PENTAGASTRIN AS A STIMULATOR OF IMMUNOGENESIS

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KEY WORDS: pentagastrin; immune response; precursor T cells.

Many regulatory peptides are polyfunctional, i.e., they are found and they perform their functions in different systems of the body [1]. One such regulatory peptide is gastrin, a hormone of the digestive system, which is also found in the CNS [5, 8-11]. Since the gastrin level depends on functional activity of the thymus [4], a study of the effect of gastrin on function of the immune system may prove useful.

The aim of this investigation was to study the ability of pentagastrin (PG), an analog of gastrin, on the level of the thymus-dependent and thymus-independent immune response in vivo, and also on differentiation of medullary precursor T cells into T lymphocytes in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on 358 male CBA mice weighing 14-16 g. PG (Boc-Ala-Trp-Met-Asp-Phe-NH_2, from Sanitas, Kaunas) was injected subcutaneously into the animals in pyrogen-free physiological saline over a wide dose range for 10 days. Control animals received pyrogen-free physiological saline in accordance with the same scheme. The mice were then immunized intravenously with sheep's red blood cells (SRBC, $2 \cdot 10^6$) or Vi-antigen (0.1 μg per mouse). On the 4th day after immunization the number of IgM-antibody-forming cells (AFC) was determined in the spleen of each mouse by the method [7] and the hemagglutinin titer was determined in the serum. To detect AFC and antibodies to Vi-antigen, the latter was loaded on SRBC. The final concentration of Vi-antigen in the solution was 20 μg/ml. To remove unbound Vi-antigen the SRBC were washed with physiological saline at least 8 times. The number of AFC was counted per 10^6 splenic karyocytes.

Differentiation of medullary precursor T cells into T lymphocytes under the influence of PG was estimated by a modified method [6], after treatment of the bone marrow cells in vitro with PG at 37°C for 1.5 h. The number of T lymphocytes in the bone marrow cell population was determined with the aid of rabbit antibrain serum in the complement-dependent cytotoxicity test [2]. The antiserum was obtained by repeated immunization of rabbits without Freund's adjuvant [2], absorbed with a mouse liver homogenate and with murine and SRBC [2], and used in a dilution of 1:50. In this dilution, in the presence of complement (fresh guinea pig serum, 1:3) the antiserum caused death of 85 ± 2.5% of thymocytes and did not interact with bone marrow cells of CBA mice. Not less than 200 cells were counted in each sample; their viability was determined with a 0.2% aqueous solution of trypan blue. The experiment was repeated at least 4-5 times.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that injection of PG into mice for 10 days in doses of 0.01 to 5 μg per animal daily, caused marked stimulation of the immune response to SRBC. The maximal effect was found after injection of PG in doses of 5 and 1 μg. Injection of PG in these doses caused an increase in the number of AFC by 2.2-2.7 times and more than doubled the antibody titer. The dose of 0.1 μg caused a weaker, but significant increase in both these parameters: the number of AFC and the hemagglutinin titer. Injection of PG in a dose of 0.01 μg stimulated AFC production only and had no significant effect on the antibody level.

The immune response of the mice to thymus-independent Vi-antigen was unchanged by the action of PG (Table 2).

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TABLE 1. Effect of PG on Immune Response of Mice to SRBC (M ± m)

Preparation	Dose, μg/mouse/day	Number of IgM-AFC per 10^6 splenic karyocytes	Hemagglutinins, reciprocal titers
Pyrogen-free physiological saline (control)	0	8,6±2,0 (45)	23,1±2,4 (43)
PG	5	24,7±3,5* (24)	46,4±5,1* (21)
	1	19,0±2,8* (13)	52,6±12,9* (18)
	0,1	11,8±1,0*** (22)	43,2±6,3* (16)
	0,01	13,2±1,3** (28)	28,2±4,0 (33)
	0,001	10,4±1,2 (20)	23,3±4,1 (21)
	0,0001	11,4±1,0 (9)	24,4±7,1 (9)

Legend. *, **, and ***) Differences significant compared with control at $p < 0.001$, $p < 0.01$, and $p < 0.05$ levels respectively. Number of animals given in parentheses.

TABLE 2. Effect of PG on Immune Response of Mice to Vi-Antigen (n = 9, M ± m)

Preparation	Dose, μg/mouse/day	Number of IgM-AFC per 10^6 splenic karyocytes	Hemagglutinins, reciprocal titers
Pyrogen-free physiological saline	0	76,7±2,6	782±76,2
PG	1	77,2±4,2	675±114,3

To study the mechanism of the immunostimulating action of PG, its ability to accelerate differentiation of medullary precursor T cells into mature T cells was investigated. For this purpose PG, in doses of 0.0001 to 1 μg/ml was added in vitro to bone marrow cells and the number of T cells was determined in the complement-dependent cytotoxic test with anti-brain serum. Treatment with PG in doses of 1, 0.1, and 0.01 μg/ml considerably increased the number of Thy-1-positive cells: the cytotoxicity index (CI) was 17.5 ± 2.7, 16.1 ± 2.1, and 16.8 ± 1.9% respectively. The dose of 0.001 μg/ml was much less effective (CI 2.8 ± 1.2%), and a dose of 0.0001 μg/ml no longer stimulated differentiation of precursor T cells into T lymphocytes (CI = 0).

These results are evidence that PG, a synthetic analog of gastrin, a stimulator of gastric secretion, has marked immunostimulating activity on the thymus-dependent immune response. The fact that PG does not affect the level of the thymus-independent response shows that it has no direct action on B cells and that its immunologic activity is linked with the function of T lymphocytes. The mechanism of the immunostimulating action of PG was based on its ability to accelerate differentiation of precursor T cells into T lymphocytes. The results characterize PG as a peptide which participates not only in digestion, but also in immunogenesis. A further study of the immunologic properties of PG and the mechanisms of its action and, in particular, of the role of the active amino acids which compose it [3], could yield promising results for the creation of new immunomodulating preparations and to improve our understanding of the principles of regulation of homeostasis.

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IMMUNOCORRECTIVE THERAPY OF THE TRAUMATIC SYNDROME

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Mechanical trauma leads to marked changes in the immune system which can be interpreted as inhibition of immunobiological defense [1, 5, 9, 10, 13]. This has provided the basis for the inclusion of immunostimulators of the thymalin type in the combined treatment of such patients, with good therapeutic effects [4]. Detailed studies in experiments on animals have shown that the functional activity of the immune system changes in phases during the course of traumatic shock and the post-traumatic period [2], and this must be taken into account when different immunomodulators are prescribed. In turn, estimation of the effectiveness of immunomodulators can provide definite information for the elucidation of the mechanisms of immunologic changes in the traumatic syndrome.

The aim of this investigation was to study the effectiveness of the combined use of thymalin and interferon for the prevention and treatment of the traumatic syndrome in rats. The survival rate was chosen as the criterion of efficacy, and the mechanisms of this phenomenon were assessed at the level of immunobiological defense factors.

EXPERIMENTAL METHOD

Traumatic shock was produced by Cannon's method in male Wistar rats weighing 200-250 g [3]. The development of a post-traumatic syndrome in the course of traumatic shock was studied until the 7th day. The survival rate, in per cent, was determined from the number of surviving animals on the 7th day. The animals as a whole were divided into four groups with six or seven rats in each group: 1 (basic) — traumatized animals treated with thymalin combined with interferon; 2, control — traumatized, untreated animals; 3) control — uninjured animals receiving thymalin and interferon; 4) traumatized animals receiving thymalin alone. The scheme of administration of the immunomodulators was as follows: 1 h after trauma thymalin was injected intramuscularly in a dose of 0.02 mg/100 g body weight, after which thymalin was given in the same dose together with interferon (10 U/100 g intramuscularly) for 6 days. On the 7th day the animals were decapitated. The functional state of the immune system was estimated by means of the following parameters: the percentage of T lymphocytes in the rosette-forming test with sheep's red blood cells, the number of theophylline-sensitive T lymphocytes (TS-lymphocytes) in the test with treatment of T-RFC with theophylline, the level of circulating immune complexes (CIC) by precipitation in a 3.76% solution of polyethylene-glycol, and the serum lysozyme activity, by a nephelometric method [6].

EXPERIMENTAL RESULTS

Combined administration of thymalin and interferon to the injured animals give a marked increase of survival rate during the first 7 days of the traumatic syndrome. Among injured animals of group 1 receiving the immunomodulators (55 rats) the mortality was 13.3%, compared

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