3. B. L. Largent, A. L. Gundlach, and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 81, No. 15, 4983 (1984).
4. M. J. Marks and F. Medzihradsky, Mol. Pharmacol., 10, No. 5, 837 (1974).
5. W. R. Martin, C. G. Eades, J. A. Thompson, et al., J. Pharmacol. Exp. Ther., 197, No. 3, 517 (1976).
6. R. Pechnick, R. George, and R. E. Poland, J. Pharmacol. Exp. Ther., 232, No. 1, 163 (1985).
7. R. Pechnick, R. George, and R. E. Poland, J. Pharmacol. Exp. Ther., 232, No. 1, 170 (1985).
8. C. B. Pert, M. R. Ruff, R. J. Weber, and M. Herkenham, J. Immunol., 135, No. 2, 820 (1985).
9. R. Sircar, R. Nichtenhauser, J. R. Ieni, and S. R. Zukin, J. Pharmacol. Exp. Ther., 237, No. 2, 681 (1986).
10. T.-P. Su, J. Pharmacol. Exp. Ther., 223, No. 2, 284 (1982).
11. S. W. Tam, Proc. Natl. Acad. Sci., USA, 81, No. 17, 5618 (1984).
12. S. W. Tam, Eur. J. Pharmacol., 109, No. 1, 33 (1985).

ACTION OF MYELOPEPTIDES ON DNA AND TOTAL PROTEIN SYNTHESIS IN CELLS OF MOUSE LYMPHOID ORGANS

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Great interest has recently been aroused by the special class of low-molecular-weight (mol. wt. about 2 kilodaltons) peptides of bone marrow origin, namely myelopeptides (MP), discovered by Petrov and co-workers [2-4, 8]. The antibody-stimulating and opioid properties of MP are well known [3, 4, 8], although there are virtually no data on the possible action of MP on integral parameters of cell metabolism such as synthesis of chromosomal DNA and of total protein. In view of the realistic prospects for the use of MP as a therapeutic preparation (B-activin, myelopid), the need for obtaining such data will be evident. At the same time, there is reason to suppose that MP may also have diverse functions [2, 4, 8].

The aim of this investigation was to study the action of MP on replicative DNA synthesis and protein synthesis (without histones) in cells of mouse lymphoid organs.

EXPERIMENTAL METHOD

Cells from nonimmune (except lymph nodes) lymphoid organs of (CBA × C57BL/6)F_1 mice were used. MP were isolated from pig bone marrow by the usual method [1]. The cells were incubated in a concentration of $3 \cdot 10^6$/ml for 2 days in medium RPMI-1640, with essential additives [5] in the presence of MP. The dose of MP in the incubation medium (50 μg/ml) was chosen experimentally within the range from 1 to 100 μg/ml. In some cases 2-mercaptoethanol (ME) was added to the incubation medium in a concentration of $5 \cdot 10^{-5}$ M with or without MP. ^{3}H-thymidine (2 μCi/ml, 19 Ci/mole) together with ^{14}C-amino acids (1 μCi/ml, chlorella digest, Czechoslovakia) were added to the culture 4 h after the end of incubation in 24-well channels (Nuclon, Denmark) in an atmosphere of 5% CO_2 at 37°C. After the end of incubation the cells were transferred into centrifuge tubes and washed twice in an excess of medium 199 in the cold to remove nonspecifically adsorbed radioactive label. The washed cells were suspended in 2 ml of cold water, and the carrier protein (70 μg/ml) and TCA to a final concentration of 10% were added to the suspensions. After the residues had formed the samples were shaken vigorously in the cold for 30 min. Under these conditions histones go into

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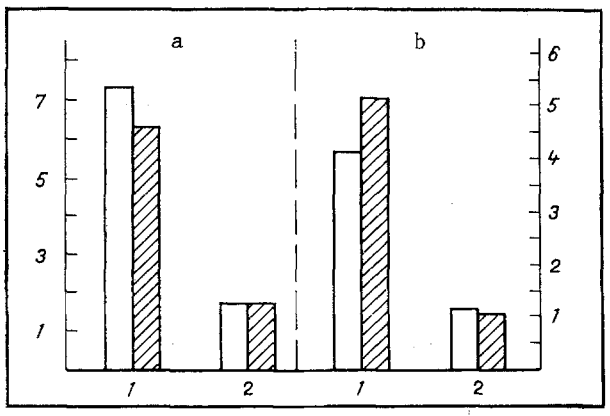

Fig. 1

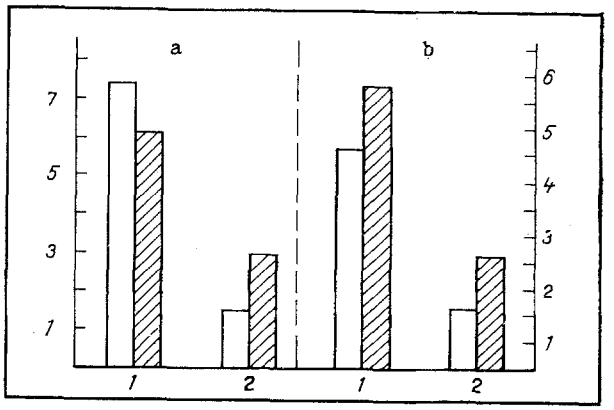

Fig. 2

Fig. 1. Action of MP on synthesis of chromosomal DNA (a) and total protein (b) in mouse bone marrow (1) and spleen (2) cells. Ordinate: on left – content of ^{3}H-label (in cpm·10^{-3}), on right – content of ^{14}C-label (in cpm·10^{-4}). Unshaded columns – control, shaded columns – action of MP.

Fig. 2. Action of ME on synthesis of chromosomal DNA (a) and total protein (b) in mouse bone marrow (1) and spleen (2) cells. Shaded columns – action of ME. Remainder of legend as to Fig. 1.

solution and the residue contains DNA and most of the cell protein. Why it is necessary to exclude histones from the total mass of newly formed cell protein will be stated below. The residues were collected on GFB glass fiber filters (Whatman, England) and washed three times with cold 10% TCA and once with 96% ethanol. The content of the $^{3}H/^{14}C$-label was measured in a scintillation mixture based on toluene, in an SL-40 counter (Intertechnique, France), using a special double label counting program.

EXPERIMENTAL RESULTS

A typical result of the action of MP on DNA and total protein (without histones) synthesis in mouse bone marrow and spleen cells is illustrated in Fig. 1. In the presence of MP in bone marrow cells the level of chromosomal DNA synthesis was reduced (by 13-25% of the control), whereas the level of protein synthesis was simultaneously raised (by 16-21%). Under these same conditions MP had no appreciable effect on these parameters in spleen cells. No changes in the level of DNA and total protein synthesis in the presence of MP likewise were found in cells of the mouse thymus and immune lymph nodes (data not given).

Selectivity of the action of MP on replicative DNA synthesis and total protein synthesis was thus demonstrated in relation to cells of mouse lymphoid organs: MP modify these two parameters only in bone marrow cells and do not affect them in cells of other lymphoid organs studied from this point of view. The action of MP on bone marrow cells under these circumstances is exhibited within a dose range of MP from 25 to 50 μg/ml, in a dose of under 25 μg/ml this effect was not observed, and in a dose of over 50 μg/ml MP had a toxic action on the cells and reduced both DNA and protein synthesis.

Incidentally, the changes in the conditions of DNA and total protein synthesis observed for mouse bone marrow cells were characteristic of the cell differentiation (maturation) process. As is well known, this process is usually accompanied by decline of DNA replication and by an increase in the level of protein synthesis, according to the "either DNA or protein" rule [7]. Clearly, it was necessary to rule out a contribution of DNA-dependent protein synthesis, which is essentially histone synthesis and may account for half of the total synthesis of cell protein [6]. The result thus indicates that MP may exhibit "differential" properties, especially when most of the cells are immature forms. However, how specific is the "differential" effect of MP observed in this case?

It is considered that MP are produced by cells of the monocytic series [2], i.e., that they may perhaps possess the properties of monokines. We also know that thiols can "replace" monocytes in activating lymphokine production, and in some other monocyte-dependent processes [9]. In this connection, to assess the specificity of the "differential" action of MP, we studied the action of ME on DNA and total protein synthesis in cells of mouse lymphoid organs under the same conditions as for MP, as a control.

TABLE 1. Action of MP on DNA and Total Protein Synthesis in Mouse Bone Marrow and Spleen Cells in the Presence of ME

Organ	^{3}H-DNA and ^{14}C-protein content in $3 \cdot 10^6$ cells, percent of control						^{3}H/^{14}C		
	^{3}H-DNA			^{14}C-protein					
	control	MP	MP + ME	control	MP	MP + ME	control	MP	MP + ME
Bone marrow	100	87	115	100	121	131	0,17	0,12	0,17
Spleen	100	100	207	100	100	240	0,14	0,14	0,12

Legend. Control – incubation of cells with neither MP nor ME. Average ^{3}H-DNA content in control for bone marrow cells was 7300 cpm, for spleen cells 1400 cpm. Average content of ^{14}C-protein was 42,000 and 10,000 cpm, respectively.

The typical result of the action of ME on DNA and protein synthesis in mouse bone marrow and spleen cells is shown in Fig. 2. This agent in culture had a significant effect on metabolism of the cells of both these organs. In bone marrow cells the level of DNA synthesis fell by 18-20% of the control, and at the same time the level of total protein synthesis rose by 20-26%; in other words, the action of ME on bone marrow cells was found to be similar to the action of MP on the same cells (Fig. 1). Meanwhile, ME almost doubled the intensity of proliferation of spleen cells (and also of mouse thymus and lymph node cells – data not given), i.e., it had a marked mitogenic action on these cells. Thus ME, like MP, exhibits selectivity of action on DNA and total protein synthesis, which is evidently determined by the nature (state) of the cells themselves. However, this selectivity of action of ME on cells of mouse lymphoid organs differs in principle from that of MP, which indicates definite specificity of action of MP as a possible factor in differentiation of bone marrow cells. Evidence in support of this conclusion is given by the results of determination of the ability of cells from mouse lymphoid organs to bind ^{14}C-labeled MP in vivo [5]. According to these data, bone marrow, which produces MP, is also the chief site of their action. This conclusion is supported by data on the effect of MP on DNA and total protein synthesis in cells of mouse lymphoid organs in the presence of ME.

Table 1 shows that in this case the level of DNA replication for bone marrow cells increased (by 15-20% of the control), whereas at the same time total protein synthesis increased by 31-35%. The ratio ^{3}H/^{14}C, which conventionally characterizes the relationship between proliferation and differentiation, remained the same in this case as in the control (0.17), whereas in the presence of MP alone it decreased (0.12), i.e., it was shifted toward "differentiation." The presence of ME in the incubation medium thus abruptly "modifies" the action of MP on the bone marrow cells which is observed in its absence (Fig. 1): ME abolishes the "differential" effect of MP relative to these cells. Meanwhile MP potentiate the mitogenic action of ME on spleen cells, but only very slightly (Fig. 2; Table 1). In the presence of ME, MP also potentiate proliferation of mouse thymus cells and immune mouse lymph node cells (data not given). Thus in the presence of ME a selective action of MP on DNA and total protein synthesis is not observed with respect to cells of mouse lymphoid organs: in this case MP exhibit their mitogenic properties for cells of all the organs mentioned above. The reasons why the presence of ME "modifies" the action of MP on cells of mouse lymphoid organs, exhibited in its absence, are not yet clar.

Simultaneous assessment of levels of chromosomal DNA and total protein synthesis in cells of mouse lymphoid organs in the presence of MP thus revealed selectivity of their action on integral parameters of cell metabolism in relation to these cells. This selectivity is evidently determined by the nature of the cells themselves. For bone marrow cells, which are mainly immature forms, MP exhibit a "differential" action, but for other lymphoid organs, in which most of the cells are differentiated, no effect of MP on DNA and total protein synthesis could be discovered. Meanwhile, our preliminary data showed that culture of mouse bone marrow cells for 48 h with MP (50 μg/ml) causes changes in the cell composition of the culture compared with the control. Analysis of the myelograms shows that under these conditions the main event taking place is accumulation of mature lymphoid and erythroid forms. The results thus indicate that MP possess the properties of cell differentiation factors.

LITERATURE CITED

1. L. A. Zakharova, A. V. Katlinskii, R. N. Stepanenko, et al., Immunologiya, No. 3, 35 (1986).

2. R. V. Petrov and A. A. Mikhailova, Probl. Gematol., No. 10, 3 (1981).
3. R. V. Petrov and A. A. Mikhailova, Progress in Science and Technology. Series: Immunology [in Russian], Vol. 12, Moscow (1983), pp. 63-85.
4. R. V. Petrov, L. A. Zakharova, and A. A. Mikhailova, Gematol. Transfuziol., No. 2, 43 (1984).
5. L. A. Strelkov, N. B. Chiaureli, and K. A. Kafiani, Mol. Biol., 17, No. 5, 1077 (1983).
6. L. A. Strelkov and R. N. Stepanenko, Byull. Éksp. Biol. Med. (1987).
7. T. Eremenco and P. Volpe, Eur. J. Biochem., 52, 203 (1975).
8. R. V. Petrov, A. A. Mikhailova, L. A. Zakharova, et al., Scand. J. Immunol., 24, 237 (1986).
9. E. E. Roosnek, M. C. Brouwer, and L. A. Aarden, Eur. J. Immunol., 15, 652 (1985).

OPIATE μ-RECEPTOR LIGAND ACCUMULATION IN PHYTOHEMAGGLUTININ-ACTIVATED LYMPHOCYTE CULTURE

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Facts directly or indirectly confirming synthesis and post-translation processing of high-molecular-weight precursors of opioid peptides in cells and organs of the immune system have recently been obtained. For instance, thymus cells [6] and activated lymphocytes [9] have been shown to contain fragments of proopiomelanocortin (POMC), a high-molecular-weight precursor of endorphins, lipotrophin, and corticotrophin. POMC and its fragments also are found in bone marrow [1, 4] and POMC template RNA, and POMC fragments have been identified in splenic macrophages [8]. However, of the peptides formed by POMC processing, only β-endorphin interacts relatively effectively, but with about the same affinity, with opiate receptors of μ- and δ-type. Meanwhile ligands of these receptors in experimental models both in vivo and in vitro have exhibited directly opposite effects on the state of the immunocompetent cells [3, 7]. It was accordingly decided to study the distribution not only of peptides formed as the result of POMC processing, but also of ligands selectively interacting with μ- and δ-receptors, in the immune system.

The aim of this investigation was to determine ligands of opiate receptors (LOR) of μ- and δ-type by radioreceptor analysis in different lymphocyte populations and also to examine the possibility of synthesis of these LOR in immunocompetent cells.

EXPERIMENTAL METHOD

The number of LOR in the lymphocytes was estimated by determining the ability of extracts of these cells to displace labeled LOR from rat brain opiate receptors. Lymphocytes were isolated from the peripheral blood of healthy blood donors (aged 20-30 years) by gradient centrifugation in a Ficoll–Verografin system. T lymphocytes and a population enriched with B cells were isolated by spontaneous rosette formation [10]. Some specimens of the total lymphocyte population ($1.2 \cdot 10^7$ cells/ml) were incubated with phytohemagglutinin (PHA, 6 μg/ml) for 3 h at 37°C in medium 199 with the addition of 2 mM HEPES and 16 μg/ml of gentamicin. After incubation the lymphocytes were washed to remove PHA, cultured at 37°C, and sedimented. LOR were extracted from the lymphocytes with acetic acid, under the conditions described previously [2]. The samples were boiled in 1 M acetic acid for 15 min (ratio of volume of cell residue to volume of acid 1:10). The yield of extracts was about 1 mg from $3 \cdot 10^6$ cells.

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