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REVIEW

Nonopioid Effect of β-Endorphin

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Abstract—This review presents the generalized literature data and the results of our own research of the nonopioid effect of β-endorphin, an opioid neuropeptide interacting not only with opioid but also with nonopioid (insensitive to the opioid antagonist naloxone) receptors. The roles of the hormone and its receptors in regulation of the immune, nervous, and endocrine systems are discussed. The effect of neuromediator on the immune system mediated by both opioid and nonopioid receptors is considered in detail. The data on distribution and function of the nonopioid β-endorphin receptor in human and animal organisms are presented. All available data on the characteristics of the nonopioid β-endorphin receptor obtained by means of radioligand analysis are given. The discussed information is supposed to extend our conceptions of the role of β-endorphin in mammals and to be of extensive use in medicine and pharmacology.

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β-Endorphin is a neuropeptide consisting of 31 amino acids and formed in the hypophysis as a result of cleavage of proopiomelanocortin (POMC). The peptide interacts with two types of opioid receptors: μ and δ [1]. The ability of β-endorphin to bind to several types of receptors is due to its structural peculiarities. It is considered that the molecule of this hormone contains two different sites: the *N*- and *C*-terminal fragments needed for the binding to opioid receptors (μ- and δ-, respectively) [2]. Opioid receptors have been found in the brain and spinal cord [3], on cells of the immune system, and on adrenal glands, enabling it to perform its hormonal functions. It was shown that the anesthetic effect of β-endorphin, regulation of respiration, control of the cardiovascular system, and eating behavior were mediated through the δ- and μ-opioid receptors [2]. The δ-receptors play an important role in peptide regulated motion activity, sense of smell, cognitive functions, and emotional behavior [2, 4], while μ-opioid receptors are important for controlling thermoregulation, learning, and memory by β-endorphin [2, 5].

Previously it was shown that cells of the immune system not only contain opioid receptors but also express the β-endorphin precursor (*POMC*) gene and secrete active β-endorphin. The mRNA of the precursor and the peptide itself were found in T- and B-lymphocytes, monocytes, and macrophages [6]. Besides, immunohistochemical analysis shows that macrophages, monocytes, granulocytes, and lymphocytes contain a complete enzyme complex necessary for β-endorphin synthesis and secretion [7]. In this case, the role of β-endorphin consists in regulation of cell activity of the immune system and anesthetization in an inflammatory focus [6, 8]. The effect of this opioid neuropeptide on cells of the immune system is mediated by the μ- and δ-opioid receptors found on these cells [9-12]. It should be noted that all the above effects are blocked by specific opioid antagonist naloxone.

However, some physiological activities of the hormone are not blocked by opioid antagonists and, consequently, cannot be mediated by interaction with opioid receptors. Up to now, nonopioid receptors are little studied and the relevant data are fragmentary. The goal of this review is to generalize the results of available studies of the nonopioid effect of β-endorphin and its nonopioid receptor.

EFFECT OF β-ENDORPHIN ON THE IMMUNE SYSTEM

The effect of β-endorphin as a hormone has been studied most completely in cells of the immune system: T-lymphocytes, monocytes, macrophages, and B-lym-

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phocytes. Three types of opioid receptors are expressed on the surface of immunocytes: μ, δ, and κ [9-13]. β-Endorphin has a dual effect on immune cells: the suppressing effect of β-endorphin on phagocytosis of macrophages mediated through opioid receptors is described [14]; in accordance with other investigations, the hormone has an inhibitory effect on proliferation of the donor T-lymphocytes [15]. Besides, β-endorphin was shown to decrease proliferation of T-lymphocytes of peripheral human blood *in vitro* activated by hemagglutinin [16]. This effect is typical of many endogenous opioids (endorphins, enkephalins) and morphine, the alkaloid opioid having an inhibitory effect on cells of the immune system [17]. This review [17] presents solid evidence of the fact that morphine inhibits the functions of natural killers, B-cells, T-cells, and phagocyting cells when introduced *in vivo*. The direct suppressing effect of the narcotic has been shown *in vitro* in phagocyting cells. The effect of morphine disappears in the presence of opioid blockers. This means that the inhibitory effect is realized through classical opioid receptors. The action of opioids may be direct (immediately on immunocytes) or indirect (via neuronal signals or other neuromediators). The results of the above works suggest that opioids, including β-endorphin, have an inhibitory effect on immunocytes by interacting with opioid receptors.

This suggestion is favored by the work of Refojo [18], where β-endorphin knockout mice were obtained. These mice were tested for the level of cytokines in plasma and the activity of cells of the immune system. The knockout mice were shown to have enhanced splenocyte proliferation, production of cytokines IL-2, IL-6, and TNF-α by macrophages, and the IL-6 level in plasma after the treatment with lipopolysaccharide. All tests showed the increase in immune response. These data may be indisputable evidence of the inhibitory effect of endogenous β-endorphin on the immune system at all levels.

Inhibitory effect of the hormone is confirmed by the modern data obtained in the study of the influence of the agonists of opioid receptors (μ, δ, κ) on regulation of the expression of chemokines, cytokines, and their receptors, the central component of immunomodulating activity of opioids [19]. It has been shown that β-endorphin inhibits the transcription of IL-2 and the transcription factors transactivating IL-2 in activated human T-lymphocytes. Incubation of T-lymphocytes with opioids reduced the level of cAMP in the cells. Thus, β-endorphin had an inhibitory effect on physiological regulation of the activation of T-cells [20].

However, quite a number of studies are devoted to the stimulating effect of β-endorphin on T-lymphocytes [21-23] and on macrophages and monocytes [24, 25]. This problem has to be clarified.

The existence in an organism of nonopioid receptors (i.e. insensitive to the opioid blocker naloxone) is known. The term "nonopioid" receptor was first introduced by the American scientist Hazum in 1979 [26]. It was shown that specific binding of ^{125}I-labeled β-[*D*-Ala^2]endorphin with transformed human lymphocytes was inhibited neither by naloxone (the antagonist of opioid receptors) nor by morphine, enkephalins, α-endorphin, β-lipotropin, α-melanocyte-stimulating hormone, ACTH, insulin, and glucagon [26]. However, the binding was completely inhibited by β-endorphin and β-[*D*-Ala^2]endorphin. The dissociation constant (K_d), the major characteristic describing the interaction between the ligand and the receptor, was 3 nM.

This discovery suggested the presence on human lymphocytes of unknown specific β-endorphin binding sites of nonopioid nature. It was shown that the *C*-terminal region of the β-endorphin molecule was necessary for the binding with this receptor, because no binding of α-endorphin with this receptor was revealed. Thus, the receptor studies proved the existence of nonopioid β-endorphin receptors on human T-lymphocytes, making it possible to explain the immunomodulating effect of the hormone.

The studies of Heijnen et al. imparted clearness to investigation of the problem of β-endorphin action on T-lymphocytes [15]. It was ascertained that β-endorphin has a modulating effect on T-lymphocytes. The study of the influence of the hormone on concanavalin A (Con A) induced T-lymphocytes of two donors showed that the effect of this peptide was exactly the opposite: the hormone increased the proliferation of T-lymphocytes of one donor and inhibited the proliferation of lymphocytes of the other donor in the same range of concentrations (10^{-14}-10^{-9} M). The influence of different fragments of β-endorphin on the proliferation of Con A activated T-lymphocytes of these donors was investigated. According to the data of Heijnen et al. [15], β-endorphin fragments 10-16 and 2-31 had the same activity as the intact molecule. The findings suggest that the modulating effect of β-endorphin on human T-lymphocytes is associated with the level of expression of the nonopioid receptor on cell surface.

Further studies in this field confirmed the presence of nonopioid β-endorphin receptors on T-lymphocytes. Gilman was the first to establish the influence of β-endorphin on the functional activity of immune cells [27]. In the presence of β-endorphin, the production of mitogens by human T-lymphocytes increased. The hormone also contributed to the proliferation of T-cells *in vitro*. Later it was shown that the stimulating effect of β-endorphin on T-lymphocytes was associated with its ability to enhance IL-2 production by these cells, and the subsequent interaction between cytokine and receptors resulted in cell division. This effect was not inhibited by naloxone; consequently, it was mediated by the naloxone-insensitive receptor [28].

The research of van der Bergh et al. [21] plays a special role in investigation of the effect of β-endorphin on

T-lymphocytes. This team of researchers studied the effect of five opioid peptides (α-, β-, γ-endorphins, [Met^5]- and [Leu^5]enkephalins) on Con A-induced proliferation of rat spleen T-cells. It was shown that continuous presence of any of these peptides in the cultivation medium had no effect on the proliferative response of splenocytes. At the same time, 30-min preincubation of T-cells with β-endorphin (but not with other peptides) resulted in dose-dependent increase in the level of proliferation by 50-100%. The presence of naloxone did not influence the stimulating effect of β-endorphin. Hence it follows that the effect of β-endorphin on proliferation of T-cells is mediated not by opioid receptors. Simultaneously it was shown that the continuous presence of β-endorphin (or α-endorphin) in the culture of T-cells preincubated with β-endorphin completely eliminates the stimulating effect of β-endorphin. The authors of this work suggested that in the absence of opioid peptides on the surface of rat spleen T-lymphocytes only nonopioid receptors of β-endorphin were accessible for binding. Introduction of β-endorphin into the medium resulted in enhancement of the proliferative response mediated by these receptors. It is supposed that opioid receptors are expressed on the surface of T-cells under conditions of continuous presence of β-endorphin (or another opioid peptide) in their cultivation medium; the binding of β-endorphin to these receptors inhibits its own stimulating effect, which it induces through the nonopioid receptor [21].

It has been mentioned above that the binding to the nonopioid receptor needs the *C*-terminal region of the β-endorphin molecule (sequence 6-23 [15, 27]). The influence of synthetic β-endorphin fragments 6-31, 18-31, 24-31, 28-31, and 1-27 on proliferation of T-lymphocytes (the peptides were introduced into the cultivation medium before the stimulation of T-lymphocytes with a mitogen) was studied to prove the hypothesis that the β-endorphin molecule contains two different regions for binding to opioid and nonopioid receptors [22]. These studies showed that β-endorphin fragments 6-31 and 18-31 increased the proliferation of T-cells and that the former fragment was much more active than the latter. At the same time, β-endorphin fragments 1-27, 24-31, and 28-31 proved to be inactive. Based on these results, the authors supposed the importance of the region of β-endorphin molecule (6-23) for realization of β-endorphin effect on T-lymphocytes and, hence, for interaction with naloxone-insensitive receptors. The β-endorphin fragment 18-23 was supposed to play the key role in binding. Simultaneously, the same research team showed that β-endorphin increased the production of cytokines IL-2 and IL-4 in CD4$^+$ T-cells [29]. Based on these data, the effect of the hormone was supposed to be realized through the nonopioid pathway, because β-endorphin did not influence the level of cAMP, which is typical of the cascade of classical opioid receptor [29].

Summing up the above, it may be concluded that (1) there are two types of β-endorphin receptors on the surface of T-lymphocytes and (2) the hormone has an inhibitory effect on proliferation of lymphocytes when interacting with opioid receptors and stimulates the T-cell element of immunity when binding to nonopioid receptors.

The nonopioid β-endorphin receptor was studied in detail by Schweigerer et al. [30]. In 1985, they discovered nonopioid β-endorphin receptors on the cells of several mouse thymoma cell lines. It was shown for the cell line EL4 that the binding of ^{125}I-labeled β-endorphin to the nonopioid receptor was inhibited by unlabeled β-endorphin, depended on temperature (later on, all experiments were carried out at 4°C) and pH, and was characterized by saturability and reversibility. The dissociation constant was 2.2 nM. The binding of ^{125}I-labeled β-endorphin was not inhibited by [Leu^5]- and [Met^5]enkephalins and by *N*-terminal β-endorphin fragments: β-endorphin (1-16) (α-endorphin) and β-endorphin (1-27) [30]. These studies once again substantiate the assumption that the *C*-terminal region of the β-endorphin molecule is responsible for the binding to the nonopioid receptor.

It has been established that the binding of ^{125}I-labeled β-endorphin to the nonopioid receptor on the surface of EL4 cells at 37°C was followed by internalization of a ligand–receptor complex into the cell by means of endocytosis [30]. Most of the peptide hormones and growth factors enter target cells exactly through this pathway, but their further fate inside the cell is unknown [31, 32]. Schweigerer et al., based on King's results [33] on internalization of epidermal growth factor, suggested that β-endorphin could modulate cell functions such as proliferation of T-lymphocytes by interacting with specific intracellular binding sites. Soon it was shown that the hormone was able to bind to the intracellular protein calmodulin and to influence the activity of phosphodiesterase, and the binding was observed in the case of both *N-Ac*-β-endorphin and β-endorphin fragment 14-31 [34, 35]. However, the arguments for assertion of intracellular binding sites of the hormone are still insufficient.

Investigations with synthetic β-endorphin-like peptides have a special place in the study of nonopioid β-endorphin receptor [36]. In the 1980s, the American scientist Julliard found a SLTCLVKGFYPSDI peptide in human placenta extract: fragment 364-379 of the IgG heavy chain homologous to the central part of β-endorphin molecule SQTPLVTLFKNAII by 60% (Fig. 1) [37]. This peptide was synthesized and used in receptor studies. The experiments showed that the peptide became bound to the β-endorphin receptors on rat brain membranes [38]. Later on it was established that these receptors were nonopioid. We have synthesized a peptide corresponding to fragment 364-373 of the IgG heavy chain and similar to β-endorphin sequence 10-19 (the authors named the peptide as immunorphin) and its fragments, pentarphin and a cyclic analog cyclopentarphin (Fig. 1). These pep-

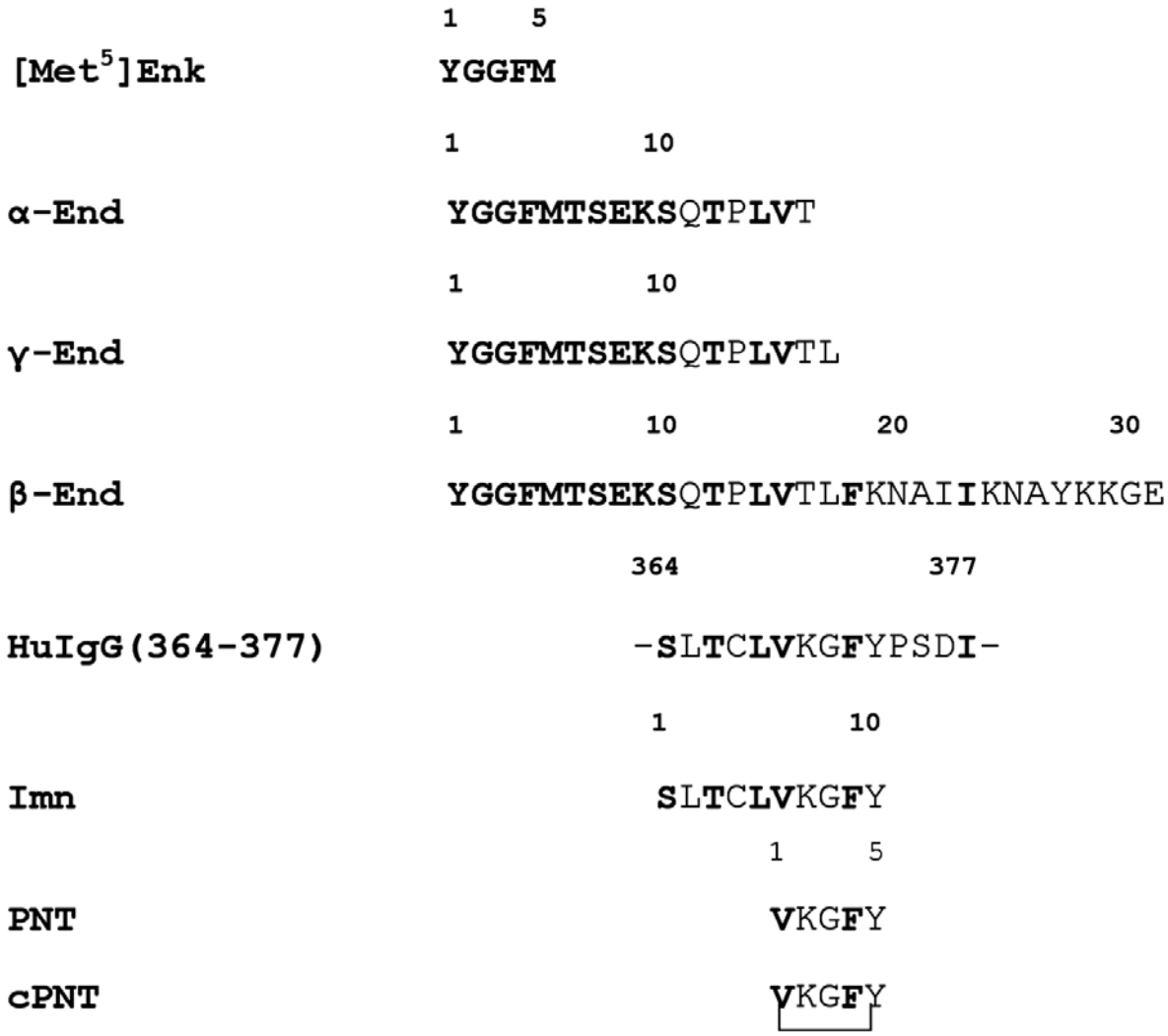

Fig. 1. Amino acid sequences of [Met^5]enkephalin, α-, γ-, β-endorphins, fragment 364-377 of the IgG heavy chain, immunorphin, pentarphin, and cyclopentarphin. The matched amino acid residues are in bold [108].

tides were shown to be selective antagonists of nonopioid β-endorphin receptor [39].

The studies with the synthetic peptides immunorphin, pentarphin, and cyclopentarphin confirm that the stimulating effect of β-endorphin on cells of the immune system is realized through the nonopioid receptor. It has been shown that β-endorphin and immunorphin in the range of concentrations 10^{-11}-10^{-9} M stimulate the Con A-induced proliferation of T-lymphocytes isolated from human peripheral blood [40-43]. The effect of β-endorphin and immunorphin on the proliferation of T-lymphocytes was not inhibited by naloxone. Thus, the effect could be mediated by the naloxone-insensitive receptor. It was also shown that β-endorphin and immunorphin stimulated the division of cells of Jurkat and MT-4 human T-lymphoblast lines [44, 45].

Radioligand analysis on human T-lymphocytes showed the presence of a single common receptor for immunorphin and β-endorphin. It was established that naloxone did not influence the binding of immunorphin and the hormone to this receptor. The dissociation constant of the β-endorphin–receptor complex is in the region of nanomolar concentrations (K_d = 0.25 nM) [41, 42], being in agreement with the results of other authors [26, 30]. Analogous experiments with immunorphin on human T-lymphocytes have shown that K_d = 7.0 nM [43].

Thus, it is demonstrated that the nonopioid receptor binds, besides β-endorphin, to synthetic β-endorphin-like peptides. The kinetic characteristics obtained by radioligand analysis for both β-endorphin and immunorphin are in the range of nanomolar concentrations, which is evidence of the high affinity of ligands to the receptor. Immunorphin can be considered as a selective agonist of the naloxone-insensitive β-endorphin receptor: in contrast to β-endorphin, it binds only to a receptor of such type, having the same effect on target cells as β-endorphin.

According to the published data, β-endorphin stimulates not only the T-cell element of immunity but also macrophages and monocytes. This peptide has chemoattractant properties and increases the phagocytosis of latex and *Candida albicans* by macrophages [24, 46, 47]. It has been shown that naloxone does not influence the β-endorphin-stimulated absorption of latex particles. Consequently, this process is realized through a naloxone-insensitive receptor [46].

Naloxone-insensitive receptors binding ^{125}I-labeled β-endorphin with high affinity have been found on mouse peritoneal macrophages and characterized [39, 48, 49]. The binding characteristics obtained in these experiments (dissociation constants) were in the range of nanomolar concentrations: K_d was found to be 8.2 nM [49]; according to other studies, K_d = 9.75 nM [48] or 6.1 nM [39]. It was noted that competitors for the binding with ^{125}I-labeled β-endorphin on macrophages were its *N*-acetylated derivative, β-endorphin, and its fragments 6-31, (1-5) and (16-31), while β-endorphin (1-27), β-endorphin (28-31), the agonists of δ- and κ-receptors, and naloxone were inactive [48, 49]. It was shown that immunorphin actively displaced ^{125}I-labeled β-endorphin from the receptor complex on mouse macrophages (inhibition constant K_i = 7.2 nM). The minimal fragment competing with the ^{125}I-labeled β-endorphin for binding with the cells was immunorphin (7-10) (K_i = 5.6 nM) [39]. The characteristics of the nonopioid β-endorphin receptor obtained on macrophages coincide with those described previously on T-lymphocytes.

The effects of β-endorphin and immunorphin on the functional activity of macrophages and reception of [^{3}H]immunorphin and ^{125}I-labeled pentarphin with mouse peritoneal macrophages were studied at our laboratory. β-Endorphin and immunorphin were shown to stimulate all stages of phagocytosis in macrophages: migration, adhesion, spreading, absorption, and digestion of bacterial agents [50, 51]. Besides, the study of phagocytosis of bacteria of the virulent strain *Salmonella typhimurium* 415 by mouse peritoneal macrophages *in vitro* showed 100-fold more effective action of cyclopentarphin compared to the patented peptide tuftsin (Fig. 2) [50, 51]. The binding of labeled ligands ([^{3}H]immunorphin and ^{125}I-labeled pentarphin) to mouse peritoneal macrophages was characterized by saturability and high affinity: K_d was 3.6 nM for the ^{125}I-labeled pentarphin and 2.4 nM for [^{3}H]immunorphin. These values correspond to the data obtained by other researchers [39, 48, 49] for macrophages with application of ^{125}I-labeled β-endorphin. The Scatchard graphs describing the interaction between the labeled ligands and the receptor are shaped as a straight line in both cases, indicating the presence of a single type of receptors on the cell surface (Fig. 3). These results confirm that the stimulating effect of β-endorphin and immunorphin on macrophages is realized via nonopioid β-endorphin receptors. Particularly, it should be

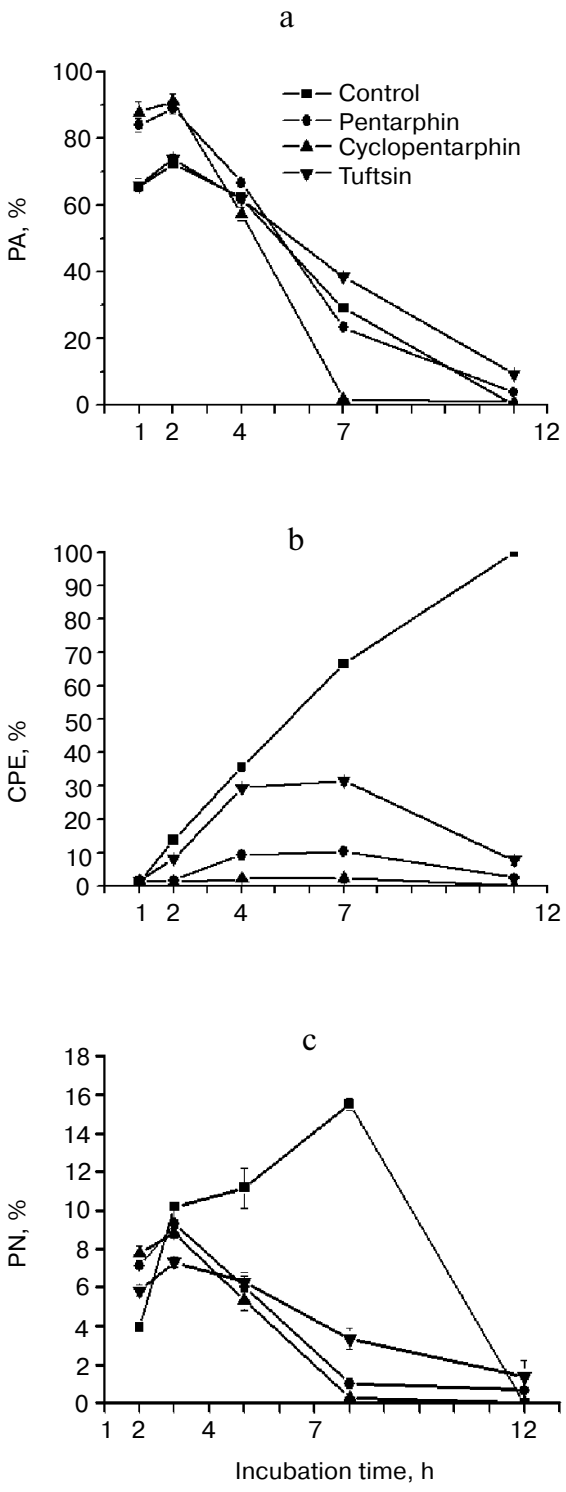

Fig. 2. Effects of pentarphin, cyclopentarphin (1 nM), and tuftsin (100 nM) on phagocytosis of bacteria of the virulent strain *Salmonella typhimurium* 415 by mouse peritoneal macrophages *in vitro*. a) PA (phagocytic activity), percentage of macrophages participating in phagocytosis; b) CPE (cytopathic effect of the bacteria), percentage of phagocytes destroyed by intracellular bacteria; c) PN (phagocytic number), the average number of microbes per macrophage (the graphs were plotted from the data of Tables 2 and 3 [51]).

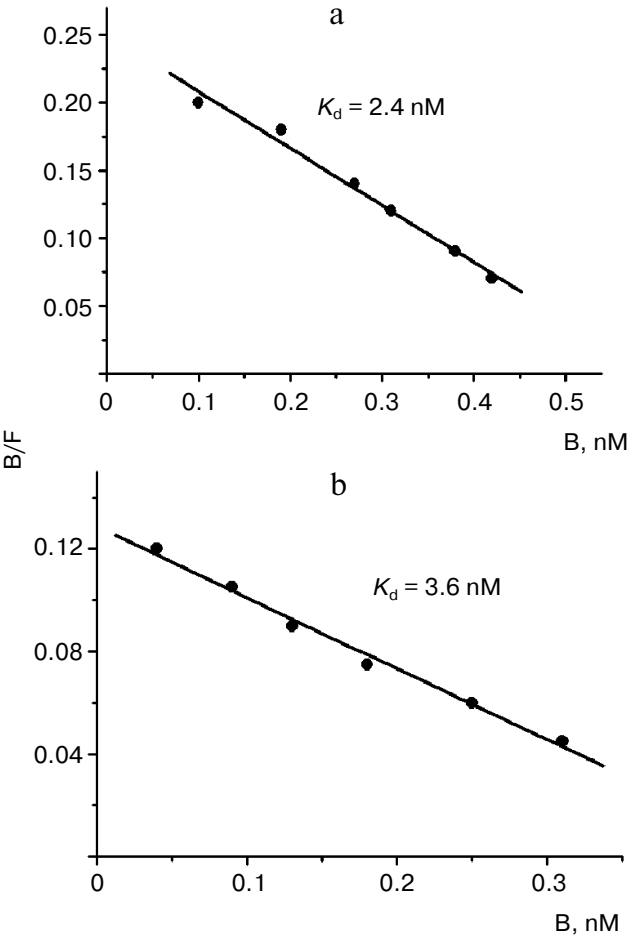

Fig. 3. Analysis in Scatchard coordinates of specific binding of [^{3}H]immunorphin (a) and ^{125}I-labeled pentarphin (b) to mouse peritoneal macrophages. B and F, molar concentrations of the bound and free labeled peptide, respectively [51, 104].

noted that in two different test systems using ^{125}I-labeled pentarphin and [^{3}H]immunorphin, in the experiments on binding to mouse peritoneal macrophages, we have obtained practically the same values of receptor density: 28,000 per macrophage. This means that the characteristics obtained in the experiments are close to the true values.

Naloxone-insensitive receptors have been found on the cells of diffuse histiocytic lymphoma U-937 [49]. The cells of this line are precursors of monocytes by origin. Experimental results showed that the binding of ^{125}I-labeled β-endorphin to the receptor was characterized by saturability and high affinity ($K_d = 1.2 \cdot 10^{-8}$ M). The binding of ^{125}I-labeled β-endorphin to the cells of human monocytic cell line U-937 was inhibited by β-endorphin and *N-Ac*-β-endorphin, while naloxone, morphine, and other selective opioid agonists were inactive. The effects of ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}) and guanosine triphosphate on the binding of ^{125}I-labeled β-endorphin to the membranes of U-937 cells has been studied for the first time. Enhancement of concentrations of these ions was shown to decrease the binding. The presence of guanosine triphosphate (10^{-4} M) also decreased the binding by 25% [49].

We have also studied the effect of β-endorphin and immunorphin on the growth of U-937 cells and shown that both peptides in a concentration of 10^{-8}-10^{-6} M increase the rate of cell division by 30-40% [52].

When making conclusions, one may emphasize that the high-affinity naloxone-insensitive β-endorphin receptors have been shown to exist on T-lymphocytes and macrophages, the cells of diffuse histocytic lymphoma U-937, the Jurkat and MT-4 T-lymphoblast cell lines, and mouse thymoma EL4; affecting them, the hormone stimulates the functional activity of these cells. Besides the hormone, β-endorphin-like peptide immunorphin interacts with the nonopioid receptor on these cells, having a similar stimulating effect.

The data on the effect of β-endorphin on B-lymphocytes are contradictory. According to the results presented in the works [13, 17], the hormone has no effect on B-lymphocytes. However, Shahabi et al. showed the existence of the nonopioid β-endorphin receptor on intact mouse splenocytes cultivated *in vitro* [53]. It was found that the binding of ^{125}I-labeled β-endorphin to splenocytes was characterized by saturability; the Scatchard graph was shaped as a straight line, demonstrating the presence of a single type of receptors on cell surface, K_d = 4.1 nM. Besides, the binding of ^{125}I-labeled β-endorphin to splenocytes was equally inhibited by *N-Ac*-β-endorphin and β-endorphin, while β-endorphin fragments 6-31 and 28-31 were less active than the hormone (10 and 1000 times, respectively). Naloxone and β-endorphin fragment 1-27 were inactive. Thus, the characteristics of the nonopioid β-endorphin receptor on mouse splenocytes coincide with those obtained for other target cells. Since splenocytes are a population of T- and B-lymphocytes, the described receptor is most probably localized on T-lymphocytes. Shaker et al. also published data concerning naloxone-insensitive receptors on the B-cells of mouse lymphoma A20 [54]. The dissociation constant was $2.2 \cdot 10^{-8}$ M. However, the authors carried out their research with Con A-stimulated cells. This fact gives rise to doubt whether the cells under study were B-lymphocytes, because Con A induces blast transformation of only T-cells. It seems that the mouse B lymphoma A20 is a heterogeneous population, and the naloxone-insensitive receptor described for this cell line is actually localized on T-lymphocytes.

In accordance with the works of Gilman and Morgan, β-endorphin has no effect on splenocyte proliferation *in vitro* induced by LPS or dextran sulfate, which are specific mitogens of B-lymphocytes [27, 55]. The data obtained with immunorphin are in agreement with the results of other researchers: immunorphin has no effect on B-lymphocytes *in vitro*. The absence of effects of β-

endorphin and immunorphin on the growth of B-lymphoblast line RPMI-1788 may be due to the fact that the surface of such cells has no naloxone-insensitive receptors [52]. Although immunorphin did not influence the activity of B-lymphocytes *in vitro*, the LPS-stimulated spleen cells isolated from mice receiving intraperitoneally pentarphin contained 2.2-fold more [methyl-H^3]thymidine compared to the control [52]. Such effect is apparently associated with the stimulating action of pentarphin on T-cells and macrophages *in vivo*, which, in their turn, activated B-cells. Thus, it has been shown that β-endorphin and immunorphin have no direct influence on the proliferation of B-cells *in vitro*.

The presence of nonopioid binding regions was also established for other constituents of the immune system, e.g. for two complexes of the human complement system: cytolytic membrane complex C5b-9(m) and cytolytically inactive serum complex SC5b-9. The interaction between ^{125}I-labeled β-endorphin and the receptor on C5b-9(m) was not affected by ACTH, insulin, the releasing factor of luteinizing hormone, dynorphin (1-13), and β-casomorphin [56]. The interaction between the hormone and the nonopioid receptors was observed in the components of human blood plasma and serum in the presence of heparin [57]. At the same time, it was temperature-dependent and not observed in the presence of other anticoagulants. The binding of ^{125}I-labeled β-endorphin to the components of human blood plasma and serum was characterized by saturability and reversibility and was not inhibited by naloxone, morphine, and a number of other opioid peptides being fragments of the *N*-terminal region of β-endorphin. The *C*-terminal fragment of β-endorphin completely inhibited the specific binding of ^{125}I-labeled β-endorphin to the components of human blood plasma and serum, substantiating the nonopioid nature of the receptor.

Particular attention should be paid to the mechanisms of peripheral analgesia performed by immune cells. The results obtained in animals and the human clinical data confirm the involvement of peripheral opioid receptors in analgesia, particularly during inflammation. Under inflammatory stress, the expression of opioid receptors increases manifold [58, 59]. Leukocytes containing opioids are drawn into the inflammatory focus due to the expression of extracellular adhesion molecules ICAM-1 in blood vessels of the tissue. Modern methods have shown that under inflammation the peripheral nerves and sympathetic nerve fibers increase the expression of vesicular ICAM-1, thereby enhancing the migration of opioid-containing leukocytes to neurons in peripheral inflamed tissues [60]. In the inflammatory focus, under the action of corticotrophin releasing factor (CRF), noradrenalin, and IL-1β, cells of the immune system secrete β-endorphin. The hormone interacts with peripheral opioid receptors and, as a result, inhibits local pain during inflammation [58, 59, 61]. It is shown that surgical stress, like inflammatory one, causes the release of endogenous opioids from immune cells followed by analgesia through activation of peripheral opioid receptors [62]. Under CRF stimulation of T-lymphocytes accumulated near the damaged (denuded) nerve fibers, the cells secrete β-endorphin for the activation of local neuronal opioid receptors to reduce neuropathic mechanical hypersensitivity [63].

Besides, it is believed than β-endorphin is secreted by sarcoma cells and provides antinociception through interaction with peripheral opioid receptors located in the tumor microenvironment [64].

In the absence of inflammation, hydrophilic opioid peptides cannot penetrate the perineural barrier and induce antinociception. However, the study of conditions when this process is possible has shown that antinociception mediated by endogenous opioids in uninflamed tissues has two important requirements: the opening of perineural barrier for penetration of opioid peptides and release of the latter from neutrophils with the involvement of p38 MARK (mitogen-activated kinase). Thus, anesthesia can also take place in uninflamed tissues owing to endogenous opioids [65, 66].

Thus, it is possible to draw a conclusion about the role of β-endorphin in the immune system. According to the data of [7], macrophages and monocytes, and granulocytes and lymphocytes possess a complete mechanism for the synthesis, posttranslational processing, and secretion of biologically active POMC-peptides including β-endorphin. The synthesis and secretion of POMC-derivative β-endorphin from the secretory granules of immunocytes proceeds by the classical pathway like that in hypophysis [7]. The number of adhesion molecules ICAM-1 (CD54), which contribute to the enhancement of migration of opioid-containing immunocytes into inflamed tissues, on the surface of vesicular endothelium increases under inflammatory stress [67]. Under inflammatory conditions, the number of immunocytes in the inflammation focus increases manifold, while the level of hormone secretion becomes considerably higher. Besides, IL-1β, IL-2, IL-6, interferons, tumor necrosis factor α, corticotropin-releasing hormone, which is also secreted by cells in the inflammation focus [68], noradrenalin, and potassium and calcium ions additionally activate the immune cells for β-endorphin secretion. The latter, acting as a tissue hormone, in its turn inhibits pain senses due to local interaction with the opioid receptors of inflamed tissues. Besides, the hormone influences immunocytes. The opioid and nonopioid β-endorphin receptors are expressed on the cells of the immune system (macrophages, monocytes, granulocytes, and T-lymphocytes). The hormone interacts with the opioid receptors and thereby has an inhibitory effect on immunocytes. On the contrary, the effects realized through the high-affinity nonopioid receptor (insensitive to the opioid blocker naloxone) are stimulating. In the inflammation focus, β-

endorphin concentration increases manifold due to the synthesis and secretion of the hormone by the cells of the immune system; thus, immunocytes provide themselves with additional stimulation for controlling infectious agents.

EFFECT OF β-ENDORPHIN ON THE NERVOUS AND ENDOCRINE SYSTEMS

β-Endorphin is localized not only in immune cells, but also in many other tissues of an organism: placenta, thyroid gland [69], adrenal medulla [70], pancreas [71], gastrointestinal tract, and reproductive organs. In this context, β-endorphin plays a key role in the interrelationship between the nervous, endocrine, and immune systems.

The endocrine system is represented by central organs (hypothalamus and hypophysis) and peripheral organs (thyroid gland, the cortex and medulla of adrenal glands, pancreas, ovaries, and testicles).

As is known, opioid peptides (α- and β-endorphins) participate in the regulation of thyrotropin secretion by influencing the secretion of thyrotropin-releasing hormone in the hypothalamus [72, 73]. This effect is mediated through opioid pathways. Catecholamines acting directly on the α-1 or β-adrenergic receptors of the adenohypophysis increase the level of β-endorphin in the plasma of unstressed rats, while thyroid hormones regulate the expression of α-1 adrenergic receptors [74]. Thus, β-endorphin regulates the functions of the thyroid gland, while its hormones indirectly influence the level of β-endorphin in blood.

The results of investigation of the effect of β-endorphin on the release of pancreatic hormones (insulin and glucagon) are contradictory. The modern studies still cannot elucidate this question [75]. Previously it has been shown that β-endorphin has an inhibitory effect on the cells of the pancreas. Stimulation of the α-2 adrenoreceptor of the pancreas inhibits glucose-induced insulin secretion by releasing endogenous opioids (β-endorphin). This process is performed due to activation of μ-opioid receptors and the opening of K^+ (ATP-dependent) channels [76]. However, there are literature data on the stimulating effect of β-endorphin on pancreatic cells: introduction of the hormone considerably increases the levels of insulin and glucagon in plasma and decreases the level of glucose. In one case, naloxone did not influence the sensitivity of insulin and glucagon to β-endorphin [77]. In the other case, naloxone and naltrexone considerably reduced the insulin response to the increase in blood glucose under hyperinsulinemia [78].

Besides, there is information about the modulating effect of β-endorphin on the functions of pancreas. The hormone inhibited or stimulated insulin secretion after intravenous introduction in low (0.25 to 1 nM/kg) or high (64 nM/kg) doses, respectively [79]. The effect of the hormone was neutralized by naloxone. A similar situation was observed in immunocompetent cells; in contrast to these, there are no data on the expression of nonopioid receptors in pancreas, but the presence of opioid receptors has been shown for this organ [80]. Specific binding of ^{125}I-labeled β-endorphin was shown for rabbit pancreas. It was blocked by unlabeled β-endorphin, opioid antagonists of the μ- and δ-receptors [80]. Specific binding was localized mainly on α-glucagon- and δ-somatostatin-containing cells and, to a lesser extent, on insulin-containing β-cells. The positions of opioid binding sites and the results of previous studies suggest that β-endorphin regulates the secretion of pancreatic hormones by a paracrine or autocrine pathway.

It is interesting how β-endorphin influences the hypothalamus–hypophysis–adrenal system responsible for the response of the organism to the impact of stressors. Activation of the stress system of the organism induced by any (emotional or physical) stressors stimulates the activity of POMC-derived peptides of the hypothalamus such as α-melanocyte-stimulating hormone and β-endorphin, which mutually inhibit the activity of central components of the hypothalamus stress system, provide analgesia through the release of hormones into the paleoencephalon and spinal cord, where they inhibit the ascending pain stimuli [81, 82]. In accordance with the results of Szalay's work [83] devoted to investigation of the effect of POMC peptides on the functional activity of adrenal cortex, β-endorphin can stimulate, inhibit, or have no effect on steroidogenesis depending on its dosage and the functional state of cortical cells. According to the data of Kapas et al. [84], the stimulating effect of β-endorphin on aldosterone secretion by the cells of zona glomerulosa of the adrenal cortex is mediated by μ-opioid receptors, while corticosterone secretion by the cells of zona fasciculata and zona reticularis of the adrenal cortex is mediated by the μ- and κ-receptors. The work also shows that the binding of β-endorphin to the μ- and κ-opioid receptors of cells of the adrenal cortex results in the activation of phospholipase C.

Results of the studies from our laboratory [85] show that nonopioid β-endorphin receptors are also expressed on the surface of adrenal glands in addition to opioid receptors. The analysis of specific binding of [^{3}H]immunorphin with the membranes of adrenal cortex in Scatchard coordinates revealed the presence of two types of binding sites (receptors) with different affinity (K_{d1} = 40.0 nM, K_{d2} = 0.25 μM) and density (B_{max1} = 40.7 pmol/mg protein, B_{max2} = 187.8 pmol/mg protein). Immunorphin, when binding to nonopioid β-endorphin receptors on the membranes of rat adrenal cortex, inhibited the adenylate cyclase activity and reduced the secretion of 11-oxycorticosteroids (corticosterone) from adrenal glands into blood (table). Thus, the effect of immunorphin is opposite to the effect of ACTH: the lat-

Effect of immunorphin on the level of 11-oxycorticosteroids (CS) in rat adrenal glands and blood plasma 1.6 and 24 h after intramuscular injection [85]

Immunorphin dosage, μg/kg	Level of 11-oxycorticosteroids, experiment/control					
	1 h		6 h		24 h	
	adrenal gland	plasma	adrenal gland	plasma	adrenal gland	plasma
10	1.32 ± 0.08*	0.67 ± 0.14*	1.29 ± 0.09*	0.65 ± 0.10*	1.07 ± 0.08*	0.59 ± 0.12*
100	1.49 ± 0.06**	0.56 ± 0.11*	1.52 ± 0.09*	0.49 ± 0.08*	1.09 ± 0.09*	0.51 ± 0.09*

Note: CS content in plasma and adrenal glands of control animals was on average 0.3 μg/ml and 35 μg/g of tissue, respectively; * $p < 0.02$; ** $p < 0.001$.

ter stimulates the synthesis and secretion of glucocorticoids by the cells of zona fasciculata and zona reticularis of the adrenal cortex through activation of adenylate cyclase and enhancement of intracellular cAMP [86].

Our results lead to a conclusion that the inhibitory effect of β-endorphin on the stress system involves both opioid and nonopioid receptors.

Quite a lot of works are devoted to investigation of the effect of β-endorphin on the reproductive system. As is shown, the hormone is secreted by follicular cells surrounding the ovule and thereby regulates the maturation and ovulation of oocytes [87]. The level of the hormone in blood plasma of pregnant women continually changes [88]. Besides, β-endorphin is secreted by the placenta [89] and endometrial cells during implantation of the embryo into the uterus wall [90]. In male mice and rats, Leydig cells (interstitial glandular cells of testis) secrete β-endorphin, which may affect the quality of spermatozoons [91]. The presence of opioid receptors on the 2- and 8-cell mouse embryos was shown [92]. However, the effect of the hormone on early embryos was not studied.

At present, we have demonstrated that β-endorphin and β-endorphin-like peptides (immunorphin, pentarphin, and cyclopentarphin) may act as growth factors stimulating the processes of cell division in early mouse embryos (2-, 4-, and 8-cell embryos), formation and "hatching" of mature blastocysts *in vitro*. Cyclopentarphin most completely manifests its properties as a nonspecific growth factor enhancing the viability of early mouse embryos. The stimulating effect of β-endorphin, immunorphin, pentarphin, and cyclopentarphin on embryos is not blocked by naloxone [93-95]. The interaction of pentarphin and β-endorphin with the nonopioid receptor on mouse embryos results in enhancement of intracellular calcium [96]. The presented data demonstrate that β-endorphin participates in the regulation of early development of mammals; the hormone acts through opioid and specific naloxone-insensitive receptors.

We have studied the binding of ^{125}I-labeled β-endorphin to rat brain membranes. [Met^5]Enkephalin, [Leu^5]enkephalin, and naloxone had no effect on the binding of ^{125}I-labeled β-endorphin. Thus, β-endorphin was shown to interact with the high-affinity specific naloxone-insensitive receptor on rat brain membranes. The dissociation constant characterizing the hormone binding to the receptor was $K_d = 1.87$ nM, $B_{max} = 144$ fM/mg. Besides, it was shown that immunorphin and pentarphin successively competed with ^{125}I-labeled β-endorphin for the binding to nonopioid receptor on the membranes of rat brain cortex [97]. Inhibition constants were 1.18 and 1.58 nM, respectively. The K_d values characterizing the specific binding of ^{125}I-labeled immunorphin and pentarphin to brain cortex membranes were determined as well: $K_{d1} = 2.93$ and $K_{d2} = 3.17$ nM, respectively. Thus, β-endorphin, immunorphin, and pentarphin interact with the nonopioid receptor on rat brain cortex membranes, and the characteristics of their specific binding coincide with those obtained previously for β-endorphin and peptides on other objects.

Now it has been shown at our laboratory that fragment 12-19 (TPLVTLFK, named by the authors as octarphin) is responsible for binding to the nonopioid receptor in the β-endorphin molecule. The study of octarphin reception also confirms the presence of nonopioid binding sites on rat brain membranes, $K_d = 2.6$ nM [98].

The main function of β-endorphin in an organism is provision of anesthesia (antinociception) and the state of euphoria. The theory of stereochemical basis of structural activity of aromatic and heterocyclic rings in a number of opioids, particularly β-endorphin, which explains the analgesic effect of the hormone, is now under discussion [99]. Joint localization of the μ- and δ-opioid receptors providing analgesia on the nociceptive neurons of small diameter in the roots of spinal ganglions has been shown. This finding explains that joint localization of the receptors is a basis for direct interaction of opioid receptors in the modulation of nociceptive afferent transmission and provision of opioid analgesia [100].

Besides the opioid receptors, the chain of analgesia provision by the hormone includes also a series of non-

opioid components. The researchers investigating the properties of biphalin (an opioid peptide analog) have ascertained that it is a stronger analgesic than morphine. It may be due to the fact that biphalin activates three types of opioid receptors at once (μ, δ, κ). Besides, it has been shown that NMDA receptors play a key role in providing anesthesia by biphalin and, probably, all opioids. Blockade of NMDA receptors significantly enhances the antinociception of biphalin or morphine, unmasks the antinociception caused by endogenous opioids in peripheral tissues and, besides, increases the level of β-endorphin in the roots of dorsal ganglions and saphenous nerves [101, 102]. As is shown, the joint introduction of IFN-α and β-endorphin produces antinociception by means by interaction between IFN-α and supraspinal β-endorphin-sensitive opioid receptors [66]. Previously it was established that antinociception caused by N_2O in the rat hot plate test depends on β-endorphin antagonists. It has been shown in a rat model *in vivo* that N_2O stimulates the NO-dependent neuronal release of β-endorphin, while the hormone, in its turn, provides antinociception [103]. Since the involvement of nonopioid elements in analgesia provision by β-endorphin has been demonstrated, it seems worthwhile to study the effect of immunorphin on antinociception.

The existence of nonopioid β-endorphin receptors is shown for the adrenal cortex (inhibition of glucocorticoid secretion into blood), rat brain cortex (unstudied function, supposedly antinociception), and on early mouse embryos, for which the neuropeptide plays the role of a growth factor. It may be concluded that, like many peptide hormones, β-endorphin participates in regulation of the functions of the immune system, the organs of the endocrine systems, and the central nervous system. It should be emphasized that realization of the interrelationship between the nervous, endocrine, and immune systems involves, besides classical opioid, also high-affinity specific nonopioid receptors of β-endorphin.

INVESTIGATION OF THE NONOPIOID β-ENDORPHIN RECEPTOR

In spite of a significant role in the regulation of functions of the nervous, endocrine, and immune systems, the nonopioid β-endorphin receptor is still little studied. It should be noted that the studies of naloxone-insensitive β-endorphin receptors are descriptive. The main characteristics given by all researchers who have studied the nonopioid receptor are as follows: high specificity to β-endorphin and its *N*-acetylated derivative; K_d in the range of nM concentrations; and the binding not blocked by naloxone.

The first attempt of studying this receptor at the molecular level was made by Shahabi et al. [49, 53]. They isolated from splenocyte membranes the complex of a receptor chemically cross-linked to ^{125}I-labeled β-endorphin, and the molecular weights of proteins of this complex were determined by polyacrylamide gel electrophoresis. It was shown that the hormone was bound to proteins with molecular weights of 66 and 57 kDa. Similar studies were carried out also with the monocytic cell line U-937 and the following values were obtained: ^{125}I-labeled β-endorphin was bound to proteins of 66 and 44 kDa [49]. In both cases, the binding was characterized by high specificity and insensitivity to naloxone. In other studies, investigation of the binding of β-endorphin to the nonopioid receptor of EL4 mouse thymoma cells showed that the hormone in the presence of naloxone interacted with two binding sites different in the affinity and molecular weight of receptor complex proteins. Molecular weights of the proteins of high-affinity and low-affinity receptor complexes were 72 and 40 kDa, respectively. Further studies showed that only β-endorphin and its *C*-terminal fragments inhibited the binding of ^{125}I-labeled β-endorphin to the high-affinity binding site of EL4 cells, which was evidence of the nonopioid nature of the receptor [30]. Some discrepancy between the molecular weights of receptor complex proteins is most likely associated with different methods of receptor complex analysis during the experiment.

In the course of investigation of the binding of [^{3}H]immunorphin to the membranes from different rat organs (liver, kidneys, lung, myocardium, spleen, adrenal glands, intestines, and brain), we have ascertained distribution of the nonopioid β-endorphin receptor in a mammalian organism. [^{3}H]Immunorphin made it possible to reveal and characterize the nonopioid receptors on membranes isolated from the rat myocardium, spleen, adrenal glands, and brain. The value of specific binding of 8.4 nM [^{3}H]immunorphin to the membranes of adrenal glands, spleen, myocardium, and brain in rats was 1146.0 ± 44.7, 698.6 ± 28.1, 279.1 ± 15.4, and 172.2 ± 1.8 fmol/mg protein, respectively. The ability to inhibit the binding of [^{3}H]immunorphin to membranes of the above organs was shown to be characteristics of unlabeled β-endorphin, pentarphin, cyclopentarphin, and the Fc-fragment of IgG1 [104].

We suppose that the naloxone-insensitive β-endorphin receptor is an Fc receptor. This assumption is favored by the following facts: first, distribution of this type of receptor in an organism (it was found mainly on the cells of the immune and complement systems); second, this receptor consists of two subunits, which is typical of most Fc receptors; and, third, immunorphin, the highly specific ligand of the nonopioid receptor, is a region of the IgG Fc fragment.

We have investigated the question of whether there is a relation between the nonopioid β-endorphin receptor and FcγR. The study of the region of the human IgG1 Fc fragment (Fig. 4), containing the sequences of immunorphin and endogenous macrophage-stimulating peptide

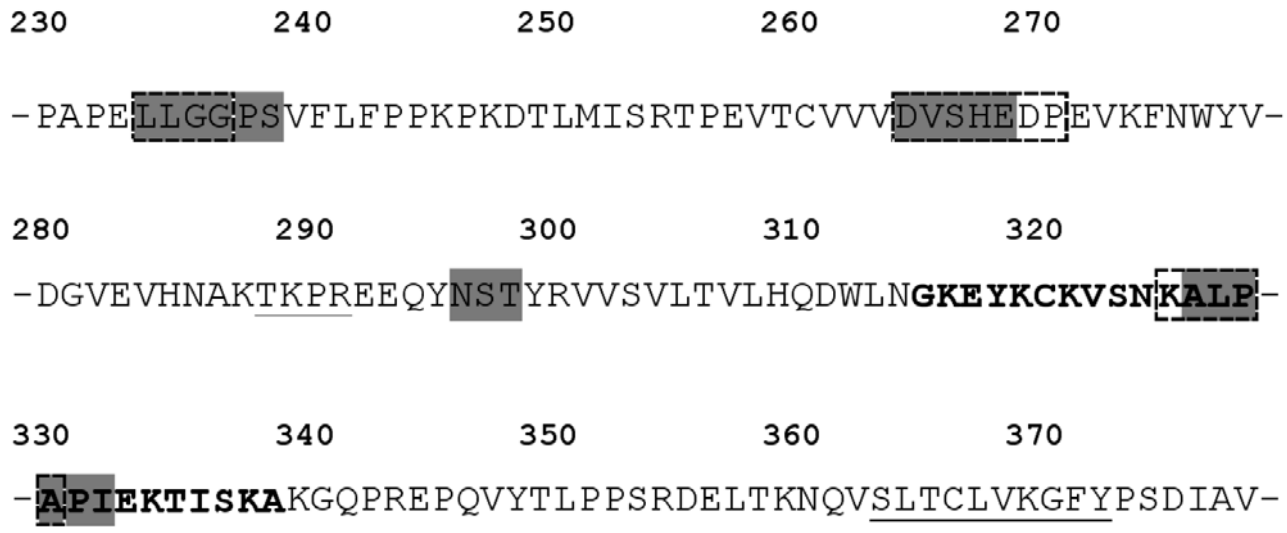

Fig. 4. Amino acid sequence of the region of the human IgG1 Fc fragment including the tuftsin (289-292) and immunorphin (364-373) sequences (underlined) and the regions of binding to FcγRI (bold), FcγRII (framed), and FcγRIII (filled) [104].

tuftsin [105] and the sites of binding to FcγRI and FcγRIII [106, 107], showed that the sequences of the Fc fragment corresponding to tuftsin and immunorphin were not included in the FcγRI and FcγRIII binding sites and, consequently, could not directly participate in the binding to the Fc receptors of class I and III. However, the results of our work show that the unlabeled Fc fragment competitively inhibits the specific binding of [^{3}H]immunorphin to mouse peritoneal macrophages and the value of K_i 6.0 pM is evidence of high affinity of the Fc fragment to the nonopioid β-endorphin receptor of macrophages [104]. However, previously it has been shown that unlabeled immunorphin weakly (by less than 10%) displaces ^{125}I-labeled IgG1 from the receptor complex on macrophages [39]. Hence it follows that the nonopioid β-endorphin receptor is different from FcγR, and the ability of the IgG Fc fragment to bind to the nonopioid β-endorphin receptor is due to the presence of immunorphin sequence in the Fc fragment.

Besides, we have studied the ability of 30 unlabeled synthetic fragments of β-endorphin to inhibit the specific binding of [^{3}H]immunorphin to mouse peritoneal macrophages and identified a shorter fragment capable of binding to the nonopioid β-endorphin receptor with high affinity. It was shown that the shortest peptide with practically the same inhibiting activity as β-endorphin (K_i 2.9 nM) was its fragment (12-19): octarphin (TPLVTLFK) (K_i 3.1 nM) (Fig. 5) [108]. [^{3}H]Octarphin was obtained and its specific binding to mouse peritoneal macrophages was studied. It was shown that [^{3}H]octarphin bound to one type of receptors on mouse peritoneal macrophages with high affinity (K_d = 2.3 nM) and this interaction was not blocked by naloxone. Besides, it was shown that the specific binding of [^{3}H]octarphin to mouse peritoneal macrophages was inhibited by unlabeled immunorphin and β-endorphin (K_i = 2.4 and 2.7 nM, respectively) [109]. Previously it was established that the region (6-23) of the β-endorphin molecule is responsible for binding to the nonopioid receptor [15, 22, 27, 45]. Using the selective agonist of the nonopioid β-endorphin receptor (immunorphin), we could exactly localize the region of binding to the nonopioid receptor in the β-endorphin molecule: fragment 12-19.

Thus, in spite of the numerous works devoted to the naloxone-insensitive β-endorphin receptor, it is still a puzzle for researchers. Immunorphin binds to the naloxone-insensitive receptors with high affinity, so it can be

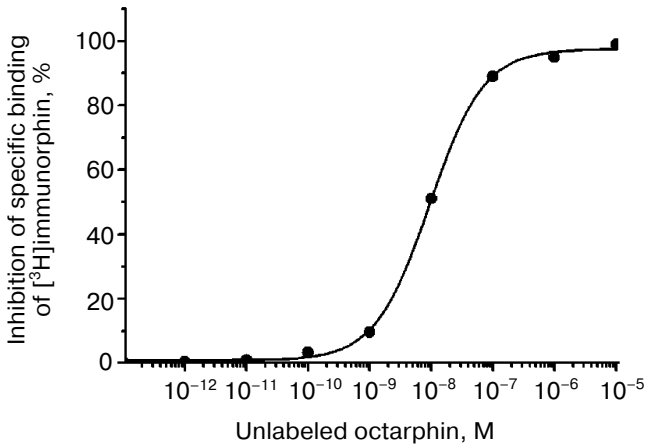

Fig. 5. Inhibition of specific binding of [^{3}H]immunorphin (5 nM) to mouse peritoneal macrophages by unlabeled octarphin [108].

used as a selective agonist for the study of this type of receptors. Immunorphin is more preferable for such studies than β-endorphin, because the former binds to this receptor only, while the latter binds, besides the nonopioid receptors, also to the μ-, δ-, and ε-opioid receptors.

NONOPIOID RECEPTORS

The publications of recent years demonstrate increasing interest in the study of the nonopioid effects of opioid peptides and the receptors mediating these effects. A nonopioid receptor (the orphanin FQ receptor coupled with G proteins) has been found on frog brain membranes. G proteins are activated by the nonopioid mechanism [110]. The receptors of this type have been cloned; they show a high degree of homology with the κ-opioid receptors. The endogenous ligand of the orphanin FQ receptor is a natural heptapeptide, nociceptin, which is similar to dynorphin A [111]. Nociceptin has anesthetic effect when introduced into the spinal cord of frogs [112]. It is also known that dynorphin has nonopioid effect realized through the glutamate receptor subtype *N*-methyl-D-aspartate being receptor channels (NMDA receptors), the κ_2 opioid receptors, and through unknown receptors of nonopioid nature. At this stage of research, it has been reliably established that the opioid peptides [Met^5]enkephalin, BAM22, nociceptin, and endomorphin-1 and -2 possess their own nonopioid receptors [111].

The σ opioid receptor has interesting properties. Wollemman, Benyhe, and their coworkers studied the binding of [^{3}H]MERF ([Met^5]-enkephalin-Arg-Phe) to frog, rat, and guinea pig brain membranes and showed that naloxone and opioid peptides also competed for the binding to the receptor along with MERF, but naloxone only partially displaced [^{3}H]MERF from the ligand–receptor complex, while dynorphin and β-endorphin provided complete inhibition of the binding. The discovered nonopioid component of the receptor is of low-affinity; K_d is in the range of μM concentrations [113]. The effects mediated by interaction with the σ_2 receptor are little studied, but sedative effect, the influence on motor activity, and potentiation of NMDA receptors have been described. The presented facts seem to be obscure, but the studies of recent years make it possible to explain these effects. At present it has been shown that σ receptors may be physically associated with μ-opioid receptors and can modulate opioid transduction not affecting the binding of the opioid receptor but modulating the signalization of G protein-bound receptors [114]. It is quite possible that this very complex binding center demonstrates the low-affinity naloxone-insensitive component (σ-receptor) and the binding of opioid agonists and antagonists (μ-receptor).

The nonopioid nature of σ receptors may be confirmed by the results of Fontanilla [115, 116]. The work describes the hallucinogen *N*,*N*-dimethyltryptamine, being an endogenous ligand for the σ_1 receptor. It is typical that this type of receptors does not bind other opioid peptides and the K_d value of binding to mouse brain membranes is in the range of μM concentrations. The work also describes the effects of *N*,*N*-dimethyltryptamine realized through the naloxone-insensitive σ_1 receptor, e.g. high cytotoxicity is demonstrated by growth inhibition for quite a number of tumor cell lines. In this work it is emphasized that both σ_1 and σ_2 receptors are different from the orphanin FQ receptor and, although we can speak about the nonopioid component of the σ_2 receptor binding center, σ-receptors are nonopioid receptors. Some researchers have noted the interrelation between the σ_1 and NMDA receptors. The activation of spinal σ_1 receptors results in enhancement of phosphorylation of the NR1 subunit of NMDA receptors in the mouse spinal cord followed by potentiation of receptor functions [117]. It has been shown that NMDA receptors facilitate the production of IL-8 and reduce the secretion of IL-10 by the Jurkat leukemic line cells and lymphocytes of human peripheral blood, while σ_1-ligands modulate the NMDA activity [118].

Thus, one may say that there are quite a lot of publications on the opioid-binding receptors insensitive to naloxone. It seems that some of these effects may be explained by formation of associations and dimers of opioid receptors, which has been widely discussed recently [119]. It has been shown that *in vivo* opioid receptors can interact with each other and form new functional structures, the simplest of them being a dimer; however, the formation of heterodimers is tissue-specific and the binding of the resulting structure is characterized by a number of new properties [120]. Other effects may be due to nonopioid receptors proper, e.g. the orphanin FQ receptors that still need detailed investigation. Probably, the structure of uncloned nonopioid receptors will have a certain percentage of homology with opioid receptors, like in case of the orphanin FQ receptors.

The above data demonstrate that the nonopioid effect of β-endorphin is realized through a specific binding site: the nonopioid receptor. Its characteristic features have been revealed in the studies of the past 30 years. These studies have shown the existence of naloxone-insensitive binding sites that bind β-endorphin, the *C*-terminal fragments of β-endorphin, *N*-*Ac*-β-endorphin, and β-endorphin-like peptide immunorphin with high affinity (K_d in the range of nM concentrations). This binding region was found on rat brain membranes, mouse peritoneal macrophages, human T-lymphocytes, transformed human and mouse cell lines, rat adrenal cortex, and mouse embryos. The nonopioid binding site is of protein nature and probably consists of two subunits. When interacting with this binding site, β-endorphin stimulates the proliferation of Con A-induced human T-lymphocytes, the growth of cell lines *in vitro*, the functional activ-

ity of macrophages, inhibits glucocorticoid secretion from the adrenal cortex into blood, and contributes to successful development of early mouse embryos *in vitro*. All the above is evidence of the key role of nonopioid binding sites in human and animal cells and suggests that these binding sites are the nonopioid β-endorphin receptor. The nonopioid effect of β-endorphin is spread via this specific high-affinity receptor located on target cells to the immune, nervous, and endocrine systems.

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