immunomodulating activity; this paves the way for targeted addition of reagents containing reactive platinum groups to immunologic preparations, with the aim of obtaining biologically active complexes.

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EFFECT OF STIMULATION OF OPIOID RECEPTORS ON LYMPHOCYTE FUNCTION IN VITRO

D. D. Kharkevich and Z. G. Kadagidze

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The list of mediators of neuroimmune interactions includes opioid peptides (enkephalins, endorphins, dynorphins, etc.) which participate in the regulation of emotional responses, the functioning of peripheral organs, development of the adaptation syndrome, and potentiation of immune responses [1]. In particular, Met-enkephalin has been shown to stimulate activity of natural killer cells [5], EA-rosette formation [7], antibody-dependent cellular cytotoxicity [6], and leukocyte migration [10].

The aim of this investigation was to study the effect of Met-enkephalin on spontaneous adhesion and on phytohemagglutinin- (PHA-) stimulated proliferative activity of healthy human lymphocytes in the blast transformation reaction (BTR) in vitro, using naloxone, a blocker of opioid receptors.

EXPERIMENTAL METHOD

Cells were isolated and spontaneous adhesion of healthy human peripheral blood lymphocytes were studied by the method described previously [2, 3]. A suspension of healthy human lymphocytes in a concentration of $2 \cdot 10^6$ cells/ml in medium 199 with 15% embryonic calf serum, inactivated by heating at 56°C for 30 min, in a volume of 0.1 ml, 0.05 ml of a solution of Met-enkephalin ("Serva," West Germany) and, if necessary, 0.05 ml of naloxone solution ("Hoffmann-LaRoche, USA) were added to the wells of 96-well flat-bottomed plastic panels ("Falcon Plastics," USA). The panels were incubated at 37°C in an atmosphere of 5% CO_2 in a humid chamber for 1.5 h. Cells nonadherent to the bottom of the wells were then separated and counted, as described previously [2, 3]. The reaction was assessed by the usual formula for studying inhibition of lymphocyte adhesion (ILA):

Laboratory of Clinical Radioimmunology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

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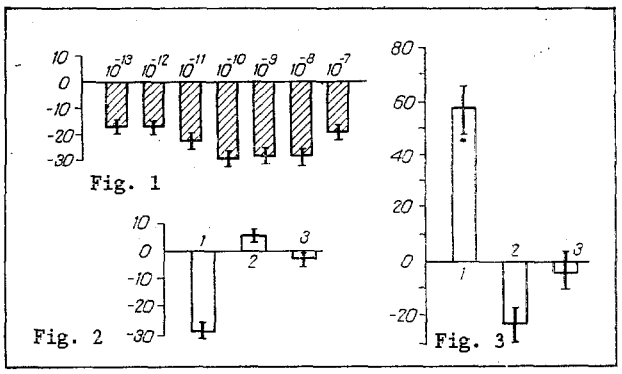

Fig. 1. Effect of Met-enkephalin on spontaneous lymphocyte adhesion. Abscissa, molar concentrations of Met-enkephalin; ordinate, ILA-index (in %).

Fig. 2. Specificity of enhancement of spontaneous lymphocyte adhesion by Met-enkephalin. 1) Met-enkephalin (10^{-9} M); 2) Met-enkephalin (10^{-9} M) + naloxone (10^{-8} M); 3) naloxone (10^{-8} M). Ordinate, ILA index (in %).

Fig. 3. Specificity of enhancement of BTR with PHA by Met-enkephalin. 1) Met-enkephalin (10^{-9} M); 2) Met-enkephalin (10^{-9} M) + naloxone (10^{-8} M); 3) naloxone (10^{-8} M). Ordinate, enhancement index of BTR (in %).

$$\text{ILA index} = (a - b)/b \cdot 100\%,$$

where a denotes the average number of nonadherent cells in the experimental tests and b the average number of nonadherent cells in the control tests. A negative value of the ILA index corresponds to stimulation of lymphocyte adhesion. BTR of the lymphocytes with PHA was set up by the standard method with ^{3}H-thymidine [4]. The enhancement index (EI) of the BTR was calculated by the equation:

$$\text{EI} = (a - b)/b \cdot 100\%,$$

where a is the number of counts per minute in the experimental tests and b the number of counts per minute in the control tests. The significance of the results was estimated by Student's t test.

EXPERIMENTAL RESULTS

Figure 1 gives the results of a study of the action of a Met-enkephalin concentration gradient in vitro, obtained by the study of a group of 16 healthy blood donors. As Fig. 1 shows, addition of Met-enkephalin enhanced spontaneous lymphocyte adhesion within the concentration range studied (10^{-13}-10^{-7} M); the optimal concentration of the preparation for a stimulating effect was 10^{-10}-10^{-8} M.

To study the specificity of action of Met-enkephalin, the opioid receptor blocker naloxone was added to the culture wells. As Fig. 2 shows, addition of naloxone completely abolished the effect induced by Met-enkephalin, whereas addition of naloxone alone, without Met-enkephalin, had no effect on spontaneous lymphocyte adhesion.

Figure 3 shows the effect of Met-enkephalin and naloxone on PHA-stimulated proliferative activity of healthy human lymphocytes. It will be clear from Fig. 3 that the addition of naloxone abolished enhancement of BTR induced by Met-enkephalin. The addition of naloxone alone caused no change in BTR during the response of the lymphocytes to PHA in vitro (Fig. 3).

Met-enkaphalin, incidentally, is destroyed quite quickly by the serum enkephalinase and aminopeptidase, and for that reason the stimulating effects discovered, like the action of acetylcholine [2], are among the early responses of the immune system.

Met-enkephalin thus has a stimulating action on spontaneous lymphocyte adhesion and on the BTR in vitro. This stimulating effect of Met-enkephalin is abolished by naloxone, and this confirms data in the literature indicating the presence of opioid receptors on T cells [1].

The results are in agreement with data obtained by other workers on the stimulating action of Met-enkephalin on a whole range of immunologic functions [5, 7]. Met-enkephalin has been shown to increase the percentage of active T-rosettes, to potentiate expression of OKT 10 and Leu 11 markers and of receptors to interleukin-2, to increase interleukin-2 production, and to increase natural killer cell activity and to raise the *c*AMP level. Other agonists of *c*AMP formation, such as prostaglandin E and forskolin had an additive effect with Met-enkephalin [9].

The study of immunomodulating properties of endogenous opioid neuropeptides is very promising, for they are not compounds that are foreign for the body [8, 9]. Meanwhile their physiological properties can be potentiated by chemical modifications and the optimal substances chosen for adequate immunocorrection.

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