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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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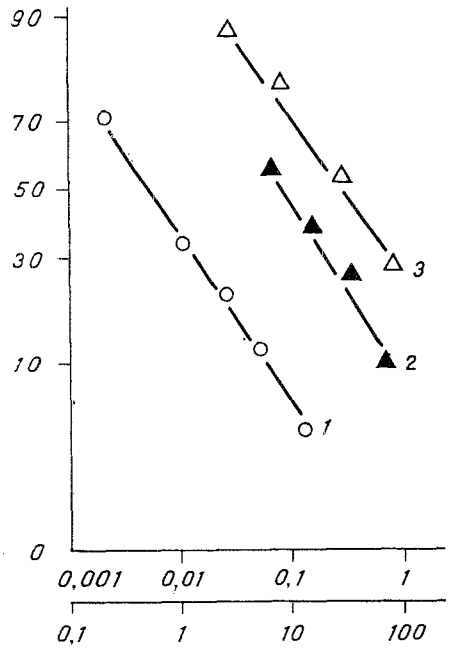

Fig. 1

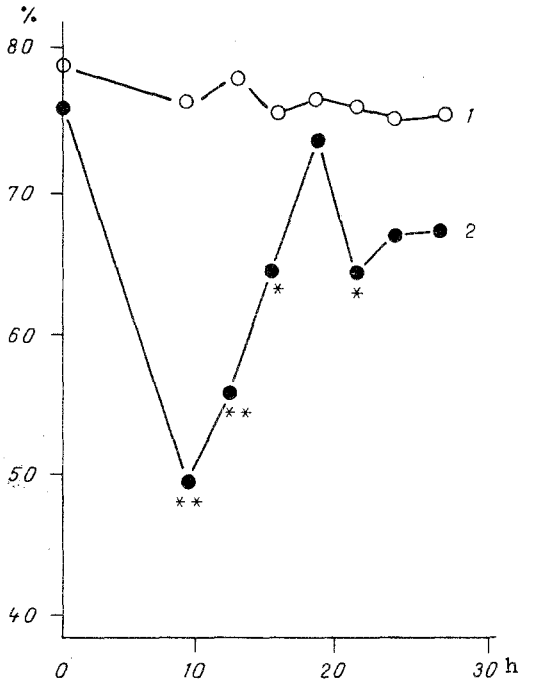

Fig. 2

Fig. 1. Comparative ability of D-Ala^2-Met-enkephalinamide (1) and of extracts of activated (2) and unactivated (3) lymphocytes to displace ^{3}H-D-Ala^2-Met-enkephalinamide from opiate receptors. Abscissa: top – concentration of D-Ala^2-Met-enkephalinamide (in μM), bottom – number of cells ($\cdot 10^6$) used to obtained extracts, added to 1 ml of incubation mixture; ordinate, level of specific binding (in % of control). Duration of culture of lymphocytes 9 h after rinsing to remove PHA. Results of one of four independent experiments are shown.

Fig. 2. Displacement of ^{3}H-DAMEA by extracts of unactivated lymphocytes (1) and of lymphocytes cultured after activation by PHA (2). Abscissa, duration of culture after rinsing to remove PHA (in h); ordinate, level of specific binding (in % of control). Extracts obtained from 10^7 cells were added to each sample. Each point on the curves in the mean value of four independent experiments, conducted in two parallel samples. *$p < 0.05$, **$p < 0.01$ (Student's t test) compared with specific binding determined in the presence of extracts from unactivated cells.

Membrane fraction of rat brain containing opiate receptors was isolated and radioreceptor analysis carried out as described previously [2]. The reaction mixture, in a volume of 1 ml, contained 0.8-1.2 mg protein of the membrane fraction, 50 μg bacitracin, and one of the labeled LOR (in a concentration of 4 nM): ^{3}H-morphine – a selective LOR of μ-type, ^{3}H-D-Ala^2-enkephalin (5-D-leucine; ^{3}H-DADLE) – an LOR interacting mainly with receptors of δ-type, or ^{3}H-D-Ala^2-enkephalin (5-D-methioninamide; ^{3}H-DAMEA) – an unselective LOR of μ- and δ-type. The extracts were used in concentrations of 0.02 to 4 mg/ml. Specific binding was determined as the difference between binding of labeled LOR in the presence and in the absence of the unlabeled LOR in the reaction mixture in a concentration of 2 μM. To prove the peptide nature of the LOR studied, the effect of treatment with pronase E (isolated from Streptomyces griseus, 6DMC U/mg, from Serva, West Germany) on the displacing activity of the extract was determined: $[(B_o - B)/B_o] \cdot 100\%$, where B_o and B denote specific binding of labeled LOR in the absence and in the presence, respectively, of the extract in the medium. The extract obtained from 10^8 cells was incubated for 1.5 h at 37°C in 1 ml of 50 mM Tris-HCl buffer (pH 7.7), containing 0.2 mg of pronase. The enzyme was then inactivated by boiling the sample for 15 min. The control series of experiments showed that the enzyme, when inactivated in this way, did not change the level of specific binding of LOR. Labeled LOR were obtained from Amersham International, (England) and the corresponding unlabeled peptides were synthesized and generously provided by M. I. Titov (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR).

TABLE 1. Content of LOR (in pmole-eq/10^7 cells) in Different Lymphocyte Populations (M ± m, n = 34)

Cells tested	Ligands		
	morphine	DAMEA	DADLE
Total lymphocyte population (control)	2,36±0,25	1,19±0,14	0,66±0,37
T lymphocytes	2,43±0,14	—	0,18±0,02*
Population enriched with B cells	2,72±0,18	—	2,07±0,34**
Total lymphocyte population activated by PHA	7,23±0,42*	4,28±0,22*	0,63±0,24

Legend. *$p < 0.01$ compared with control, **$p < 0.01$ compared with T cells. Lymphocytes cultured for 9 h after rinsing to remove PHA.

EXPERIMENTAL RESULTS

Lymphocyte extracts in the concentrations used did not change the level of nonspecific binding of labeled LOR. Meanwhile substances contained in the extracts displaced labeled LOR from the receptors. Curves showing competition between substances contained in the extracts and LOR for binding with receptors were parallel (Fig. 1), evidence that LOR were present in the extracts. Treatment with pronase led to a significant ($p < 0.01$, Student's t test) decrease in displacing activity of the extract obtained from a total peripheral blood lymphocyte population. Thus, when ^{3}H-morphine was used, the displacing activity of extract obtained from $28 \cdot 10^6$ cells and boiled, but not treated with pronase, was 55%, compared with 33% when treated with pronase. When ^{3}H-DADLE was used the displacing activity of the extract in the same concentration was 39% without treatment with pronase and 26% with treatment. Thus at least some of the LOR found in the lymphocyte extracts are peptide in nature. It follows from Table 1 that the number of LOR of μ-type did not differ significantly in extracts of the total lymphocyte population, of T lymphocytes, and of a population enriched with B cells. Meanwhile the concentration of LOR of δ-type was 15 times less in the T cells than in populations rich in B lymphocytes.

If it is assumed that irreversible binding of LOR with lymphocytes may take place during isolation of the cells and extraction, the results can be explained on the grounds that LOR from the plasma passed into the test extracts. To prove that LOR are in fact formed in the lymphocytes, the time course of changes in the LOR content in these cells was therefore studied during culture. The results showed that the LOR content in unactivated lymphocytes did not change significantly during 25 h of culture. Meanwhile activation by PHA led to an increase in the content of LOR in the cells, which reached a maximum after 9 h of culture. Later, the LOR content in the lymphocytes again fell (Fig. 2). As Table 1 shows, after 9 h of incubation the content of LOR displacing ^{3}H-morphine from the receptors was trebled. Activity of the extract estimated on the basis of displacement of ^{3}H-DADLE, however, did not change significantly. Thus the increase in the content of LOR in the lymphocytes was probably due to synthesis of LOR of the μ-type. This result is perfectly logical, because under the experimental conditions used, PHA stimulates secretory activity of T cells, in which the LOR are mainly of the μ-type (Table 1). The results are in agreement with those showing that the maximal ratio of concentrations of LOR of μ- and δ-types among organs of the immune system is observed in the thymus [5], 90% of the cells of which are T lymphocytes.

No endogenous LOR of μ-type is yet known. Moreover, of three high-molecular-weight opioid precursors which have been isolated and characterized, namely POMC and proenkephalins A and B, none is a precursor of a selective LOR of μ-type. The data described above may therefore provide a basis for the discovery for elucidation of a new endogenous LOR.

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RESPONSE OF PULMONARY MACROPHAGES TO HYDROCORTISONE AND ADRENALECTOMY IN RATS

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The mononuclear phagocyte system (MPS) is under endocrine control [4]. In this connection macrophages have receptors for insulin, glucagon, somatomedin, parathormone, estrogens, and adrenalin [4, 7]. The writers showed previously that alveolar macrophages (AM) of rats possess a saturable (receptor) corticosterone binding system, and that the number of binding sites depends on the degree of activation of MPS [6]. Meanwhile reactivity of pulmonary macrophages during changes in the glucocorticoid level in animals has virtually not been studied, although the character of injury to lung tissue depends essentially on the functional state of the phagocytic cells of the bronchoalveolar tract [9, 10].

The aim of the present investigation was to study changes in the pulmonary compartment of MPS in response to injection of large doses of hydrocortisone and to bilateral adrenalectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 95 Wistar rats of both sexes weighing 180-250 g. Subcutaneous injections of hydrocortisone (HC) acetate were given in a daily dose of 125 mg/kg subcutaneously for 7 days to the animals of group 1 in order to form a depot and to maintain a consistently raised glucocorticoid level in the animal [11]. Rats receiving daily injections of 1 ml of 0.85% NaCl solution in accordance with the same schedule served as the control for this group. The rats were killed 24 h after the last injection. Rats of group 2 underwent adrenalectomy, and the control group the corresponding mock operation, 7 days before investigation [15]. To stimulate MPS, animals of group 3 received an intravenous injection of zymosan in a dose of 0.1 g/kg, and the corresponding control group received 1 ml of 0.85% NaCl solution; the animals were killed 5 days after the injection. Rats of group 4 were stimulated with zymosan 24 h after the last injection of HC, and in the control the rats received 0.85% NaCl solution instead of HC. Blood clearance of colloidal carbon was estimated by the method in [12] and the number of phagocytic cells in the interstices of the

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