IMMUNOSTIMULATING PROPERTIES OF THE OCTAPEPTIDE CHOLECYSTOKININ AND ITS FRAGMENTS

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In previous investigations [3, 6] the thymus-dependent immune response was shown to be stimulated by pentagastrin (PG), which had no action on the level of the thymus-independent response. While stimulating the immune response pentagastrin did not affect phagocytic activity of neutrophils [3].

No information on the action of the octapeptide cholecystokinin (CK-8), which shares a common C-terminal tetrapeptide with PG, on the above-mentioned parameters could be found in the accessible literature. It is not known whether any correlation exists between the hormonal and immunomodulating activity of CK-8.

The aim of this investigation was to study the action of various forms of CK-8 and its fragments on immunologic parameters of intact and thymectomized mice.

EXPERIMENTAL METHOD

Experiments in vivo were carried out on 313 intact, 59 thymectomized, and 20 mock-thymectomized male CBA mice weighing 14-16 g. The thymectomized and mock-thymectomized animals were used in the experiments 1-1.5 months after the operation. Thymectomy was performed surgically under superficial ether anesthesia [2]. In the mock operation all steps were carried out except removal of the thymus.

The following substances were tested: PG (Boc-β-Ala-Trp-Met-Asp-Phe-NH_2, from "Sanitas," Kaunas), the sulfated form of CK-8 [Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2, obtained from the Cardiologic Scientific Center, Russian Academy of Medical Sciences, Moscow , the nonsulfated form of CK-8 (Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2), and fragments of the nonsulfated form of CK-8 (Asp-Tyr-Met-Gly; Trp-Met-Asp-Phe-NH_2; from St. Petersburg University).

The preparations were made up in pyrogen-free physiological saline ("Polfa," Poland) subcutaneously for 5 days within the dose range of 10^{-9}-10^{-14} M per mouse per day. The control animals were given pyrogen-free physiological saline alone by the same schedule, The animals were then immunized intravenously with sheep's red blood cells ($2 \cdot 10^6$ SRBC) or with 0.001 μg of Vi-antigen. On the 4th day after immunization the number of IgM antibody-forming cells (AFC) was counted in the spleen of each mice and the result expressed per 10^6 karyocytes [7].

The effect of the peptides on medullary precursor T-cells was estimated on the basis of the appearance of Thy-I-antigen on the cells after incubation with the preparations at 37°C for 90 min. To assess interaction between the preparations and mature splenic T cells the phenomenon of screening (reducing the sensitivity of peptide-treated Thy-I-cells to the action of anti-Thy-I-antibodies was used [4]). In both cases the number of Thy-I-cells was determined with the aid of antibone-marrow serum [1] in the complement-dependent cytotoxic test [1, 4].

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TABLE 1. Effect of Octapeptide Cholecystokinin and Its Fragments on Immune Response to SRBC (M ± m, n = 10)

Parameter of immune response	Preparation	Dose of preparation, M/mouse/day						Injection of pyrogen-free physiologycal saline (control)
		10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}	10^{-14}	
Number of IgM-AFC per 10^6 splenic karyocytes	Sulated CK-8	12.9±0.9*	14.7±1.1*	18.4±2.7*	15.1±1.7*	11.9±0.9*	8.6±0.8	8.5±0.7
	Nonsulfated CK-8	13.3±1.4*	15.5±2.0*	24.5±5.0*	16.7±2.3*	11.3±1.0*	9.1±1.1	8.3±0.6
	C-terminal tetrapeptide	15.8±1.6*	13.9±1.5*	21.7±3.7*	14.3±1.7*	9.9±0.9	—	8.4±0.6
	N-terminal tetrapeptide	12.3±0.8*	10.1±1.2	9.8±1.0	9.6±1.1	9.0±1.5	—	8.7±0.7

Legend. *$p < 0.05$: Significant difference from control.

TABLE 2. Effect of Octapeptide Cholecystokinin and Its Fragments on Number of Splenic Thy-I^+-Cells and on Immune Response of Thymectomized Mice to SRBC (M ± m)

Mice	Preparation	Number of Thy-I^+-cells in spleen (cytotoxicity index of anti-bone-marrow serum, %)	Number of IgM-AFC/10^6 splenic karyocytes
Undergoing mock operation	Pyrogen-free physiological saline	18.4±2.0	14.0±0.8 (20)
	Pyrogen-free physiological saline	1.0±0.7	6.4±0.8 (20)
Thymectomized	Sulfated CK-8	11.0±1.8*	12.1±1.4 (10)*
	Nonsulfated CK-8	10.6±1.7*	11.7±1.3 (9)*
	C-terminal tetrapeptide	10.8±1.8*	12.1±1.1 (10)*
	N-terminal tetrapeptide	3.2±1.1	7.9±0.7 (10)

Legend. Asterisk indicates significant difference compared with corresponding parameter for thymectomized mice receiving pyrogen-free physiological saline ($p < 0.01$). Number of animals given in parentheses.

To assess the effect of the peptides on phagocytic activity of neutrophils peritoneal exudate cells were used; the cells were obtained 2.5 h after injection of sterile 10% peptone solution. The object of phagocytosis was a 24-h culture of *Staphylococcus aureus* strain 9198. The phagocytic number and phagocytic index were determined [5]. The reference preparation used was 0.005% lipopolysaccharide (prodigiosan).

EXPERIMENTAL RESULTS

As Table 1 shows, the sulfated and nonsulfated forms of CK-8 do not differ in their ability to stimulate the thymus-dependent immune response The C-terminal tetrapeptide of the nonsulfated form of CK-8 within the dose range 10^{-9}-10^{-12} M increases the number of IgM-AFC compared with the control by 1.7-2.5 times in the same way as both forms of CK-8. The N-terminal tetrapeptide stimulates IgM-AFC production only in a dose of 10^{-9} M, and only by 1.4 times.

The above-mentioned peptides, tested in a dose of 10^{-11} mole/mouse, did not act on the immune response to Vi-antigen: the number of IgM-AFC varied between limits of 10.0 ± 0.6 and 11.6 ± 1.4 compared with 13.4 ± 3.0 in the control (10 animals were tested in each group).

Injection of CK-8 into thymectomized mice in a dose of 10^{-9} M increased the number of Thy-I^+-cells and restored the level of the immune response to SRBC to the level found in animals undergoing the mock operation. The C-terminal tetrapeptide in the same dose also had a significant ($p < 0.01$) effect on the level of immunity in thymectomized mice. The N-terminal tetrapeptide CK-8 did not exhibit any activity (Table 2).

TABLE 3. Effect of Octapeptide Cholecystokinin and Its Fragments on Phagocytic Activity of Peritoneal Neutrophils in Vitro (M ± m)

Preparation	Phagocytosis of staphylococci	
	phagocytic index	phagocytic number
Hanks' solution	25,5±1,2	1,7±0,09
LPS	41.2±1.7*	2,0±0,04*
Sulfated CK-8	26,3±1,5	1.70±0.07
Nonsulfated CK-8	23,5±2,1	1,64±0,06
C-terminal tetra peptide CK-8	26,4±2,0	1,90±0,1
N-terminal tetra-peptide CK-8	26,1±1,2	1,90±0,09

Legend. Peptides tested in a concentration of $1.4 \cdot 10^{-11}$ mole/ml. Each number is result of counting at least 900-1000 neutrophils. Asterisk indicates significant difference compared with corresponding parameters obtained on treatment of cells in vitro with Hanks' solution ($p < 0.05$).

Treatment of bone marrow cells in vitro with the test peptides (10^{-9} mole/ml) likewise did not give a consistent result: CK-8 and its C-terminal fragment increased the number of Thy-I^+-cells from 0% in the control to 6.3 ± 1.3 and 4.3 ± 1.1% respectively. The N-terminal tetrapeptide caused virtually no change in the number of Thy-I^+-cells in the bone marrow (1.3 ± 0.8 compared with 0% in the control). Exposure of the above-mentioned peptides in vitro with splenocytes did not affect the content of Thy-I^+-cells in this population: during treatment with the preparations the number of Thy-I^+-cells varied between limits of 30.6 ± 2.7 and 34.1 ± 2.3% compared with 32.0 ± 3.3% in the control (not shown in Table 2).

CK-8 and its fragments did not affect the phagocytic activity of the neutrophils (Table 3).

The results show that CK-8, like PG [3, 6], stimulates the thymus-dependent, but does not affect the thymus-independent response or phagocytic activity of the neutrophils. Stimulation of the thymus-dependent immune response by CK-8, in the absence of any effect on the thymus-independent response, is evidence of its connection with the function of T-, but not of B-cells. Induction of Thy-I-antigen on precursor T cells, coupled with inability to interact with mature T lymphocytes, by CK-8 shows that the immunostimulating activity of CK-8 is based on its effect on the immature T-cell population. The virtual absence of activity of the N-terminal tetrapeptide is evidence that the immunostimulating activity of CK-8 is mainly associated with the function of the C-terminal tetrapeptide, which is identical to that of the tetrapeptide pentagastrin. The immunostimulating action of CK-8 is unconnected with its hormonal activity.

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