dence of the existence of a specialized transport process, characterized by saturation. Several transport systems are known to exist in the small intestine, each of them specific for the absorption of a concrete class of natural compounds: monosaccharides, amino acids, pyrimidines and purines [6]. E is structurally similar to the xanthines, and its molecule, moreover, is similar to that of adenine [4]. The existence of two processes, responsible for transport through the intestinal epithelium, has been demonstrated for purine and pyrimidine bases: 1) a specialized transport process characterized by saturation and competition with certain other substances; 2) passive diffusion, the rate of which is unaffected by the presence of other compounds [8, 9]. It can be tentatively suggested that in dogs at least some E passes through the intestinal epithelium by means of the specialized transport system for structurally similar natural compounds, most probably purine bases.

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EFFECT OF β-ENDORPHIN AND MYELOPEPTIDES ON cAMP LEVEL AND PROLIFERATION OF LYMPHOCYTES IN VITRO

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Endogenous opioids, and β-endorphin in particular, are secreted into the blood stream and take part in regulating the functional state of immunocompetent cells [1, 3, 10], although data on this matter are highly contradictory [10, 11, 14]. Meanwhile there are grounds for considering that opioids synthesized actually in the cells and organs of the immune system participate in the regulation of immunogenesis. In particular, myelopeptides (MP), which are produced by bone marrow cells and stimulate antibody production [1, 7], contain substances capable of interacting with opiate receptors [8] and with sera against α-, β-, and γ-endorphins, and β-lipotropin [4]. There is also evidence suggesting that opioids are synthesized in the thymus [5], spleen [13], and circulating leukocytes [9].

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Accordingly, the investigation described below was undertaken, with two aims: the continuous study of the immunomodulating properties of β-endorphin and to test the hypothesis that opioids may contribute to the mechanism of the effects of MP, which belong to the class of immunologically active preparations.

EXPERIMENTAL METHOD

Lymphocytes were isolated from heparinized peripheral blood of healthy donors aged from 20 to 40 years by gradient centrifugation in a Ficoli—Verografin system. The cells were then washed twice with medium 199. The cAMP level in the samples ($2 \cdot 10^6$-$4 \cdot 10^6$ lymphocytes in 0.3 ml of medium 199, made up with 10 mM HEPES) was determined after incubation of the lymphocytes for 5 min at 37°C, by means of a kit of reagents from Amersham International (England). To determine their proliferative activity lymphocytes ($2 \cdot 10^5$ in 0.2 ml of medium) were cultured at 37° in medium 199, made up with 2 mM HEPES, 10% fetal calf serum, 16 μg gentamicin, and 0.4 mM glutamine. Phytohemagglutinin (PHA, from Sigma, USA) in a dose of 0.625-15 μg/ml or pokeweed mitogen (PWM, from Serva, West Germany) in a dose of 10^{-3}-1 μg/ml, was added to some of the samples. Proliferative activity of lymphocytes stimulated by PHA and PWM was estimated by a radioisotope method based on incorporation of ^{3}H-thymidine after 72 and 120 h, respectively. The effects of MP and β-endorphin were studied by adding them to the medium simultaneously with the mitogens or, in the case of cAMP determination, immediately before incubation of the cells. The dose of MP was estimated in microgram-equivalents of protein, determined by Lowry's method. To study the possibility of blocking the effects of the preparations by an opiate antagonist, simultaneously with MP and β-endorphin, naloxone (10^{-6} M) was added to the medium. The viability of the cells, assessed by incorporation of trypan blue, was the same in the experimental and control series, and was not less than 90%.

The preparation of MP used in the work [1, 7] was generously provided by A. A. Mikhailova (Institute of Immunology, Ministry of Health of the USSR); the β-endorphin was synthesized and presented by M. I. Titov (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR).

EXPERIMENTAL RESULTS

β-Endorphin did not change spontaneous or PHA-induced proliferative activity of the lymphocytes. Meanwhile, in concentrations of 10^{-11}, 10^{-10}, and 10^{-9} M, the opioid significantly ($P < 0.05$) reduced incorporation of ^{3}H-thymidine into cells stimulated by optimal doses of PWM (10^{-3}-1 μg/ml) by 79, 75, and 69%, respectively. Although the character of the dose-dependent curves for the action of β-endorphin was identical for all 12 donors tested, the maximal effect of the opioid was observed in one donor with β-endorphin in a concentration of 10^{-12} M, in three with 10^{-11} M, and in two groups (with four donors in each) with 10^{-10} and 10^{-9} M. The effect of β-endorphin was not blocked by naloxone.

Individual differences also were found when the effect of β-endorphin on the intracellular cAMP level was studied. Just as with its action on proliferative activity, β-endorphin had a sufficiently similar effect on lymphocytes from all donors studied. However, the concentration (C_m) with which the strongest action of the opioid was observed (elevation of the cAMP level by 15-43% of the control) varied from 10^{-10} to 10^{-8} M. Accordingly, it was only by using C_m as a conventional starting point for counting concentration that the significance of the effect of the opioid in the whole group could be found on statistical analysis of the data (Fig. 1b). As will be clear from Fig. 1 naloxone blocked this action of β-endorphin. It is an interesting fact that β-endorphin, depending on its concentration in the medium, can either raise or lower the cAMP level in cells (Fig. 1). This is probably to do with the ability of the opioid to interact with opiate receptors of μ- and δ-types, which exert opposite effects on the cAMP level in lymphocytes [3, 6].

It can be concluded from these results and data in the literature that β-endorphin interacts with a heterogeneous population of lymphocyte opiate receptors. Receptors of one type (ε-receptors), interacting with the C-terminal region of the peptide [12], are evidently involved in naloxone-independent regulation of proliferative activity of lymphocytes. Evidence of this is given both by our own data and those published previously [11, 14]. Receptors of other types (μ- and δ-receptors), which interact with the N-terminal fragment of β-endorphin, participate in regulation of the cAMP level in lymphocytes (Fig. 1) and various effector functions of these cells [10], which are blocked by naloxone.

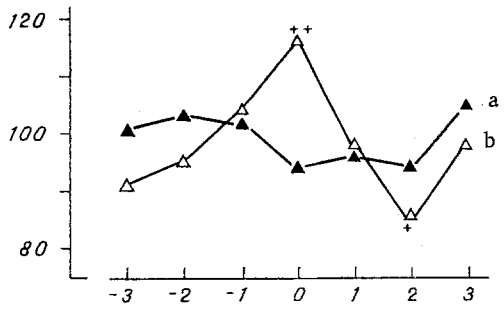

Fig. 1

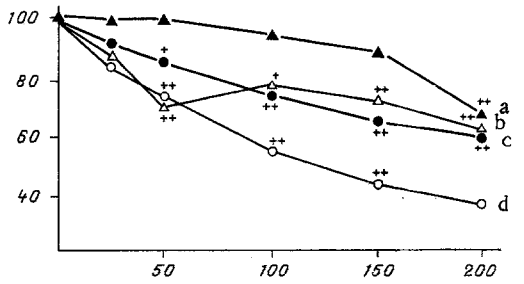

Fig. 2

Fig. 1. Dependence of cAMP level in human peripheral blood lymphocytes on β-endorphin concentration in medium with naloxone 1 μM (a) and in the absence of naloxone (b). Abscissa, log C_m − log C (where C_m is the β-endorphin concentration with which maximal elevation of the cAMP level is observed, C is the β-endorphin concentration in medium). Ordinate, cAMP level in lymphocytes (in % of control, namely 5-15 pmoles/10^6 cells). Each value is mean of 11 independent experiments. Here and in Fig. 2: *P < 0.05, **P < 0.01.

Fig. 2. Effect of MP on cAMP level in lymphocytes in medium containing 1 μM naloxone (a), or without naloxone (b) and on proliferative activity of cells stimulated by PWM (c) and PHA (d). Abscissa, concentration of MP (in μg-eq protein/ml). Ordinate, value of test parameter (in % of control, amounting to 500-700 and 500-1000 cpm, respectively, when PHA and PWM were used). Each value is mean of nine and five independent experiments to determine the corresponding level of cAMP and incorporation of ^{3}H-thymidine into stimulated lymphocytes.

The data given above, together with elevation of the blood β-endorphin level observed during the development of reactions to stress [15], suggest that β-endorphin is involved in the pathogenesis of the immunodepression observed during the development of stress [1].

It was shown in the second part of the investigation (Fig. 2c, d) that MP, while not affecting spontaneous proliferation of lymphocytes, significantly reduces incorporation of ^{3}H-thymidine into cells stimulated by optimal doses of PHA (1.25-10 μg/ml) and PWM (10^{-3}-1 μg/ml). Naloxone did not block these effects. MP causes the cAMP level in the lymphocytes to fall, and this effect is blocked by naloxone (Fig. 2a, b). Addition of bacitracin (50 μg/ml) to the reaction medium did not affect the intracellular cAMP level but depressed the effect of MP. In the presence of bacitracin, for instance, MP (50 μg/ml) lowered the cAMP level to 86% of the control value, and in its absence, to 73% (Fig. 2b). The effects found were observed in the presence of concentration of MP with which their antibody-stimulating action is observed in vitro [1, 7].

The similarity between the action of MP and β-endorphin on the state of the lymphocytes is thus limited to an effect on proliferation of PWM-stimulated cells, which is not blocked by naloxone (Fig. 2c) and to the effect on antibody production described previously [1, 7]. However, the fact that the opiate antagonist blocks the effect of MP on the cAMP level (Fig. 2a, b), which coincides with the action of Met-encephalin [3], suggests that ligands of opiate receptors of the δ-type take part in the mechanism of the immunomodulating effects of MP. This hypothesis is confirmed also by data published previously on the content of ligands with greater ability to interact with opiate receptors of δ-type, than of μ-type, in the composition of MP [8].

In view of these results, in the writers view investigations of the immunomodulating properties of synthetic ligands of opiate receptors of δ-type would be promising. Besides, further analysis of the causes of the marked individual sensitivity of lymphocytes from different blood donors to the action of β-endorphin may provide an approach to the development of a method of predicting the action of opioids on development of immune responses in vivo.

Effects of β-endorphin on the state of lymphocytes are mediated by several types of receptors on cells, but opioids present in the composition of MP take part in the mechanism of the effects of this preparation on the functional state of immunocompetent cells.

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CHANGES IN EFFECTS OF SODIUM ARACHIDONATE IN VITRO AND IN VIVO PRODUCED BY NONSTEROID ANTIINFLAMMATORY AGENTS

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KEY WORDS: nonsteroid antiinflammatory agents; arachidonic acid; mediators of inflammation.

In the modern view an essential role in the mechanism of action of nonsteroid antiinflammatory agents (NSAIA) is played by their inhibitory effect on prostaglandin (PG) biosynthesis [3, 5, 6], in which arachidonic acid is an endogenous precursor [2, 4].

In experiments in vitro and in vivo the writers have compared the effects of various NSAIA [acetylsalicylic acid (aspirin), ibuprofen, dichlofenac sodium, butadione, indomethacin] on the effects of sodium arachidonate (SA), a water-soluble salt of arachidonic acid.

EXPERIMENTAL METHOD

The effect of NSAIA on the spasmogenic effect of SA was studied on isolated segments of the ileum from guinea pigs of both sexes weighing 250-400 g. The tone of the smooth-muscle organs was recorded under isotonic conditions by mechano-electronic transducers (Hugo Sachs Elektronik, West Germany). The NSAIA (10^{-9}-10^{-5} g/ml), dissolved in Ringer's solution, were added to the jar containing the organ 3 min before SA ($5 \cdot 10^{-8}$ g/ml).

The effect of NSAIA on the spasmogenic action of other "mediators" of inflammation [histamine ($5 \cdot 10^{-8}$ g/ml), serotonin ($2 \cdot 10^{-6}$ g/ml), bradykinin (10^{-8} g/ml), PGE_2 ($5 \cdot 10^{-4}$ g/ml)] was studied in experiments on isolated segments of guinea pig ileum, and the effect of NSAIA on the inflammatory action of these "mediators" was studied on a model of acute inflammation

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