STIMULATION OF IMMUNOGENESIS BY NEUROTENSIN, PENTAGASTRIN, AND THYMOPENTIN, AND WAYS OF ITS REALIZATION

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Previous investigations [5, 6] showed that a fragment of gastrin, a hormone belonging to the digestive system, namely pentagastrin, and also the thymus peptide thymopentin, can accelerate maturation of precursor T cells into T lymphocytes and, correspondingly, can stimulate the thymus-dependent immune response, without affecting the level of the thymus-independent response. Information on the effect of neurotensin, found mainly in the intestines [8], on the above-mentioned parameters of immunogenesis cannot be found in the accessible literature. Effects of these peptides on factors of nonspecific defense and, in particular, on phagocytic activity of the neurophils, are unknown.

The aim of this investigation was to compare the action of neurotensin, pentagastrin, and thymopentin on the immune response and the phagocytic function of the neurophils and to analyze the ways of realization of the effects of these peptides.

EXPERIMENTAL METHOD

Experiments were carried out in vivo on 380 male CBA mice weighing 14-16 g. Neurotensin and thymopentin were obtained from the Department of Natural Compounds, Research Institute of Chemistry, Leningrad University, and pentagastrin from "Sanitas," Kaunas, by the classical method of synthesis in solution.

The preparations were injected subcutaneously in the course of 5 days in pyrogen-free physiological saline, over a wide range of doses. Immunization was carried out intravenously with sheep's red blood cells (SRBC, 2×10^6). On the 4th day after immunization the number of IgM-antibody-forming cells (AFC) in the spleen of each mouse was determined by the method of Jerne and Nording [9], and the hemagglutinin titer was determined in the serum.

In the experiments in vitro, after incubation of the preparations with lymphocytes at 37°C for 1.5 h, expression of the Thy-1-antigen on precursor T cells in the bone marrow [7] and interaction between the preparations and T cells of different origin in the test involving screening them against the action of anti-Thy-1-antibodies [2] were determined. Thy-1-positive cells were detected by the complement-dependent cytotoxic test [1, 2] using rabbit antiserum against cerebral cortical cells of CBA mice, absorbed with mouse liver and with mouse and sheep's red blood cells [1].

Peritoneal exudate cells of CBA mice in a final concentration of 12.5×10^6/ml were used to assess the phagocytic and enzymic activity of the neutrophils. The exudate was obtained 2.5 h after injection of 10% peptone solution. The object of phagocytosis was a 24-h culture of Staphylococcus aureus, strain 9188, in a final concentration of 250×10^6 cells/ml. The intensity of intracellular digestion was estimated in the test with nitro-BT [3], using a 0.2% solution of nitro-BT with mol. wt. of 817.63. The peptides were tested in the optimal

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TABLE 1. Effect of Neurotensin, Pentagastrin, and Thymopentin on Immune Response to SRBC (M ± m)

Preparation	Concentration of preparation, nmoles/mouse					Injection of pyrogen-free physiological saline (control)
	1	$1 \cdot 10^{-1}$	$1 \cdot 10^{-2}$	$1 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	
	Number of IgM-AFC per 10^6 splenic karyocytes					
Neurotensin	9,4±0,9 (10)	9,1±1,4 (10)	10,6±1,5 (10)	11,4±2,2 (10)	12,7±1,1 (10)	10,5±1,2 (38)
Pentagastrin	19,0±2,8* (13)	11,8±1,0* (22)	13,2±1,3* (28)	14,1±1,2* (16)	10,4±1,2 (20)	8,9±1,1 (20)
Thymopentin	20,5±2,4* (11)	15,3±2,1* (12)	20,7±3,3* (8)	16,1±2,2* (11)	6,9±1,2 (12)	8,3±0,6 (20)
	Hemagglutinins, reciprocal titers					
Neurotensin	19,1±1,8 (10)	16,5±1,0 (10)	17,2±3,0 (10)	16,0±2,6 (10)	15,2±2,6 (10)	21,5±2,7 (37)
Pentagastrin	52,6±12,9* (18)	43,2±6,3* (16)	28,2±4,0 (33)	31,4±4,3 (16)	23,3±4,1 (21)	23,3±2,3 (20)
Thymopentin	53,1±10,2* (23)	37,3±4,5* (7)	33,1±4,1* (7)	—	—	22,2±2,5 (20)

Legand. Here and in Tables 2 and 3, asterisk indicates significant differences compared with control at $p < 0.01$ level. —) Not tested. Number of animals given in parentheses.

TABLE 2. Effect of Neurotensin, Pentagastrin, and Thymopentin on Phagocytic Activity of Neutrophils (M ± m)

Preparation	Phagocytosis of staphylococcus		Nitro-BT test
	phagocytic index, %	phagocytic number	Number of diformazan-positive cells, %
Hanks' solution (control)	26,6±2,0	1,96±0,10	5,2±0,4
LPS (prodigiosan)	38,2±2,0*	2,20±0,01*	25,9±1,3*
Thymopentin	42,0±1,9*	2,06±0,13	16,9±1,3*
Neurotensin	37,2±0,8*	2,02±0,09	15,6±0,3*
Pentagastrin	26,7±1,7	1,8±0,01	9,3±0,9*

Legend. Peptides were tested in a dose of 0.014 nmole/ml. Each value is the result of counting no fewer than 900-1000 neutrophils.

dose, namely 0.014 nmole/ml, discovered by preliminary experiments to test different concentrations of the preparations. The lipopolysaccharide (LPS) prodigiosan (0.005%) was used as the reference preparation. The phagocytic index, phagocytic number [4], and the percentage of diformazan-positive cells [3] were determined

EXPERIMENTAL RESULTS

The data in Table 1 show that pentagastrin and thymopentin have a stimulating action on the immune response. Both preparations, over a wide range of doses ($1\text{-}1 \times 10^{-4}$ M) stimulate AFC and antibody formation. Neurotensin has no effect on parameters of the immune response under these conditions.

The study of the action of the preparations on phagocytic activity of the neutrophils revealed marked stimulation of phagocytosis of staphylococci by neurotensin and thymopentin, but not by pentagastrin. Meanwhile all three preparations tested (and also the reference preparation LPS) stimulated enzyme activity of the neutrophils, although in this case pentagastrin gave quite weak stimulation ($p < 0.01$; Table 2).

TABLE 3. Action of Neurotensin, Pentagastrin, and Thymopentin on Lymphocytes of Different Origin (M ± m)

Preparation	Number of Thy-1-positive cells (cytotoxicity index of antibrain serum %) at undermentioned concentration of preparation, mmoles/ml								Number of cells in Hanks' solution (control)
	7,0	1,4	$1,4 \cdot 10^{-1}$	$1,4 \cdot 10^{-2}$	$1,4 \cdot 10^{-3}$	$1,4 \cdot 10^{-4}$	$1,4 \cdot 10^{-5}$	$1,4 \cdot 10^{-6}$	
				In bone marrow					
Neurotensin	12,0±2,3*	8,6±2,0*	0	—	—	—	—	—	0
Pentagastrin	17,3±2,5*	17,5±2,7*	16,1±2,1*	16,8±1,9*	2,8±1,2*	0	—	—	
Thymopentin	0	0	0	0	6,9±1,8*	6,5±1,7*	5,8±1,6*	2,9±1,2*	
				In spleen					
Neurotensin	60,8±3,7*	51,5±3,5*	46,1±3,2*	26,5±3,1	—	—	—	—	32,0±3,3
Pentagastrin	20,3±2,8*	22,6±2,9*	26,5±4,4	27,7±4,4	—	—	—	—	
Thymopentin	0*	4,3±1,3*	4,8±1,2*	5,1±1,4*	5,5±1,6*	4,8±1,5*	6,1±1,2*	36,1±3,4	
				In thymus					
Neurotensin	55,5±3,5*	56,7±3,5*	58,5±3,5*	49,5±3,2*	44,6±3,5*	34,7±3,4*	39,6±3,4*	50,0±3,5*	87,5±2,3
Pentagastrin	27,7±3,1*	28,5±3,2*	29,9±3,2*	30,3±3,2*	29,5±3,2*	48,3±3,5*	50,0±3,5*	84,5±2,5	
Thymopentin	56,6±3,6*	55,2±3,5*	59,3±3,6*	60,7±3,7*	57,3±3,7*	49,5±3,5*	56,9±3,4*	67,2±3,3*	

Legend. Each value is the result of 5-6 experiments (no fewer than 1000-1200 cells were counted). Viability of cells in Hanks' solution in the presence of complement (without antibrain serum) was 85-90%.

Treatment of bone marrow cells in vitro with neurotensin, pentagastrin, or thymopentin facilitated expression of Thy-1-antigen on them. Their minimally effective doses differed significantly. Treatment with preparations of thymocytes, on the other hand, reduced their sensitivity to antibrain, whereas pentagastrin and thymopentin, conversely, screened the T cells against the action of antibrain serum (Table 3). Elimination of adherent cells abolished the effect of neurotensin, but did not destroy the ability of pentagastrin and thymopentin to screen the splenocytes against the action of antibrain serum.

Treatment of single cells from the axillary and mesenteric lymph nodes with the peptides (0.014 nmole/ml) showed that the same peptide reacts differently with lymph node cells from different locations. Neurotensin screened mesenteric lymph node cells: the index of cytotoxicity of the antibrain serum fell from 54.1 ± 3.5% in the control to 26.3 ± 3.1%, but it did not react with cells of the axillary lymph nodes. Pentagastrin screened cells from both axillary and mesenteric lymph nodes: the indices of cytotoxicity of the antiserum fell from 70 ± 3.5 and 54.1 ± 3.5% in the control cell populations to 31.6 ± 2.7 and 37.1 ± 3.4% respectively ($p < 0.01$). Thymopentin did not react at all with the lymph node cells. Treatment of cells of the axillary or mesenteric lymph nodes with this preparation did not change the values of the cytotoxicity indices of the antibrain serum. Differences in the ability not only of different peptides, but also of the same peptide, to react differently with T cells of lymph nodes from different locations are probably evidence of differences in the receptor apparatus of these cells.

The results show that the effects of these three peptides have similar features but also certain differences. All the preparations can accelerate maturation of precursor T cells into T lymphocytes, but at the same time they differ in their influence on Thy-1-positive cells of different origin, and on different stages of immunogenesis.

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