*Biochemistry (Moscow), Vol. 69, No. 4, 2004, pp. 394-400. Translated from Biokhimiya, Vol. 69, No. 4, 2004, pp. 488-496.*

*Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM03-125, November 30, 2003.*

# Characteristics of Non-opioid β-Endorphin Receptor

**E. V. Navolotskaya[1]*, Yu. A. Kovalitskaya[1], Yu. A. Zolotarev[2], A. A. Kolobov[3], E. A. Kampe-Nemm[3], N. V. Malkova[1], V. V. Yurovsky[4], and V. M. Lipkin[1]**

*[1]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, pr. Nauki 6, Pushchino 142290, Moscow Region, Russia; fax: (0967) 33-0527; E-mail: navolots@fibkh.serpukhov.su*
*[2]Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova 2, Moscow 123182, Russia; fax: (7-095) 196-0221*
*[3]State Research Center, Institute of Highly Pure Biopreparations, Ministry of Health of the Russian Federation, ul. Pudozhskaya 7, St. Petersburg 197110, Russia; fax: (7-812) 235-5504*
*[4]Department of Medicine, University of Maryland, Baltimore, MD 21201, USA; fax: (410) 706-0231*

Received May 13, 2003
Revision received June 2, 2003

**Abstract**—Tritium-labeled selective agonist of non-opioid β-endorphin receptor, the decapeptide immunorphine ([$^3$H]SLTCLVKGFY) with specific activity of 24 Ci/mmol has been prepared. By its use, non-opioid β-endorphin receptors were revealed and characterized on mouse peritoneal macrophages and rat myocardium, spleen, adrenal, and brain membranes. The non-opioid β-endorphin receptor of macrophages has in addition to immunorphine ($K_d$ of the [$^3$H]immunorphine–receptor complex was 2.4 ± 0.1 nM) and β-endorphin ($K_i$ of the [$^3$H]immunorphine specific binding was 2.9 ± 0.2 nM) a high affinity for Fc-fragment of human IgG1, pentarphine (VKGFY), cyclopentarphine [cyclo(VKGFY)], and [Pro$^3$]pentarphine (VKPFY) ($K_i$ values were 0.0060 ± 0.0004, 2.7 ± 0.2, 2.6 ± 0.2, and 2.8 ± 0.2 nM, respectively) and is insensitive to naloxone and [Met$^5$]enkephalin ($K_i$ > 100 μM). Treatment of macrophages with trypsin resulted in the loss of their ability for the specific binding of [$^3$H]immunorphine. Values of the specific binding of 8.4 nM [$^3$H]immunorphine to rat adrenal, spleen, myocardium, and brain membranes were determined to be 1146.0 ± 44.7, 698.6 ± 28.1, 279.1 ± 15.4, and 172.2 ± 1.8 fmol/mg protein, respectively. Unlabeled β-endorphin, pentarphine, [Pro$^3$]pentarphine, cyclopentarphine, cyclodipentarphine [cyclo(VKGFYVKGFY)], and Fc-fragment of IgG1 inhibited the binding of [$^3$H]immunorphine to membranes from these organs. No specific binding of [$^3$H]immunorphine to rat liver, lung, kidney, and intestine membranes was found.

*Key words*: β-endorphin, naloxone, immunoglobulin G, peptides, receptors

In 1980 Julliard et al. found that the heavy chain of IgG contains sequences similar to adrenocortical hormone (ACTH) and β-endorphin [1]. Houck et al. synthesized the tetradecapeptide SLTCLVKGFYPSDI, which corresponds to the β-endorphin-like sequence of IgG (the 364-377 fragment of the heavy chain $C_{H3}$-domain) and showed its competition with $^{125}$I-labeled β-endorphin for binding to membranes of rat brain [2]. We have synthesized a β-endorphin-like decapeptide immunorphine SLTCLVKGFY, which corresponds to the 364-373 sequence of the H-chain (1-4) of human IgG [3]. Biological effects of this peptide were studied, and it was found to activate human and mouse immunocompetent cells *in vitro* and *in vivo* [4-8], increase the *in vitro* growth of T-lymphoblast human leukemia cell lines Jurkat and MT-4 [9], stimulate the division of blastomeres, and accelerate the development of preimplantational mouse embryos [10]. Concurrently, immunorphine was found to bind with high affinity and specificity to the non-opioid (naloxone-insensitive) β-endorphin receptors on human T-lymphocytes [4-6], mouse peritoneal macrophages [3, 11, 12], and synaptic membranes from rat brain [8]. The specificity of the non-opioid β-endorphin receptor of human T-lymphocytes and mouse peritoneal macrophages was studied, and it was found to be insensitive to endogenous enkephalins and endorphins (α and γ) [3-7]. By data of Hazum et al., the non-opioid β-endorphin receptor of human lymphocytes failed to bind β-lipotropin, insulin, glucagon, and α-melanocyte-stimulating hormone [13]. Thus, at present, there are some data on the receptor specificity, but its structure, functions, and distribution in the body are still unknown.

The purpose of this work was to prepare [$^3$H]immunorphine and characterize it using the non-opioid β-endorphin receptor on mouse peritoneal macrophages and membranes isolated from various rat organs.

* To whom correspondence should be addressed.

0006-2979/04/6904-0394 ©2004 MAIK "Nauka/Interperiodica"

## MATERIALS AND METHODS

[Met$^5$]Enkephalin, β-endorphin, naloxone, trypsin, sucrose, BSA, EDTA, EGTA, Tris-HCl, phenylmethylsulfonyl fluoride (PMSF), sodium azide ($NaN_3$), and alumina ($Al_2O_3$) were from Sigma (USA); $Rh/Al_2O_3$ was from Fluka (USA); Unisolv 100 scintillator was from Amersham (England). All other reagents and solvents used in the work were of domestic production and were used after appropriate purification.

BALB/c mice and Wistar rats (males of 10-12 weeks) were from the nursery of the Branch of the Institute of Bioorganic Chemistry, Russian Academy of Sciences.

Fc-Fragment of human IgG1 was presented by Dr. V. M. Tishchenko (Institute of Protein, Russian Academy of Sciences, Pushchino, Moscow Region); the molecular weight of the protein was 50 kD. The isolation of Fc-fragment and its structure have been described [14].

Peptides were synthesized with automatic synthesizers (Applied Biosystems model 430A and Vega Coupler model C250, USA) and purified by reverse-phase chromatography using a Gilson chromatograph (France) on a Waters Symmetry Prep C18 column (19 × 300 mm) (Malva, Greece) as described in [11]. The resulting peptides were characterized by analytical reverse-phase HPLC (Gilson chromatograph, France) on an XTerra RP18 column (Malva), amino acid analysis (hydrolysis with 6 M HCl for 24 h at 110°C, an LKB 4151 Alpha Plus amino acid analyzer (Sweden), and mass-spectrometry (Finnigan mass-spectrometer, USA).

[$^3$H]Immunorphine was prepared by high-temperature solid-phase catalytic isotope exchange (HTCIE) [15, 16]. A solution of 2.0 mg immunorphine in 0.5 ml of water was supplemented with 50 mg alumina and evaporated on a rotary evaporator. Alumina with the peptide applied on it was mixed with 10 mg catalyst (5% $Rh/Al_2O_3$). The resulting solid mixture was placed into a 10-ml ampoule. The ampoule was vacuum-treated, filled with gaseous tritium to pressure of 250 mm Hg, heated to 170°C, and kept at this temperature for 20 min. Then the ampoule was cooled, vacuum-treated, purged with hydrogen, and vacuum-treated repeatedly. The labeled peptide was extracted from the solid reaction mixture with two 3-ml portions of 50% aqueous ethanol, and the resulting solutions were combined and evaporated. To remove labile tritium, the procedure was repeated twice. [$^3$H]Immunorphine was purified by high performance liquid chromatography (HPLC) using a Beckman spectrometer at wavelengths of 254 and 280 nm, with a Kromasil column (4 × 150 mm), 5 μM graining, at 20°C. Elution was performed with 0.1% trifluoroacetic acid with methanol gradient of 42-70% for 20 min at the flow rate of 3 ml/min. The incorporation of tritium into the peptide was determined using liquid scintillation counting.

Mouse peritoneal macrophages were isolated and cultured as recommended in [17]. The viability of the cells determined with acridine orange was 93-95%. Membrane fractions from rat myocardium, spleen, liver, lungs, kidneys, adrenals, intestine, and brain were prepared as described in [18]. The protein concentration was determined by the Lowry method with BSA as the standard [19].

The reaction of [$^3$H]immunorphine binding with mouse peritoneal macrophages was performed in 199 medium supplemented with HEPES (25 mM), $NaN_3$ (20 mM), and PMSF (0.6 mg/ml) (pH 7.4) by the following protocol: siliconized tubes were supplemented with 100 μl of the labeled peptide ($10^{-10}$-$10^{-7}$ M, three parallel samples for every concentration), 100 μl of the medium (the total binding), or 100 μl of $10^{-3}$ M of the unlabeled peptide in the medium (the nonspecific binding), and 800 μl of cell suspension ($1.3 \cdot 10^7$ cells per 1 ml of the medium). The tubes were incubated at 4°C for 1 h. After the incubation, to separate the labeled peptide bound to the cells from the unbound (free) peptide, the reaction mixture was filtered through GF/C fiberglass filters (Whatman, England). The filters were washed thrice with 5 ml of ice-cold saline. Radioactivity on the filters was counted with an LS 5801 liquid scintillation counter (Beckman, USA). The specific binding of [$^3$H]immunorphine to the cells was determined by the difference between its total and nonspecific binding, the nonspecific binding of [$^3$H]immunorphine was determined in the presence of $10^{-4}$ M of unlabeled pentarphine (1000-fold excess relative to the highest concentration ($10^{-7}$ M) of labeled immunorphine). Parameters of the specific binding of labeled immunorphine to macrophages (the equilibrium dissociation constant $K_d$ and the receptor density $n$ (the number of the specific binding sites for the peptide per cell)) were determined by plotting the dependence of molar concentration of the bound (B) and free (F) labeled immunorphine on molar concentration of the bound labeled peptide (B). The receptor density ($n$) was determined by the formula:

$$n = (R_0 \times A)/N,$$

where $R_0$ is the molar concentration of receptors, A is Avogadro's number, and $N$ is the number of cells per liter [20].

The reaction of [$^3$H]immunorphine binding to membrane fractions of various rat organs was performed in Tris-HCl buffer (pH 7.4) by the following protocol: glass tubes were supplemented with 100 μl of the labeled peptide from solution with the concentration of $8.4 \cdot 10^{-8}$ M, 100 μl of the buffer (the total binding), or 100 μl of the unlabeled peptide ($10^{-3}$ M) in the buffer (the nonspecific binding), and 800 μl of freshly prepared membrane suspension (1-5 mg protein depending on the tissue). The tubes were incubated at 4°C for 1 h. After the incubation, the reaction mixture was filtered through GF/C fiberglass filters (Whatman). The filters were washed thrice with 5 ml

of ice-cold buffer. Radioactivity was counted on the filters with an LS 5801 liquid scintillator (Beckman). The specific binding of [$^3$H]immunorphine to the membranes was determined by the difference between its total and nonspecific binding and expressed in moles per 1 mg protein.

The ability of naloxone, unlabeled peptides, and Fc-fragment of IgG to inhibit the nonspecific binding of [$^3$H]immunorphine to macrophages was studied on incubation of cells ($3.5 \cdot 10^6$ cells/ml) with labeled pentarphine (8.4 nM) and one of possible competitors (concentrations in the range from $10^{-13}$ to $10^{-7}$ M, three replicates for every concentration), as described above. The inhibition constant ($K_i$) was determined by the formula: $K_i = [I]_{50}/(1 + [L]/K_d)$ [21], where $[L]$ is the molar concentration of [$^3$H]immunorphine, $K_d$ is the equilibrium constant of dissociation of the [$^3$H]immunorphine–receptor complex, $[I]_{50}$ is the concentration of an unlabeled ligand responsible for 50% inhibition of the specific binding of labeled pentarphine. The $[I]_{50}$ value was determined from the curve of inhibition (the plot of dependence of inhibition percent versus the molar concentration of inhibitor). The $K_d$ value was determined preliminarily as described above.

To determine the ability of unlabeled peptides and Fc-fragment to inhibit the specific binding of [$^3$H]immunorphine to membranes from various rat organs, freshly isolated membranes (1-5 mg protein) were incubated with the labeled peptide (8.4 nM) and possible inhibitors (the concentration of peptides and Fc-fragment was $10^{-4}$ and $1.5 \cdot 10^{-9}$ M, respectively) as described above.

Macrophages were treated with trypsin as described earlier [22]. Macrophages ($10^7$ cells/ml) were incubated in medium 199 supplemented with trypsin (5 mg/ml) for 30 min at 37°C. The reaction was stopped by addition of a large volume of 199 medium. The cells were washed thrice with a tenfold volume of the medium and the reaction of binding was performed as described above.

## RESULTS

The main characteristics of the peptides (% purity, data of amino acid analysis, and molecular weight) are presented in Table 1.

The HTCIE reaction and purification resulted in 2 mCi [$^3$H]immunorphine with the specific activity of 24 Ci/mmol. The retention time of [$^3$H]immunorphine and unlabeled immunorphine on a Kromasil C18 column (the conditions of chromatography are presented in "Materials and Methods") was 18 min for both; the extinction ratios at 254 and 280 nm were also the same for the labeled and unlabeled peptide that suggested the maintaining of the chemical structure of immunorphine on the substitution of hydrogen by tritium.

**Binding of [$^3$H]immunorphine to mouse peritoneal macrophages.** Under the conditions of our experiments (see "Materials and Methods"), [$^3$H]immunorphine specifically bound to mouse macrophages. The specific binding of [$^3$H]immunorphine was determined as the difference between its total and nonspecific binding. The nonspecific binding of [$^3$H]immunorphine was determined in the presence of $10^{-4}$ M unlabeled immunorphine, and it was 9.7 ± 0.5% of the total binding of the labeled peptide. Figure 1 presents the dependence of the specific binding of [$^3$H]immunorphine to mouse peritoneal macrophages on incubation time at 4°C. The dynamic equilibrium in the system of [$^3$H]immunorphine-receptor was established after 1 h and was retained for at least 2 h; therefore, kinetic characteristics of the [$^3$H]immunorphine binding to macrophages were determined within 1 h.

The specific binding of [$^3$H]immunorphine to macrophages in Scatchard coordinates (Fig. 2) is a straight line, thus, there is one type of binding sites (receptors) for this peptide on the surface of macrophages. The value of $K_d = 2.4 \pm 0.1$ nM shows a high affinity of immunorphine for the receptors. The

**Table 1.** Main characteristics of the peptides

| Peptide | Purity, % | Amino acid analysis | Molecular weight, daltons |
|---|---|---|---|
| SLTCLVKGFY (immunorphine) | >99 | Thr 0.89; Ser 0.92; Gly 1.00; Val 1.00; Leu 1.94; Tyr 1.03; Phe 1.00; Lys 0.91 | 1129.3 (calculated value – 1130.0) |
| VKGFY (pentarphine) | >99 | Gly 1.04; Val 1.00; Tyr 0.96; Phe 1.00; Lys 0.96 | 613.4 (calculated value – 613.33) |
| VKPFY [Pro$^3$]pentarphine | >99 | Pro 0.91; Val 1.00; Tyr 0.96; Phe 1.00; Lys 0.95 | 652.9 (calculated value – 652.85) |
| Cyclo(VKGFY) (cyclopentarphine) | >99 | Gly 1.04; Val 1.06; Tyr 0.98; Phe 1.00; Lys 1.16 | 595.4 (calculated value – 594.32) |
| Cyclo(VKGFYVKGFY) (cyclodipentarphine) | >99 | Gly 2.02; Val 2.08; Tyr 1.88; Phe 2.02; Lys 2.36 | 1188.7 (calculated value – 1188.64) |

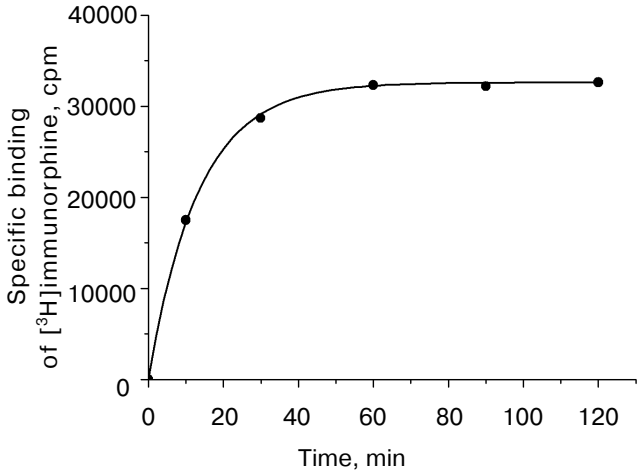


**Fig. 1.** Time dependence of the specific binding of [$^3$H]immunorphine (250,000 cpm). The specific binding of [$^3$H]immunorphine was calculated as the difference between its total and nonspecific binding. The nonspecific binding of [$^3$H]immunorphine was determined in the presence of $10^{-4}$ M unlabeled immunorphine.

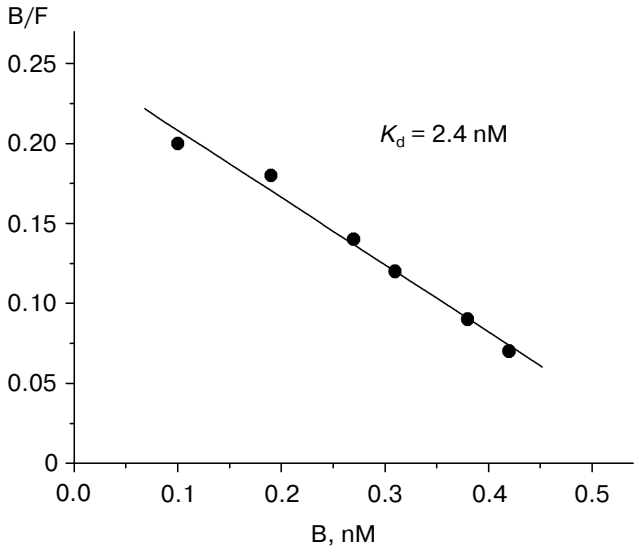


**Fig. 2.** Scatchard analysis of the specific binding of [$^3$H]immunorphine to mouse peritoneal macrophages. B and F are molar concentrations of the bound and free peptide, respectively.

receptor density, i.e., the number of specific binding sites for the labeled peptide per macrophage is 27,780 ± 560.

To characterize the specificity of [$^3$H]immunorphine binding to macrophages as possible competitors the following unlabeled compounds were tested: β-endorphin, Fc-fragment of human IgG1, pentarphine (fragment 6-10 of immunorphine), cyclopentarphine, [Pro$^3$]pentarphine, naloxone, and [Met$^5$]enkephalin. The results are presented in Table 2. Fc-fragment, β-endorphin, pentarphine, cyclopentarphine, and [Pro$^3$]pentarphine actively inhibited the binding of [$^3$H]immunorphine to macrophages: the corresponding values of $K_i$ were as follows: 0.0060 ± 0.0004, 2.9 ± 0.2, 2.7 ± 0.2, 2.6 ± 0.2, and 2.8 ± 0.2 nM. Thus, the receptors detected using [$^3$H]immunorphine displayed high affinity for β-endorphin, Fc-fragment of human IgG1, pentarphine, cyclopentarphine, and [Pro$^3$]pentarphine, but did not interact with naloxone and [Met$^5$]enkephalin.

Treatment of macrophages with trypsin resulted in their loss of ability for specific binding of [$^3$H]immunorphine. This suggested that the receptor should be a protein (at least, its part directly involved in the binding).

**Inhibition of specific binding of [$^3$H]immunorphine by Fc-fragment of human IgG1.** Figure 3 presents curves of inhibition of the specific binding of 4.2 and 8.4 nM [$^3$H]immunorphine (curves *1* and *2*, respectively) in the presence of unlabeled Fc-fragment of human IgG1. Fc-Fragment 100% inhibited the binding of the labeled peptide: both curves flattened out at the Fc-fragment concentration of about $10^{-7}$ M. The displacement of the inhibition curve to the left (to lower concentrations of Fc-fragment) with decrease in the concentration of [$^3$H]immunorphine suggests a competitive inhibition [20]. Thus, Fc-fragment of IgG1 competes with [$^3$H]immunorphine for the binding to the non-opioid β-endorphin receptor on mouse peritoneal macrophages ($K_i$ = 6.0 ± 0.4 pM).

**Binding of [$^3$H]immunorphine to membranes from various rat organs.** No specific binding of [$^3$H]immunorphine was detected to membranes from rat liver, lungs, kidneys, and intestine. Specific binding sites for [$^3$H]immunorphine were found on membranes isolated from rat adrenals, spleen, myocardium, and brain. The specific binding of 8.4 nM [$^3$H]immunorphine to the membranes (fmol/mg membrane protein) decreased in

**Table 2.** Inhibition of [$^3$H]immunorphine binding to mouse peritoneal macrophages by unlabeled Fc-fragment of human IgG1, peptides, and naloxone

| Ligand | $K_d$, nM | $K_i$, nM |
|---|---|---|
| [$^3$H]Immunorphine | 2.4 ± 0.1 | – |
| Fc-Fragment | – | 0.0060 ± 0.0004 |
| β-Endorphin | – | 2.9 ± 0.2 |
| Pentarphine | – | 2.7 ± 0.2 |
| Cyclopentarphine | – | 2.6 ± 0.2 |
| [Pro$^3$]Pentarphine | – | 3.0 ± 0.2 |
| Naloxone | – | > $10^5$ |
| [Met$^5$]Enkephalin | – | > $10^5$ |

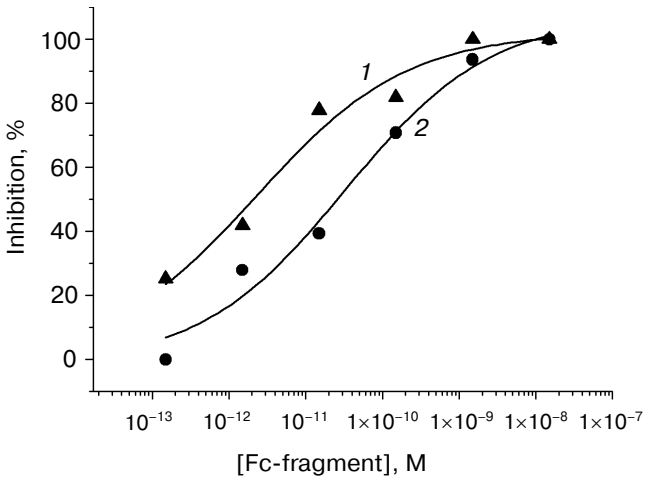


**Fig. 3.** Inhibition by unlabeled Fc-fragment of human IgG1 of the specific binding of $4.2\cdot10^{-9}$ and $8.4\cdot10^{-9}$ M [$^3$H]immunorphine to mouse peritoneal macrophages (curves *1* and *2*, respectively).

the series as follows: adrenals > spleen > myocardium > brain (Table 3). Table 4 presents data on inhibition of the specific binding of [$^3$H]immunorphine to the membranes from the above-listed organs by unlabeled naloxone, Fc-fragment of IgG1, and peptides. All compounds tested except naloxone displayed inhibitory activity. Peptides (β-endorphin, pentarphine, [Pro$^3$]pentarphine, cyclopentarphine, cyclodipentarphine) virtually completely inhibited the binding of [$^3$H]immunorphine. The inhibitory activity of Fc-fragment of IgG1 in the case of membranes from spleen and brain was also close to 100% (98.6 ± 9.9 and 98.6 ± 8.6, respectively); in the case of membranes from adrenals and brain, this parameter was 80.3 ± 7.3 and 67.5 ± 3.9%.

## DISCUSSION

We have earlier shown that the decapeptide immunorphine (SLTCLVKGFY), which corresponds to the 364-373 sequence of H-chain of human IgG (1-4) is a selective agonist of the non-opioid (insensitive to naloxone) β-endorphin receptor [7]. To detect and characterize such receptors, in our previous studies we used $^{125}$I-labeled immunorphine, and for the present work [$^3$H]immunorphine with the specific activity of 24 Ci/mmol was prepared by the HTCIE reaction. The specificity of the non-opioid β-endorphin receptor and its distribution in the rat body were studied using this preparation.

**Table 3.** Specific binding of 8.4 nM [$^3$H]immunorphine to membrane fractions from various rat organs

| Organ | Specific binding of [$^3$H]immunorphine, fmol/mg protein ± SEM |
|---|---|
| Adrenals | 1146.0 ± 44.7 |
| Spleen | 698.6 ± 28.1 |
| Myocardium | 279.1 ± 15.4 |
| Brain | 172.2 ± 1.8 |

The Scatchard plot presented in Fig. 2 shows that [$^3$H]immunorphine is bound to one type of high affinity receptors on mouse peritoneal macrophages ($K_d$ = 2.4 ± 0.1 nM). The receptor density ($n$) was 27,780 ± 560 binding sites for the labeled peptide per macrophage. Earlier we have prepared the 6-10 fragment (pentarphine) of immunorphine and its cyclic analog (cyclopentarphine), and both pentarphine and cyclopentarphine, similarly to immunorphine, stimulated the *in vitro* bactericidal activity of macrophages [11, 12]. $^{125}$I-Labeled pentarphine bound to mouse peritoneal macrophages with high affinity ($K_d$ = 3.6 ± 0.3 nM, $n$ = 28,000 ± 300). The virtually complete coincidence of $n$ values obtained in two different test-systems suggested that the resulting kinetic characteristics of the non-opioid β-endorphin receptor of macrophages should be near to their true values.

As to specificity of the [$^3$H]immunorphine binding to macrophages, it was insensitive to naloxone and [Met$^5$]enkephalin, but was 100% inhibited by unlabeled β-endorphin ($K_i$ = 2.9 ± 0.2 nM), pentarphine (2.7 ± 0.2 nM), cyclopentarphine (2.6 ± 0.2 nM), and [Pro$^3$]pentarphine (3.0 ± 0.2 nM) (Table 2). It was earlier shown that unlabeled immunorphine, β-endorphin, and cyclopentarphine completely inhibited the binding of $^{125}$I-labeled pentarphine to mouse peritoneal macrophages (the corresponding values of $K_i$ were 3.2 ± 0.3, 2.8 ± 0.2, and 2.6 ± 0.3 nM) [11]. Thus, immunorphine, cyclopentarphine, and [Pro$^5$]pentarphine display a high (approximately the same) affinity for the non-opioid β-endorphin receptor; therefore, any of these peptides may be used as a marker of this receptor. Cyclopentarphine and [Pro$^5$]pentarphine, which are less sensitive to proteases, are the most interesting in this relation.

At present, it is unclear whether the non-opioid β-endorphin receptor is a so far unknown β-endorphin receptor, or it is an already known receptor, which in addition to its own endogenous ligand can also bind β-endorphine. On consideration that the immunorphine sequence is a constituent of Fc-fragment of IgG (1-4) and that IgG Fc receptors (FcγR) play the key role in the regulation of phagocytic activity of macrophages [23, 24], we tried to determine whether the non-opioid β-endorphin receptor and FcγR are related. The FcγR family includes three types of receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). Macrophages express both high affinity FcγRI and low affinity FcγRII and FcγRIII [23]. Figure 4 presents the region of the Fc-fragment of human

**Table 4.** Inhibition by various unlabeled ligands of the specific binding of 8.4 nM [$^3$H]immunorphine to membranes from rat adrenals, spleen, myocardium, and brain

| Ligand | Inhibition, % ± SEM | | | |
|---|---|---|---|---|
| | adrenals | spleen | myocardium | brain |
| Naloxone (1 μM) | 0 | 0 | 0 | 0 |
| β-Endorphin (0.1 mM) | 93.2 ± 5.3 | 93.7 ± 5.0 | 99.5 ± 2.3 | 100.0 ± 6.4 |
| Fc-fragment (1.5 nM) | 80.3 ± 7.3 | 98.6 ± 9.9 | 67.5 ± 3.9 | 98.6 ± 8.6 |
| Pentarphine (0.1 mM) | 97.2 ± 4.4 | 100.0 ± 3.0 | 100.0 ± 4.8 | 100.0 ± 8.4 |
| Cyclopentarphine (0.1 mM) | 89.2 ± 5.3 | 98.8 ± 9.9 | 93.3 ± 4.8 | 100.0 ± 7.1 |
| Cyclodipentarphine (0.1 mM) | 72.0 ± 4.2 | 100.0 ± 7.9 | 79.9 ± 1.9 | 100.0 ± 11.6 |
| [Pro$^3$]Pentarphine | 93.0 ± 8.8 | 100.0 ± 7.4 | 100.0 ± 5.3 | 100.0 ± 6.3 |

IgG1, which contains sequences of immunorphine and the endogenous macrophage-stimulating peptide tuftsin [25] and also binding sites for FcγRI (Gly316-Ala339, data of mutagenesis) [26] and FcγRIII (Leu234-Ser239, Asp265-Glu269, Asn297-Thr299, Ala327-Ile332, X-crystallography data for the complex of Fc-fragment of human IgG1 with FcγRIII) [27]. The sequences of Fc-fragment corresponding to tuftsin and immunorphine are not constituents of the binding sites for FcγRI and FcγRIII and, consequently, cannot be directly involved in the binding to Fc-receptors of types I and III. The sequences of Fc-fragment corresponding to tuftsin and immunorphine cannot also interact with FcγRII because the binding of IgG1 to this type of Fc-receptors is provided for by the $C_{H1}$ and $C_{H2}$ domains (the sites Ser109-Val116, Phe129-Thr135, Asn154-Ser161 [28], Leu234-Gly237 [29] and, possibly, the sites Asp265-Pro271 and Lys326-Ala330 [30, 31]) (Fig. 4). However, findings of the present work show that unlabeled Fc-fragment competitively inhibits the specific binding of [$^3$H]immunorphine to mouse peritoneal macrophages, and the value of $K_i$ = 6.0 ± 0.4 pM suggests the high affinity of Fc-fragment for the non-opioid β-endorphin receptor of macrophages. However, unlabeled immunorphine was earlier shown to weakly (less than by 10%) replace $^{125}$I-labeled IgG1 from the complex with the receptor on macrophages [3]. Consequently, the non-opioid β-endorphin receptor is unlike FcγR and the ability of Fc-fragment of IgG for binding to the non-opioid β-endorphin receptor is explained by the presence of the immunorphine sequence in the Fc-fragment. A similar situation has been described for the reception of tuftsin: Fc-fragment actively displaced [$^3$H]tuftsin from its complex with the receptor on macrophages [32], but unlabeled tuftsin virtually did not inhibit the binding of $^{125}$I-labeled IgG1 to macrophages and monocytes [33]. Tuftsin is shown to have its own receptor not of the Fc-receptor family [34, 35]. Thus, in addition to Fc-receptors, the tuftsin and non-opioid β-endorphin receptors are involved in the regulation of functional activity of macrophages.

The distribution of the non-opioid β-endorphin receptor in the rat body was studied, and it was found on membranes of adrenals, spleen, myocardium, and brain. The binding of [$^3$H]immunorphine to membranes of these organs was inhibited by unlabeled β-endorphin, pentarphine, [Pro$^3$]pentarphine, cyclopentarphine, cyclodipentarphine, and Fc-fragment of IgG1. Thus, the

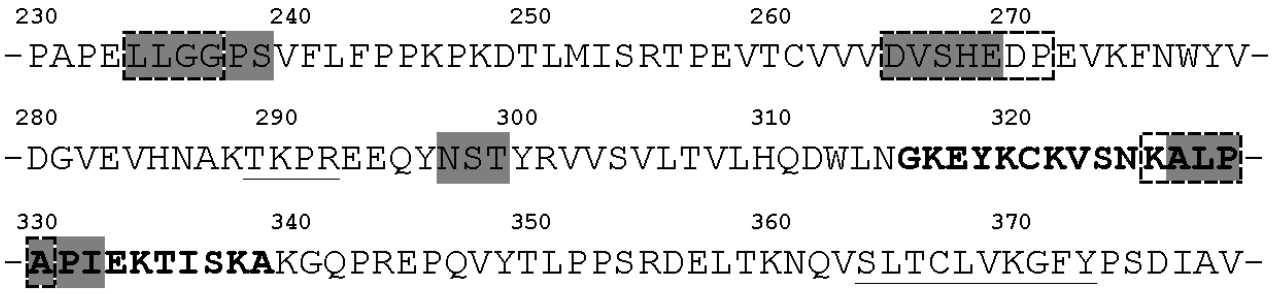


**Fig. 4.** Amino acid sequence of the region of Fc-fragment of human IgG1 [25] which includes the sequences of tuftsin (289-292) and immunorphine (364-373) (underlined) and also the binding sites for FcγRI (bold type), FcγRII (framed), and FcγRIII (shaded).

non-opioid β-endorphin receptors of immunocompetent cells, brain, myocardium, spleen, and adrenals have the same specificity. No specific binding of [$^3$H]immunorphine was detected on membranes from rat liver, lungs, kidneys, and intestine.

In total, the non-opioid β-endorphin receptor is rather widely distributed in the body: it is found on cells of the immune (macrophages and lymphocytes), nervous (brain), endocrine (adrenals), and cardiovascular (myocardium) systems. It should be also noted that, in addition to β-endorphin, Fc-fragment of IgG is also an endogenous ligand of this receptor.

This work was supported by the Russian Foundation for Basic Research (project No. 02-04-48424) and the International Science and Technology Center (project No. 1462).

## REFERENCES

1. Julliard, J. H., Shibasaki, T., Ling, N., and Guilemin, R. (1980) *Science*, **208**, 183-185.
2. Houck, J. C., Kimball, C., Chang, C., Pedigo, N. W., and Yamamura, H. I. (1980) *Science*, **207**, 78-80.
3. Zav'yalov, V. P., Zaitseva, O. R., Navolotskaya, E. V., Abramov, V. M., Volodina, E. Yu., and Mitin, Y. V. (1996) *Immunol. Lett.*, **49**, 21-26.
4. Navolotskaya, E. V., Malkova, N. V., Lepikhova, T. N., Krasnova, S. B., Zargarova, T. A., Zav'yalov, V. P., and Lipkin, V. M. (2001) *Bioorg. Khim.*, **27**, 359-363.
5. Navolotskaya, E. V., Zargarova, T. A., Malkova, N. V., Lepikhova, T. N., Zav'yalov, V. P., and Lipkin, V. M. (2001) *Peptides*, **22**, 2009-2013.
6. Navolotskaya, E. V., Malkova, N. V., Zargarova, T. A., Krasnova, S. B., and Lipkin, V. M. (2002) *Biochemistry (Moscow)*, **67**, 357-363.
7. Navolotskaya, E. V., Zargarova, T. A., Malkova, N. V., Krasnova, S. B., Zav'yalov, V. P., and Lipkin, V. M. (2002) *Biochem. Biophys. Res. Commun.*, **292**, 799-804.
8. Navolotskaya, E. V., Zargarova, T. A., Malkova, N. V., Krasnova, S. B., Zav'yalov, V. P., and Lipkin, V. M. (2002) *Peptides*, **23**, 1115-1119.
9. Malkova, N. V., Krasnova, S. B., Navolotskaya, E. V., Zargarova, T. A., and Prasolov, V. S. (2002) *Rus. J. Immunol.*, **7**, 231-237.
10. Sakharova, N. Yu., Lepikhova, T. N., Lepikhov, K. A., Malkova, N. V., Navolotskaya, E. V., and Chailakhyan, L. M. (2002) *Dokl. Akad. Nauk*, **385**, 258-261.
11. Navolotskaya, E. V., Kolobov, A. A., Kampe-Nemm, E. A., Zargarova, T. A., Malkova, N. V., Krasnova, S. B., Kovalitskaya, Yu. A., Zav'yalov, V. P., and Lipkin, V. M. (2003) *Biochemistry (Moscow)*, **68**, 34-41.
12. Navolotskaya, E. V., Zargarova, T. A., Malkova, N. V., Zharmukhamedova, T. Yu., Kolobov, A. A., Kampe-Nemm, E. A., Yurovsky, V. V., and Lipkin, V. M. (2003) *Biochem. Biophys. Res. Commun.*, **303**, 1065-1072.
13. Hazum, E., Chang, K. J., and Cuatrecasas, P. (1979) *Science*, **205**, 1033-1035.
14. Tischenko, V. M., Abramov, V. M., and Zav'yalov, V. P. (1998) *Biochemistry*, **37**, 5576-5581.
15. Zolotarev, Yu. A., Dorokhova, E. M., Nezavibatko, V. N., Borisov, Yu. A., Rosenberg, S. G., Velikodvorskaia, G. A., Neumivakin, L. V., Zverlov, V. V., and Myasoedov, N. F. (1995) *Amino Acids*, **8**, 353-365.
16. Zolotarev, Yu. A., Dadayan, A. K., Bocharov, E. V., Borisov, Yu. A., Vaskovsky, B. V., Dorokhova, E. M., and Myasoedov, N. F. (2003) *Amino Acids*, **24**, 325-333.
17. Uchitel, I. Ya. (1978) *Macrophages in Immunity* [in Russian], Meditsina, Moscow, pp. 168-180.
18. Farra, C. D., Zsurger, N., Vincent, J.-P., and Cupo, A. (2000) *Peptides*, **21**, 577-587.
19. Lowry, O. H., Rosenbrough, N. J., Farr, O. L., and Randall, R. J. (1951) *J. Biol. Chem.*, **193**, 265-275.
20. Chang, K.-J., Jacobs, S., and Cuatrecasas, P. (1975) *Biochim. Biophys. Acta*, **406**, 294-303.
21. Chang, Y. C., and Prusoff, W. H. (1973) *Biochem. Pharmacol.*, **22**, 3099-3108.
22. Kostanyan, I. A., Merkulova, M. I., Navolotskaya, E. V., and Nurieva, R. I. (1997) *Immunol. Lett.*, **58**, 177-180.
23. Gessner, J. E., Heiken, H., Tamm, A., and Schmidt, R. E. (1998) *Ann. Hematol.*, **76**, 231-248.
24. Fitzer-Attas, C. J., Lowry, M., Crowley, M. T., Finn, A. J., Meng, F., DeFranco, A. L., and Lowell, C. A. (2000) *J. Exp. Med.*, **191**, 669-682.
25. Shields, R. L., Namenuk, A. K., Hong, K., Meng, Y. G., Rae, J., Briggs, J., Xie, D., Lai, J., Stadlen, A., Li, B., Fox, J. A., and Presta, L. G. (2001) *J. Biol. Chem.*, **276**, 6591-6604.
26. Najjar, V. A., and Nashioka, K. (1970) *Nature*, **228**, 672-673.
27. Sondermann, P., Huber, R., Oosthuizen, V., and Jacob, U. (2000) *Nature*, **406**, 267-273.
28. Hulett, M. D., Witort, E., Brinkworth, R. I., McKenzie, I. F. C., and Hogarth, P. M. (1995) *J. Biol. Chem.*, **270**, 21188-21194.
29. Shields, R. L., Namenuk, A. K., Hong, K., Meng, Y. G., Rae, J., Briggs, J., Xie, D., Lai, J., Stadlen, A., Li, B., Fox, J. A., and Presta, L. G. (2001) *J. Biol. Chem.*, **276**, 6591-6604.
30. Maxwell, K. F., Powell, M. S., Hulett, M. D., Barton, P. A., McKenzie, I. F. C., Garrett, P. T. J., and Hogarth, P. M. (1999) *Nat. Struct. Biol.*, **6**, 437-442.
31. Sondermann, P., Huber, R., and Jacob, U. (1999) *EMBO J.*, **18**, 1095-1103.
32. Gottlieb, P., Stabinsky, Y., Hiller, Y., Beretz, A., Hazum, E., Tzehoval, E., Feldman, M., Segal, S., Zakuth, V., Spirer, Z., and Fridkin, M. (1983) *Ann. N. Y. Acad. Sci.*, **419**, 93-106.
33. Ratliffe, A., and Stanworth, D. R. (1982) *Immunol. Lett.*, **4**, 215-221.
34. Dagan, S., Gottlieb, P., Fridkin, M., Spirer, Z., Tzehoval, E., and Feldman, M. (1986) *Receptors*, **3**, 243-280.
35. Siemion, I. Z., and Kluczyk, A. (1999) *Peptides*, **20**, 645-674.