, enkephalins, dynorphins), the opioid system also includes relatively recently characterized FQ nociceptin/orphanin (N/OFQ), endomorphins 1 and 2, as well as a number of peptides identified in some mammals and amphibians, including casomorphins, deltorphins, dermorphins, etc. (Table 1).

The most phylogenetically ancient unit of the immune system is innate immunity responsible for recognition, abolition, and elimination of alien agents. A number of cell populations of myeloid (granulocytes, monocytes-macrophages, dendritic cells) and lymphoid (NK, NKT, γδT cells) origin, as well as some humoral factors (cytokines, antibacterial peptides, complement system) are involved in innate immunity. Besides antimicrobial function, the innate immunity cells are active in regulation and induction of adaptive response to tumor and microbial antigens.

OPIOID SYSTEM AND STRESS

Secretion of opioid peptides in the organism is an impulse process and is observed in various psychoemotional states, physical loads, but is mainly observed upon the action of various stress factors. One of the main stress indicators is increase in β endorphin content in blood plasma

Abbreviations: AC, adenylate cyclase; ACTH, adrenocorticotropic hormone; AP1, protein activator-1; ASK1, apoptosis-regulating kinase; CaM, calmodulin; cAMP, cyclic adenosine monophosphate; CBP, CREB-binding protein; ConA, concanavalin A; CREB, (cAMP-response element-binding) transcription factor; CRF, corticotropin releasing factor; ERK, kinases regulated by extracellular signals; GPCR, G-protein-coupled receptors; IFN γ, γ interferon; JNK, c-Jun N-terminal protein kinases; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; γ-MSH, melanocyte stimulating hormone γ; NF-κB, nuclear κB factor; NK, natural killers; N/OFQ, nociceptin/orphanin FQ; ORL-1, (opioid receptor-like-1) nociceptin receptor; PKA, protein kinase A; PMA, phorbol myristate acetate; POMC, proopiomelanocortin; TAK1, TGF-β-regulated kinase; TGF β, transforming growth factor β; TNF-α, tumor necrosis factor α.

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Table 1. Structure of the main endogenous opioid peptides

Receptor	Opioid peptide/ precursor	Amino acid sequence of opioid peptide	Binding to immune system cells (K_d, nM)	Sources
μ (MOP, OP_3)	β-endorphin/ POMC	Tyr–Gly–Gly–Phe–Met–Thr–Ser–Glu–Lys–Ser–Gln–Thr–Pro–Leu–Val–Thr–Leu–Phe–Lys–Asn–Ala–Ile–Ile–Lys–Asn–Ala–Tyr–Lys–Lys–Gly–Glu	0.41* (Nph) 10 (human Lph)	[105, 106]
	endomorphin-1/ unknown	Tyr–Pro–Trp–Phe–NH_2	–	[107, 108]
	endomorphin-2/ unknown	Tyr–Pro–Phe–Phe–NH_2	–	
δ (DOP, OP_1)	[Met^5]enkephalin/ proenkephalin	Tyr–Gly–Gly–Phe–Met	10 (Nph) 0.59 (mouse Sc)	[105, 109]
	[Leu^5]enkephalin/ proenkephalin	Tyr–Gly–Gly–Phe–Leu	–	
κ (KOP, OP_2)	dynorphin A (1-17)/ prodynorphin	Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln	–	[110]
	dynorphin B (1-13)/ prodynorphin	Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Gln–Phe–Lys–Val–Val–Thr	–	
	dynorphin AB (1-32)/ prodynorphin	Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Ile–Arg–Pro–Lys–Leu–Lys–Trp–Asp–Asn–Gln–Lys–Arg–Tyr–Gly–Gly–Phe–Leu–Arg–Arg–Gln–Phe–Lys–Val–Val–Thr	–	
ORL-1(NOP_1)	N/OFQ/ pronociceptin	Phe–Gly–Gly–Phe–Thr–Gly–Ala–Arg–Lys–Ser–Ala–Arg–Lys–Leu–Ala–Asn–Gln	0.021 ± 0.005; 3.4 ± 1.58** (U937)	[111-113]

Note: Nph, neutrophils; Lph, lymphocytes; Sc, splenocytes; U937, leukemic monocytic lymphoma; hyphen means absence of data.

* μM.

** Two receptor populations are present on monocytes of cell line U937, with low and high K_d.

[5-7]. Enkephalins can also be involved in the reaction of the opioid system to stress [8], but unlike β endorphin, their role in stress-dependent rearrangement is much lower. The [Met^5]enkephalin concentration in blood plasma may increase in insulin and endotoxic shock and insignificantly change upon acute hemorrhagic and surgical shock [5]. A similar state of anxiety and alert at the end of working day is accompanied by enhanced ejection of adrenocorticotropic hormone (ACTH) and β endorphin but not of [Met^5]enkephalin. This also indicates that enkephalins cannot be considered as stress hormones [9]. Moreover, the enkephalin contents in hypothalamus can decrease in stress, while concentrations of dynorphin A and β endorphin, on the contrary, increase [10]. The dynorphin A content under conditions of intermediate and severe stress increases only in hypothalamus and hypophysis without visible changes in the blood plasma [7].

In the case of the general adaptation syndrome development, β endorphin and dynorphin A exhibit inhibitory effect on the hypothalamic–hypophyseal axis via inhibition of corticotropin-releasing factor (CRF) secretion in hypothalamus via a naloxone-dependent mechanism [11]. According to other data, intravenous injection of dynorphin A into 120-day-old sheep embryos stimulated ACTH secretion during 30-60 min via enhancement of CRF formation in the hypothalamus via a naloxone-independent pathway, which resulted in increased content of corticosteroids in the blood plasma [12]. Moreover, β endorphin and dynorphin A can exert direct effects on adrenal cortex, and the pattern of their effects depends on expression of one or another receptor type [13]. Thus, upon β endorphin binding to μ receptors aldosterone is released, and activation of μ and κ receptors results in activation of phospholipase C, while inter-

action with non-opioid receptor results in inhibition of adenylate cyclase activity and decrease in corticosterone secretion [13]. Experiments on transgenic animals showed an increase in ACTH and corticosterone contents in response to lipopolysaccharide in β endorphin-deficient mice [14]. In mice with knockout at β endorphin, dynorphin, and enkephalins, a higher ACTH content was observed 10 min after 5 min stress, and in this case basal corticosterone content was decreased only in enkephalin-deficient mice, while the corticosterone secretion peak in stress conditions decreased in all mice, but it was more pronounced in β-endorphin-knockout animals [15].

Thus, endogenous opioids are directly involved in development of total adaptation syndrome via regulation of stress-induced hormonal shift at both central and peripheral levels. Among all endogenous opioid peptides, β endorphin plays the role most important in stress.

OPIOID PEPTIDE PRODUCTION BY IMMUNE SYSTEM CELLS

The ability of immune system cells to produce opioid peptides was first demonstrated in 1981. Later opioids and their precursors were identified in cells of numerous vertebrate and invertebrate species. In 1997 proopiomelanocortin (POMC) mRNA was isolated from rat mononuclear leukocytes [16]. Now the POMC gene products (β endorphin, ACTH, β lipotropin, melanocyte stimulating hormone γ (γMSH)) have been detected in Langerhans cells, keratinocytes, placenta cells, thymocytes, dendritic cells, macrophages, and lymphocytes. Enhancement of POMC gene expression is observed upon cell activation by CRF, products of microbial cell decomposition and proinflammatory cytokines [17, 18]. It should be noted that the dynamics of opioid peptide secretion by immunocompetent cells directly depends on age factors. In a group of 45-60-year-old volunteers a decrease in intracellular β endorphin concentration was observed compared to a group of donors below 30-year-old. On the contrary, lymphocyte stimulation by mitogen significantly increased this peptide amount in supernatants of cultures of peripheral blood mononuclears of donors above 45 years. Besides, the number of β-endorphin secreting cells increases as age of donors increased [19]. It was proved using immunofluorescent confocal microscopy that opioid peptides are colocalized in primary (azurophilic) granules of neutrophilic granulocytes. Ligands of chemokine receptors CXCR1 and CXCR2 stimulate p38 MAP-dependent β endorphin and enkephalin translocation and secretion by polymorphonuclear leukocytes into inflammatory tissue to abolish the nociceptive effect caused by inflammation [20].

The source of enkephalins in an organism is proenkephalin A whose mRNA was detected in cells of human and rodent immune system, in particular, in $CD4^+$-T cells, $CD4^+$ thymocytes, macrophages, monocytes, and mast cells [21]. Enkephalin secretion by peripheral blood mononuclear cells is induced to a higher extent by Th2 cytokines, in particular, by interleukins (IL-4 and IL-10) that enhance formation of preproenkephalin mRNA and increase [Met^5]enkephalin concentration in the lymphocyte cytoplasm. Cytokine TGF-β exhibits a similar effect, while Th1 cytokines IL-2 and interferon γ (IFN γ) are devoid of such activity [22].

The dynorphin family peptides have been also found in leukocytes. The mRNA of dynorphin A and its precursor prodynorphin are also detected in abdominal leukocytes under conditions of zymosan-induced peritonitis. Nevertheless, the level of secretion of this peptide by cells of the immune system is extremely low compared to [Met^5]enkephalin [23-25]. The authors of work [26] showed the ability of dynorphin A to penetrate through the cell plasma membrane, passing by opiate receptors, which may be an additional, formerly undetected mechanism of signal transduction by opioid peptides into the cell.

In addition to three main types of opiate receptors (δ, μ, κ), in 1994 the nociceptin receptor (ORL-1) was described for the first time [27], and a year later its endogenous ligand was isolated simultaneously in two independent laboratories [28, 29] and subsequently got the joint name "nociceptin/orphanin FQ" (N/OFQ). Like different opioid peptides, N/OFQ is incorporated in the high molecular weight precursor preproN/OFQ cloned in 1995-1998 in different animal species [27]. The mRNA of ORL-1 receptor and N/OFQ has been identified in different populations of immune system cells including mouse lymphocytes [30], mononuclear cells of human peripheral blood [31], cell lines of human T and B lymphocytes and monocytes [32], as well as neutrophils circulating in peripheral blood [33]. In 2002 the expression of ORL-1 mRNA was demonstrated in $CD8^+$ and $CD4^+$ lymphocytes [34]. In the absence of activation stimuli in a culture of peripheral blood lymphocytes, mRNA of precursor N/OFQ was detected in $CD19^+$ B cells, while in the case of mitogenetic stimulation by phytohemagglutinin it was also detected in $CD3^+$ T cells. Like in the case of opioid peptides of different families, the N/OFQ secretion significantly increases in the presence of lipopolysaccharide (LPS), concanavalin A (ConA), IL-1β, tumor necrosis factor α (TNF-α), dexamethasone, and CRF. Nevertheless, the N/OFQ concentrations are comparable with that of dynorphin in rat lymphocyte cultures and is 30 times lower than the concentration of [Met^5]enkephalin [23].

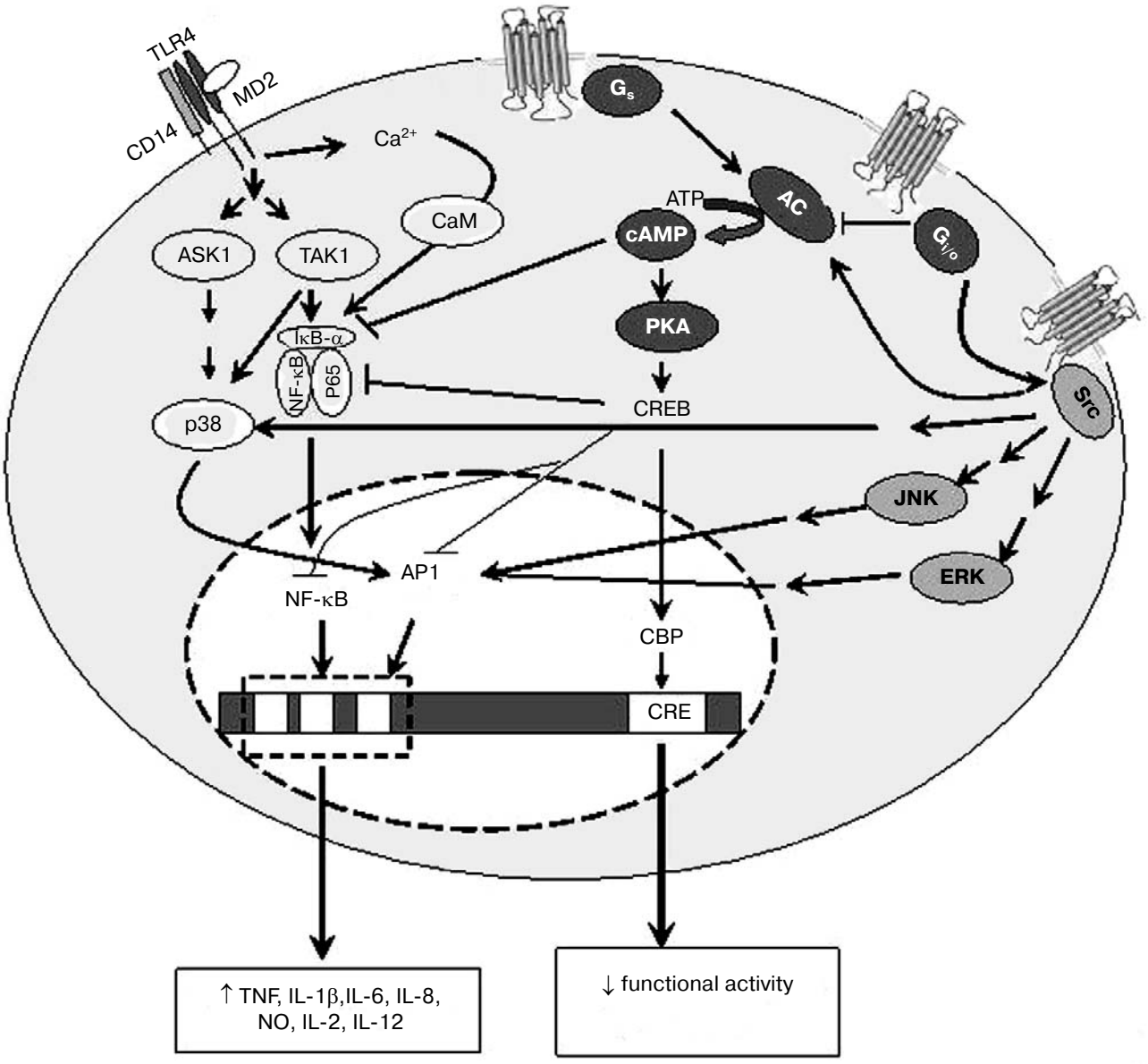
Endomorphins, highly selective endogenous ligands of μ-opiate receptors of peptide nature, were first described in 1997 [35]. In rats, endomorphin-1 and endomorphin-2 were detected in spleen and thymus, in the macrophage and B cell cytoplasms [36]. Under conditions of local inflammation induced by Freund's adjuvant, endomorphins were revealed in the cytoplasm of

monocytes/macrophages of the medullar zone of popliteal lymph nodes [37]. In humans only endomorphin-2 was initially identified in spleen cells. Later, data appeared concerning the presence of endomorphins in extracts of peripheral blood lymphocytes [36].

Thus, in inflammation focus, leukocytes actively produce opioid peptides that along with immunoregulatory activity exert analgetic effect [17, 38]. The ratio of different family peptides produced by the immune system cells can greatly vary and depends on the cell types, age, and nature of inducers.

MOLECULAR MECHANISMS OF SIGNAL TRANSDUCTION FROM OPIATE RECEPTORS

Opiate receptors belong to the family of G-protein-coupled receptors (GPCR). Signal transduction from



The main mechanisms of intracellular signal transduction from opiate receptors. cAMP is the key molecule in realization of effects from opiate receptors. Single-shot agonist binding to receptor activates heterodimeric Gi/o-proteins inhibiting cAMP formation due to decrease in AC activity. Simultaneously, Gi/o-proteins can be involved in PKC-dependent activation of Src kinases whose targets are MAPK (JNK, ERK, p38). Following transfer of MAPK into the nucleus, they phosphorylate nuclear factors AP-1 responsible for proinflammatory response. In the case of long-lasting effect, signal is transduced via Gs proteins or Src kinases with activation of the AC-cAMP-PKA-CREB cascade resulting in activation of CRE sequences in DNA. In cells activated by LPS or proinflammatory cytokines, the GPCR-induced increase in the cAMP and phosphorylated CREB contents hinders the ASK-1/CaM-induced activation of NF-κB or its (as well as AP-1) binding to DNA, which finally results in lowering of the functional activities of cells

opiate receptors is mainly mediated by heterotrimeric $G\alpha_{i/o}$ proteins [39, 40], but according to the latest data $G\alpha_s$ proteins can also be involved in signal transduction [40]. Activation of $G\alpha_s$ proteins launches classical signal cascade associated with activation of adenylate cyclase (AC-cAMP-PKA-CREB). Activation of $G\alpha_{i/o}$ proteins, on the contrary, blocks adenylate cyclase activity (Scheme). Single stimulation of opiate receptors expressed on nervous system cells results in decrease in adenylate cyclase activity. However, continuous introduction of agonists results in enhanced expression of the GNAS1 gene and subsequent increase in cAMP production [40]. cAMP production by immune system cells may increase or decrease depending on a number of accompanying conditions: species affiliation of the object of investigation or cell line, type of expressed opiate receptors, ligand concentration, duration effect, as well as the presence of additional stimuli [41].

Another important mediator in signal transduction from GPCR via $G\alpha_{i/o}$ and $G\alpha_s$ proteins includes nonreceptor tyrosine kinases of the Src family [40, 42, 43]. These kinases are directly or indirectly involved in activation of MAP cascade kinases: ERK1/2 [42-44], JNK, and p38 [42, 45, 46] that in turn phosphorylate and activate different nuclear factors (ATF2, c-fos, c-jun, etc.) involved in formation of AP1 by homo- or heterodimerization [47]. AP1 interacts with consensus sequence TGACTCA [20] appearing in promoters of many genes that modulate stress response, inflammation, and apoptosis [42, 45, 46]. Transcription factor CREB and AP1 are structural homologs and recognize the same DNA sequences. CREB is able to interact with AP1 sites but cannot activate transcription from these sites. Simultaneous presence of these factors in the nucleus inhibits AP1-induced transcription. The level of antagonism between these two factors is regulated by cAMP-dependent CREB phosphorylation.

It has been shown that δ-opiate agonists (deltorphin and DADLE) stimulate phosphorylation of kinases regulated by extracellular factors (ERK) in Jurkat cells [48]. In this case $G\alpha_{i/o}$ proteins and protein kinase C (PKC) play an important role in signal transduction. The MAP cascade activation induced by δ-agonists results in increased amount of c-fos mRNA [48]. The same authors showed that activation of δ-receptors expressed on mouse splenocytes resulted in activation of both MAP kinases associated with T cell receptor (ERK1/2) and with c-Jun of N-protein kinase (JNK) regulated, independently of T cell receptor activation, with subsequent phosphorylation of transcription factor ATF2 [49]. In the case of chronic effect of opiate agonists, Src kinases play the key role in increase in adenylate cyclase activity and accumulation of cAMP [50].

Additional stimulation of cells by LPS with subsequent ASK1 activation plays an important role in realization of effects from opiate receptors expressed on innate immunity cells. ASK-1 is activated in the presence of reactive oxygen species generated by intracellular sources (mitochondria, NAD(P)H oxidases) [51]. Thus, in the case of activation by lipopolysaccharide of splenocytes isolated from $ASK^{-/-}$ mice, kinase p38 was not activated and production of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α was disturbed [51].

Proinflammatory response is launched by transcription factor NF-κB phosphorylation by TGF-β-regulated kinase (TAK1) [51] or by a Ca^{2+}/CaM-dependent pathway [52]. NF-κB activation results in pronounced proinflammatory response with activation of IL-1β, IL-2, TNF-α, IL-6, IL-12, and NO production. The most widespread intracellular NF-κB form is the p50/p65 dimer. The cAMP/PKA signal cascade retards degradation of inhibitory protein IκBα and its disconnection from the NF-κB complex, thus limiting penetration of transcription factor into the cell nucleus. Also, increase in cAMP concentration results in formation of CREB competing with p65 subunit of NF-κB for limited amounts of transcription coactivator CBP [53].

Thus, cAMP is the main secondary messenger formed upon stimulation of opiate receptors; increase or enhancement of its production results, respectively, in inhibition or enhancement of proinflammatory activity of immune system cells via direct stimulation of transcription processes as well as by regulation of kinase cascades mediating production of proinflammatory factors.

EFFECT OF OPIOID PEPTIDES ON FUNCTIONS OF MONOCYTE-MACROPHAGE CELLS

In the current literature, immunomodulating activity of β endorphin is characterized most completely (Table 2). It has been shown that this peptide enhances phagocyte activity of monocytes and neutrophils [54] and modulates their bactericidal potential [55] and secretory activity [56]. The best coordinated literature data were obtained for IL-1β, whose production by mouse macrophages [57] and leukocytes of human peripheral blood [58, 59] is enhanced by β endorphin. In parallel, β endorphin activated synthesis of receptor antagonist IL-1β – IL-1ra [60]. Simultaneously, IL-4 production by $CD4^+$ cells was not mediated by IL-1β but was dependent on cyclooxygenase cycle products [61]. The direction of β endorphin effect on IL-6 production depended on the experimental conditions. Thus, in the presence of cortisol β endorphin inhibited IL-6 production by mouse splenocytes [62], but it stimulated IL-6 secretion by intact peritoneal mouse macrophages [57]. In IL-6-knockout mice increased basal level of β endorphin was observed compared to control animals [63], which is in general indicative of negative regulation of the opioid peptide levels by IL-6. Besides, secretion of two different proinflammatory factors (TNF-α and IL-8) was inhibited by β endorphin [59, 64, 65].

Table 2. Opioid peptide effects on functional activity of the monocyte-macrophage cells

Object of investigation	Peptide	Model	Effects	Source
Mouse splenocyte	endorphin β	*in vivo*	↓IL-6	[62]
Mouse peritoneal macrophages, mouse spleen macrophages	–"–	*in vitro*	↑IL-1, IL-6	[57]
Monocytes	–"–	–"–	↑/ – IL-1ra	[60]
Human peripheral blood leukocytes	–"–	–"–	↑IL-1β	[59]
Human chondrocytes	–"–	–"–	↑IL-1β, ↑TNF-α	[58]
Human peripheral blood leukocytes	–"–	–"–	↓TNF-α	[61]
Choriodecidual cells of placenta	–"–	–"–	↓IL-8	[65]
Human peripheral blood leukocytes	–"–	–"–	↓IL-8	[59]
Rat peritoneal macrophages	endorphin β, dynorphin	–"–	↑ROS	[83]
Mouse peritoneal macrophages	[Met^5]enkephalin	*in vivo*	↑NO	[71]
–"–	–"–	–"–	↑IL-1β, ↓PhA	[67]
–"–	–"–	*in vitro*	↑IL-6	[68]
Rat glia cells	[Met^5]enkephalin, [Leu^5]enkephalin	–"–	↑IL-1β	[66]
Mouse peritoneal macrophages	dynorphin	–"–	↑PhA	[72]
Mouse splenocyte	nociceptin/orphanin FQ	*in vivo*	↑TNF-α, IFN-γ	[76]
Rat peritoneal macrophages	–"–	–"–	↓IL-1, TNF-α	[75]
–"–	endomorphin-1, -2	*in vitro*	↓TNF-α, IL-10, 12, PhA, ROS ↑IL-1β, Mac1	[79, 80]
THP-1	–"–	–"–	↑IL-10, 12	[78]

Note: THP-1, cell line of human monocytic leukemia; ROS, reactive oxygen species; PhA, phagocytic activity; ↑, parameter stimulation; ↓, parameter inhibition; hyphen means no effect.

[Met^5]- and [Leu^5]enkephalins enhanced spontaneous and LPS-induced IL-1β production by glia cells via the naloxone-independent pathway [66] and cancelled the LPS-induced synthesis of TNF-α [67]. It was also shown that [Met^5]enkephalin stimulated independently of naloxone spontaneous and IL-1β/IFN-γ-induced IL-6 production by mouse peritoneal macrophages [68], enhanced IL-6 mRNA formation in mouse peripheral blood cells, and increased cytokine content in animal and human blood plasma [69]. Inhibition of IL-6 production by monocytes was shown under blockade of intracellular enkephalin production. Addition of synthetic [Met^5]enkephalin or δ-selective deltorphin agonist to preliminarily treated cells cancelled the inhibitory effect of blocking [70]. [Met^5]Enkephalin inhibited spontaneous and enhanced the LPS-induced

phagocyte activity of mouse peritoneal macrophages [67] and *in vivo*, but not *in vitro*, it stimulated NO formation by mouse peritoneal macrophages [71].

Peptides of the dynorphin family also significantly enhanced phagocytosis [72], IL-1 production, and superoxide secretion by macrophages [73], modulated oxidative burst, and enhanced tumoricidal activity of macrophages [74]. In this case the effect of dynorphin A on cell phagocytic activity was more pronounced compared to that of enkephalins, dynorphin B, and separate fragments of the dynorphin A molecule (1-13, 9-17, 13-17) [72].

Data from the literature concerning the effect of N/OFQ on functional and secretory activity of innate immunity effectors are extremely rare and ambiguous. On one side, intracerebroventricular introduction of this peptide to rats in traumatic shock conditions blocked IL-1β and TNF-α secretion by peritoneal macrophages [75]. On the other side, it was noted that the injection of N/OFQ into mice before introduction of staphylococcal enterotoxin A, on the contrary, increased formation of TNF-α and IFN-γ mRNA in the spleen as well as increased the TNF-α concentration in the blood plasma [76].

Endomorphins 1 and 2 decreased spontaneous production of both TNF-α and IL-1β by rat peritoneal macrophages during 12 and 24 h cultivation in the range of concentrations 10^{-6}-10^{-9} M. In this case stronger effect was registered for endomorphin-1 [77]. Both endomorphins inhibited the LPS-induced IL-10 and IL-12 production in culture of human monocytic leukemia cells THP-1 [78] and by LPS-stimulated rat peritoneal macrophages [79]. The ability of endomorphin to modulate phagocytic activity of native immunity effectors was shown in a number of works. Both peptides enhanced adhesion of rat peritoneal macrophages to fibronectin and increased density of Mac-1 (receptor for CR3 complement) molecule expression on their surface, and inhibited chemotaxis of macrophages and spontaneous production of reactive oxygen species, in particular, of superoxide-anions [79, 80]. However, despite enhancement of Mac-1 expression in response to endomorphin 2, inhibition of phagocytosis of *E. coli*, opsonized by complement, was observed [79]. Similar effects of endomorphin-2 on phagocytic activity and chemotaxis were registered during growth of THP-1 cell line in culture [78].

OPIOID PEPTIDE EFFECTS ON NEUTROPHILIC GRANULOCYTE FUNCTIONS

Opioid peptides modulate absorption, secretory, and bactericidal activities of granulocytes as well as CD-marker expression on their surface and apoptosis processes (Table 3). Thus, β-endorphin and [Met^5]enkephalin in low concentrations enhanced expression of CD11, CD18 [55, 81], CD16, and CD35 [81] molecules on the neutrophil surface via stimulation of δ-opiate receptors [81].

Table 3. Opioid peptide effects on functional activity of neutrophilic granulocytes

Object of investigation	Peptide	Model	Effects	Source
Human neutrophils	[Met^5]enkephalin	*in vitro*	↑/↓ ROS, ↑CD11, CD18	[55, 81]
–"–	[Met^5]enkephalin, β-endorphin	–"–	↑CD16, CD35, ROS	[81]
Mouse plasma	[Met^5]enkephalin	*in vivo*	↑mRNA IL-6, IL-6	[69]
Human neutrophils	β-endorphin	*in vitro*	↓IL-8	[64]
Human polymorphonuclear leukocytes	β-endorphin, dynorphin	–"–	↑ROS	[83]
–"–	β-endorphin, [Leu^5]enkephalin, dynorphin	–"–	↑ROS	[84]
Human neutrophils	dynorphin	–"–	↑TxB2, LtB4, ROS	[85]
Human polymorphonuclear leukocytes	nociceptin/orphanin FQ	–"–	↑Cht	[87]
Human neutrophils	–"–	–"–	↑lysozyme	[88]
–"–	endomorphin-1, -2	–"–	↓/↑ ROS	[89]
Rat neutrophils	–"–	–"–	↓ROS, ↑Cht, – PhA	[89, 90]

Note: Lt, leukotriene; Tx, thromboxane, Cht, chemotaxis; other designations are the same as in Table 2.

Sharp et al. [82] showed the absence of effects of β-endorphin, [Met^5]enkephalin, and dynorphin on superoxide-anion generation by neutrophils, but data obtained in some other works unambiguously point to the ability of all the above-mentioned compounds in low concentrations to enhance, in naloxone-dependent manner, the generation of reactive oxygen species by polymorphonuclear leukocytes [81, 83, 84] and to stimulate metabolism of arachidonic acid in neutrophils (dynorphin A) [85]. However, O_2^- and NO production by neutrophils, on the contrary, may decrease upon β-endorphin and [Met^5]enkephalin introduction into cultures in higher concentrations (10^{-6}-10^{-8} M) [55]. Treatment of granulocytes with [Met^5]enkephalin and β-endorphin resulted in significant increase in the number of apoptotic granulocytes [86].

N/OFQ significantly stimulated chemotaxis of polymorphonuclear leukocytes of human peripheral blood *in vitro* and enhanced infiltration by mouse leukocytes of inflammation focus caused by subcutaneous introduction into the animal's back of sterile air pouches [87]. Azuma et al. [88] showed that N/OFQ stimulated lysozyme secretion by neutrophilic granulocytes but was devoid of attractive activity towards neutrophils.

Depending on accompanying conditions, endorphins exerted differently directed effects on activity of neutrophil granulocytes. Thus, in response to endomorphin-1 and endomorphin-2 superoxide anion production decreased in neutrophils of rats, stimulated by phorbol myristate acetate (PMA), while in non-stimulated cultures the reverse situation was observed and in this case the leading role of μ-opiate receptor in this process was confirmed [89]. Endomorphins stimulated chemotaxis of polymorphonuclear leukocytes, but the neutrophil phagocytic activity did not change in this case [90].

OPIOID PEPTIDE EFFECTS ON NK CELL FUNCTIONS

β-Endorphin enhanced NK-mediated cytotoxicity *in vitro* by activation of IFN-γ, perforin, and granzyme B production (Table 4) [91]. In rotation stress conditions, the enhancement of β endorphin secretion correlated with enhanced activity of NK cells [6]. Most pronouncedly β endorphin stimulated cytolytic activity of NK cells upon intracerebroventricular introduction. However, κ agonist dynorphin A had no effect on the activity of this cell population [92, 93].

Literature data on the effects of enkephalins on NK cell activity also show the mainly stimulating trend in their actions [94]. It was shown that [Met^5]enkephalin enhanced mouse NK-cell activity *in vivo* [95] and *in vitro*, and in this case the most pronounced effect was registered upon peptide introduction at the dose of 1 μg/kg directly into a region of the central nervous system (into the cisterna cerebello-medularis) via a naloxone-dependent mechanism. Intraperitoneal and intravenous introduction of [Met^5]enkephalin did not result in such effect [96]. According to other data, [Met^5]enkephalin inhibited

Table 4. Opioid peptide effects on functional activity of NK and dendritic cells

Object of investigation	Peptide	Model	Effects	Source
Rat natural killers	β-endorphin	*in vitro*	↑IFN-γ, granzyme, perforin B	[91]
Mouse natural killers	–"–	*in vivo*	↑CTA	[92]
Rat natural killers	–"–	–"–	↑CTA	[93]
–"–	[Met^5]enkephalin, [Leu^5]enkephalin, dynorphin	–"–	– CTA	[93]
Mouse natural killers	[Met^5]enkephalin	–"–	↓/–CTA	[97]
–"–	–"–	–"–	– CTA	[92, 98]
Rat natural killers	dynorphin	–"–	– CTA	[93]
Mouse splenocytes	[Leu^5]enkephalin	–"–	↓/↑CTA	[99]
Rat natural killers	endomorphin-1, -2	–"–	– CTA, IFN-γ	[101]
Mouse Langerhans cells	β-endorphin	*in vitro*	↑IL-1β, ↑IL-10, ↓TNF-α	[106]
Mouse dendritic cells	dynorphin	–"–	↓T cell proliferation	[114]

Note: CTA, cytotoxic activity; other designations are the same as in Table 2.

activity of spleen NK cells in intact mice and had no effect upon immunization of the animals with sheep erythrocytes [97]. In athymic mice long introduction of [Met^5]enkephalin had no significant effect on the activity of NK cells [98]. Intraperitoneal introduction of [Leu^5]enkephalin at the dose of 7.5-10.0 μg/kg changed the NK cell activity in two directions by inhibiting it at the initial step via a naloxone-dependent mechanism and further stimulation in a naloxone-independent manner [99]. The peptide also enhanced NK activity in seropositive HIV patients with retained cytolytic activity of NK cells and had no effect on this index in patients with low cytolytic activity as well as in seronegative patients [100]. Some authors believe that *in vitro* opioid peptides can inhibit NK cell functions in people having in the norm high levels of this cell population activity and stimulate these functions in people with initially low NK cell activity [100, 101].

No significant influence of endomorphin on rat spleen natural killer activity and IFN γ secretion was found [102].

REGULATION OF DENDRITIC CELL FUNCTION BY OPIOID PEPTIDES

In human and mouse dendritic cells, mRNA of μ-, δ-, and κ-opiate receptors have been identified, the expression of which is enhanced during maturation, especially in the presence of proinflammatory factors such as TNF-α [103]. Simultaneously, the ability of dendritic cells to produce opioid peptides that, in turn, modulate their functional activity was shown. Dynorphin A inhibited T cell proliferation mediated by dendritic cells and had no effect on the antigen-presenting function and maturation processes [104]. Selective agonists of δ- and μ-receptors modulated in dose-dependent manner the T cell proliferation induced by dendritic cells [103], while δ-receptor stimulation mediated chemotaxis of dendritic cells [104].

Thus, opioid peptides are important factors in regulation of natural resistance. Opioid peptides are secreted by cells of very different organs and tissues, including immune system cells, and depending on the opiate receptor concentration and expression extent they modulate the innate immunity system and subsequent development of immune reactions.

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