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INTERACTION BETWEEN THE THIRD FRACTION OF THYMOSIN AND OPIATE RECEPTORS

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Proof of the existence of opiate receptors of lymphocytes is based both on data showing changes in the state of the immune system in patients with opiate addiction and in persons treated with opiates for a long time [9, 10] and on the results of experiments *in vitro*. It has been shown, in particular, that endogenous and exogenous opiates affect the level of E-rosette formation [14], the cAMP concentration in the lymphocytes [1, 3], and the proliferative response of these cells, stimulated by phytohemagglutinin (PHA) [7].

The functional role of lymphocyte opiate receptors is probably determined by interaction with opioids found in the composition of interferon [5, 6], a bone marrow humoral factor stimulating antibody production [2, 4], and also with opioids synthesized by the adrenals and other endocrine glands [13]. Meanwhile, the possible role of biologically active substances of this group in the action of the thymus on development of the immune response has not hitherto been investigated.

The aim of the present investigation was to test the hypothesis that the unpurified thymosin fraction contains substances which interact with opiate receptors.

EXPERIMENTAL METHOD

The third fraction of thymosin (T_3), isolated from calf thymus by Goldstein's method [8], was used. To analyze the ability of T_3 to interact with opiate receptors of rat brain the method of competitive replacement of ^{3}H-naloxone and ^{3}H-morphine by the T_3 preparation was used.

The membrane fraction was obtained from the brain of male Wistar rats weighing 200-250 g by a modified Simantov's method [12], as described previously [4].

The reaction mixture, in a volume of 1 ml, contained 0.7 ml of a membrane suspension of protein, 4 nM of labeled morphine or naloxone, and 50 µg of bacitracin. To displace the label with the T_3 preparation, it was used in concentrations of 5 µg/ml to 6 mg/ml. The amount of specific binding of the label was determined as the difference in binding of ^{3}H-

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TABLE 1. Effect of Enzyme Treatment on Ability of T_3 to Displace ^{3}H-Morphine from Opiate Receptors (M ± m)

Experimental conditions	Dose of enzyme, U/ml	Preparation tested		
		T_3	β-endorphin	met-enkephalin
Control	—	48,2±3,6 %	94,0±4,2 %	95,5±2,1 %
Pronase + boiling	7	19,7±3,0 %*	3,7±2,8 %*	5,8±3,1 %*
Neuraminidase + boiling	0,1	49,8±3,4 %	—	—
Boiling	—	48,8±4.2 %	87,7±6,3 %**	95,4±0,6 %

Legend. T_3, β-endorphin, and Met-enkephalin were dissolved in 50 mM Tris-HCl buffer (pH 7.4) to yield final concentrations of 0.25 mg/ml and 1 μM respectively. The samples were incubated with the above enzymes for 3 h at 37°C. The enzymes were then inactivated by placing the samples in a boiling water bath for 15 min. Each value is the mean of three independent experiments, conducted in two parallel samples. The inhibitory ability was determined as $\frac{B_0 - B}{B} \times 100\%$, where B_0 and B denote specific binding of ^{3}H-morphine in the absence and presence of T_3 or opioid peptides respectively in the medium. The value of B_0 was 1660 cpm. *P < 0.01, **P < 0.05 compared with value determined during displacement by untreated T_3 or opioid peptides.

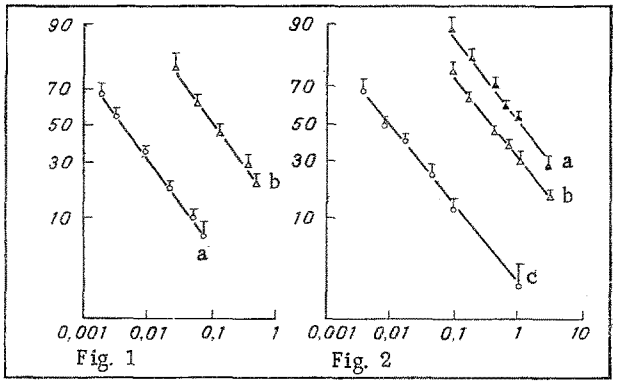

Fig. 1. Comparative ability of morphine (a) and T_3 (b) to displace ^{3}H-morphine from rat brain opiate receptors. Abscissa, concentration of morphine (in μM) and T_3 (in mg/ml) in reaction mixture; ordinate, level of specific binding of ^{3}H-morphine (in % of control). Here and in Fig. 2, each value is mean of four independent experiments conducted in two parallel samples.

Fig. 2. Comparative ability to naloxone and T_3 to displace ^{3}H-naloxone from rat brain opiate receptors: a) T_3 in medium containing 100 mM NaCl; b) T_3 in absence of NaCl from medium; c) naloxone. Abscissa, concentration of naloxone (in μM) and T_3 (in mg/ml) in reaction mixture; ordinate, level of specific binding of ^{3}H-naloxone (in % of control).

naloxone or ^{3}H-morphine in the presence and absence of 2 μM of naloxone or morphine respectively in the reaction mixture. Bound and unbound label were separated on GF/B filters (from Whatman, England). The filters were washed with 7.5 ml of cold 50 mM Tris-HCl buffer (pH 7.7). The level of radioactivity was determined on a MiniBeta counter (from LBK, Sweden). The protein concentration in the sample was determined by Lowry's method.

The following reagents were used: naloxone from Winthrop, USA; morphine — the pharmacopoeial preparation; neuraminidase type V (0.86 IU/mg) from Sigma, USA; pronase (70 U/mg, from Calbiochem, USA; bacitracin (53.5 IU/mg), methionine-enkephalin, and β-endorphin were synthesized and generously provided by M. I. Titov (All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR); ^{3}H-naloxone (55 Ci/mole) and ^{3}H-morphine (22 Ci/mole) were from Amersham Corporation, England, and Tris from Serva, West Germany. The remaining reagents were of Soviet origin and of the highest available degree of purity.

EXPERIMENTAL RESULTS

Although T_3 did not change the level of nonspecific binding, it was found to displace labeled opiates from opiate receptors. The T_3 concentration causing 50% displacement of ^{3}H-morphine from brain receptors (EC_{50}) was 244 ± 80 μg/ml (Fig. 1). EC_{50} for T_3 for displacement of ^{3}H-naloxone was 543 ± 66 μg/ml in the absence of NA in the incubation medium and 2.06 ± 0.55 mg/ml when NA was present in the medium in a concentration of 100 mM (Fig. 2).

Comparison of the curves showing displacement of ^{3}H-morphine by unlabeled morphine and T_3 (Fig. 1) and curves showing displacement of ^{3}H-naloxone by unlabeled naloxone and T_3 (Fig. 2) revealed that they were parallel between log-logit coordinates. It can accordingly be postulated that substances present in the composition of T_3 displace opiates by a competitive type of mechanism.

Considering the data on the effect of Na^+ on the displacing ability of T_3 (Fig. 2) and also the possibility of separation of ligands of opiate receptors into agonists and antagonists of morphine with respect to the coefficient of sodium displacement [11], it can be tentatively suggested that T_3 contains a substance (or substances) with the properties of mixed agonists of opiate receptors.

Neither treatment with neuraminidase nor boiling altered the ability of T_3 to displace ^{3}H-morphine from receptors. Treatment with pronase, however, considerably weakened the displacing activity of T_3 (Table 1). Under the conditions investigated, T_3 thus exhibits properties identical with those of β-endorphin and Met-enkephalin, which suggests that T_3 contains opioid peptides.

The results, together with data on the effect of opioids on the functional state of lymphocytes [1, 7, 14], can be accounted for by three hypotheses: opioid peptides determine the biological effect of some thymus preparations, they modulate these effects, or they are a new group of biologically active thymus peptides.

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