IMMUNOSTIMULATING PROPERTY OF GAMMA-AMINOBUTYRIC ACID

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The writers previously [2, 3] showed that 9 of the 20 amino acids present in the composition of proteins (Asp, Asn, Glu, Cys, Ser, Thr, Trp, Ala, Val) can induce Thy-I-antigen on bone marrow cells and can stimulate the sinus-dependent immune response in mice. The most active of these were glutamic and aspartic acids, which are neuroactive amino acids [4]. The question arises whether neuroactive amino acids which are not components of protein may also possess similar activity; one of these is gamma-aminobutyric acid (GABA), which has a marked nonspecific, protective, antistressor action [5].

The aim of this investigation was to study the ability of GABA to induce Thy-I-antigen on bone marrow cells in vitro and to affect the level of the thymus-dependent and thymus-independent immune response in vivo.

EXPERIMENTAL METHOD

Experiments were carried out on 152 male CBA mice weighing 14-16 g. GABA (Fluka, West Germany) was injected subcutaneously into the animals over a period of 10 days in pyrogen-free physiological saline, over a wide range of doses. Control animals received pyrogen-free physiological saline by the same scheme. The mice were then immunized intravenously with sheep's red blood cells (SRBC, 2×10^6 cells) or Vi-antigen (0.001 μg per mouse). 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 in [8], 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 antigen concentration was 20 μg/ml. To remove unbound Vi-antigen the SRBC were washed

TABLE 1. Increase in Number of Thy-I-Positive Lymphocytes in Bone Marrow Cell Suspension after Treatment in Vitro with GABA (M ± m)

Preparation	Dose, μg/ml	Number of Thy-I-positive lymphocytes in bone marrow cell suspension, %
GABA	40	11,0±0,4
	20	11,5±0,3
	1	7,5±0,4
	0,1	0
Hanks' solution	0	0

Legend. Each number is the result of counting at least 600-800 nucleated cells. Viability of cells in Hank's solution (without antibrain serum) was 85-90%.

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TABLE 2. Effect of GABA on Parameters of Immune Response to SRBC (M ± m)

Preparation	Dose, µg per mouse per day	Index of immune response	
		number of IgM-AFC per 10^6 splenic karyocytes	hemagglutinins, reciprocal titers
GABA	80	17,1±2,2* (16)	77,5±2,9* (16)
	40	19,6±1,7* (16)	67,5±2,9* (16)
	20	26,1±4,1* (12)	97,8±14,3* (9)
	10	15,1±2,8 (12)	30,0±6,5 (10)
	5	10,7±1,5 (12)	24,2±3,5 (12)
Pyrogen-free physiological saline (control)	0	10,8±0,7 (36)	30,1±4,6 (32)

Legend. *p < 0.01 compared with corresponding control value. Here and in Table 3, number of animals given in parentheses.

TABLE 3. Effect of GABA on Parameters of Immune Response to Vi-antigen

Preparation	Dose, µg per mouse per day	Index of immune response	
		number of IgM-AFC per 10^6 splenic karyocytes	hemagglutinins, reciprocal titers
GABA	80	7,0±1,0 (9)	60,0±9,0 (7)
	40	6,7±0,6 (10)	36,0±6,5 (10)
	20	6,3±0,7 (9)	45,0±7,9 (8)
	10	5,7±0,6 (10)	38,5±9,0 (7)
Pyrogen-free physiological saline	0	7,4±0,2	37,1±9,0 (7)

at least 8 times with physiological saline. The number of AFC was calculated per 10^6 splenic karyocytes.

Expression of Thy-I-antigen on bone marrow precursor T cells under the influence of GABA was assessed by a modified method [7] after treatment of the bone marrow cells in vitro with the test preparation at 37°C for 1.5 h. The number of T-lymphocytes in the bone marrow cell population was determined by the complement-dependent cytotoxicity test [1] using rabbit antiserum against cerebral cortical tissue of CBA mice, absorbed with mouse liver homogenate, mouse red blood cells, and SRBC [1]. After adsorption, the antigen–antibody complexes were removed from the antiserum by reprecipitation with CO_2. The antiserum was used in dilution of 1:50. In this concentration, in the presence of complement (fresh guinea pig serum, 1:3), the antiserum caused death of 88.0 ± 1.3% of thymocytes but did not interact with bone marrow cells of CBA mice. In each test no fewer than 200 cells were counted and their viability was estimated by the use of a 0.2% aqueous solution of trypan blue. The experiment was repeated at least 3 or 4 times.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that GABA, in doses of 1, 20, and 40 µg/ml, increased the number of Thy-I-positive lymphocytes in the bone marrow cell population in vitro from 0% in the control to 7.5, 11.5, and 11% respectively. In a dose of 0.1 µg/ml, however, GABA was ineffective.

Injection of GABA into the mice enhanced the immune response to SRBC (Table 2). GABA was most effective in a dose of 20 µg/ml, in which it increased AFC production by 2.4 times and antibody production by 3.2 times compared with the control (from 10.8 ± 0.7 and 30.1 ± 4.6 respectively in the control to 26.1 ± 4.1 and 97.8 ± 14.3 respectively in the experiments; $p < 0.01$). In doses of 40 and 80 µg/ml GABA stimulated the immune response significantly ($p < 0.01$), but by a rather lesser degree. In doses of 5 and 10 µg/ml, however, GABA had no effect.

The immune response to a subimmunizing dose (0.001 μg/mouse) of thymus-independent Vi-antigen was unchanged under the influence of various GABA concentrations (Table 3).

The results are evidence that, like immunoactive amino acids which are components of protein [2, 3], GABA facilitates expression of Thy-I-antigen on bone marrow cells and stimulates the thymus-dependent immune response correspondingly. The absence of effect of GABA on the level of the thymus-independent response shows that the immunostimulating action of this preparation in the doses tested is connected with T-cell, but not of B-cell function.

GABA, the principal mediator of the GABA-ergic inhibitory system [5], can thus not only take part in processes of nervous regulation of various functions of the body, but can also exert a direct influence on the receptor apparatus of lymphoid cells, thereby probably promoting intercellular cooperation and leading correspondingly to stimulation of the immune response.

The immunologic activity of substances present not only in the immune, but also in other systems, confirms the hypothesis [6] that there exists certain functional proteins, which are responsible for interaction between and regulation of the different systems of the body.

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