EFFECT OF CARNOSINE ON THE IMMUNOSUPPRESSIVE EFFECT OF HISTAMINE

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UDC 615.218.1.015.23.615.31:547.784.2

KEY WORDS: carnosine; histamine; T and B lymphocytes; proliferation.

Recent investigations have shown that the natural histidine-containing dipeptide carnosine has an antiallergic action [1, 6]. It has been suggested that the mechanism of this effect is based on the ability of carnosine to compete either with IgE or with histamine in their effect on target cells [1]. However, the direct action of carnosine on lymphocyte subpopulations and also the possibility that it may influence the immunosuppressive effect of histamine have not yet been studied.

The aim of this investigation was to study the ability of carnosine (β-imidazole-lactate) to affect histamine-induced immunosuppression of proliferative activity of various lymphocyte subpopulations and the realization of this effect through surface histamine receptors of the cells.

EXPERIMENTAL METHOD

Experiments were carried out on 60 C57BL mice weighing 25-30 g. The animals were sensitized with a single intraperitoneal injection of 0.5 ml of a solution containing 50 μg ovalbulin and 25 mg of $Al(OH)_3$ gel. The animals were used in the experiment on the 4th day of sensitization. The spleen was removed from the decapitated mice and homogenized in cold medium 199 in a Potter's homogenizer. The homogenate was freed from adherent cells [5], layered carefully on Ficoll-Verografin solution with specific gravity of 1010, and the cells were allowed to settle spontaneously at 1g [3, 4]. After 4 h fractions of immunocompetent cells with sedimentation rates of 1-4, 4-6, and 6-8 mm/h, corresponding to populations of B lymphocytes, T helper cells, and T suppressor cells [4], were collected. The lymphocytes thus obtained were washed three times and resuspended in a concentration of $5 \cdot 10^6$ cells/ml in medium 199 with 10% autologous serum and $5 \cdot 10^{-5}$ M 2-mercaptoethanol. In the experiments of group 1 lymphocytes of all fractions were incubated for 1 h at 37°C with histamine (1 mM), carnosine, mepyramine, and metiamide (all in concentrations of 0.1 mM). In the experiments of group 2 every sample also was incubated a further 1 h with 1 mM histamine. Later all lymphocytes were incubated with tritium-labeled thymidine (1 μCi per sample) for 4 h, after which their radioactivity was determined on a Mark 3 scintillation counter. In the experiments of group 1, the ability of each of the test substances to affect the proliferative activity of lymphocytes of different fractions of the mouse spleen was thus determined, whereas in group 2 the ability of mepyramine, metiamide, and carnosine to depress the inhibitory effect of histamine on proliferation of these same fractions of immunocompetent cells was compared. Inhibition of lymphocyte proliferation was calculated by the equation:

$$\% \text{ of inhibition} = \frac{\text{cpm (C)} - \text{cpm (E)}}{\text{cpm (C)}} \times 100\%,$$

where cpm (C) denotes radioactivity of the control sample, containing only lymphocytes of sensitized animals, and cpm (E) denotes radioactivity of the experimental sample, containing lymphocytes of sensitized animals together with the test substance.

The numerical results were subjected to statistical analysis by Student's t test.

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 11, pp. 603-604, November, 1984. Original article submitted January 5, 1984.

 0007-4888/84/9811-1568$08.50

TABLE 1. Effect of Histamine, Carnosine, Mepyramine, and Metiamide on Proliferation of Subpopulations of Mouse Spleen Lymphocytes (M ± m, %; n = 60)

Substance	Total pool of lymphocytes		B lymphocytes		T helper cells		T suppressor cells	
	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P
Histamine	59,4±2,2	<0,001	7,7±15,4	>0,05	8,8±5,0	>0,05	67,7±1,5	<0,001
Carnosine	0±2,8	>0,05	7,5±15,0	>0,05	0±5,4	>0,05	0,1±4,3	>0,05
Mepyramine	—2,2±2,9	>0,05	15,4±15,4	>0,05	3,2±4,5	>0,05	1,7±4,0	>0,05
Metiamide	—2,6±2,8	>0,05	23,1±15,4	>0,05	0,9±5,0	>0,05	—1,9±3,6	>0,05
Control	0±3,0	—	0±15,4	—	0±5,0	—	0±3,1	—

Legend. Here and in Table 2: concentration of histamine 1 mM, of other substances 0.1 mM.

TABLE 2. Competitive Effect of Histamine, Carnosine, Mepyramine, and Metiamide on Proliferation of Subpopulations of Mouse Spleen Lymphocytes (M ± m, %; n = 60)

Substance	Total pool of lymphocytes		B lymphocytes		T helper cells		T suppressor cells	
	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P	per cent inhibition of proliferation	P
Histamine (I mM)	59,4±2,2	—	7,7±15,4	—	8,8±5,0	—	67,7±1,5	—
Histamine (1 mM) + carnosine (0,1 mM)	37,1±2,0	<0,05	0±19,3	>0,05	10,8±3,2	>0,05	29,0±2,4	<0,05
Histamine (1 mM) + mepyramine (0,1 mM)	57,5±2,2	>0,05	15,4±19,3	>0,05	6,2±4,6	>0,05	68,3±1,9	>0,05
Histamine (1 mM) + metiamide (0,1 mM)	22,2±3,5	<0,05	7,7±19,3	>0,05	5,4±4,1	>0,05	20,5±3,6	<0,05

EXPERIMENTAL RESULTS

The investigations showed that proliferative activity of lymphocytes from the mouse spleen reached a maximum on the 4th day after sensitization by ovalbumin, when it was increased by 10-12 times. Addition of 1 mM histamine to the culture medium led to inhibition of the intensity of proliferation of the total lymphocyte pool by 59.4 ± 2.2% ($P < 0.001$) and of the T suppressor cell fraction by 67.7 ± 1.5% ($P < 0.001$). Since histamine did not significantly affect proliferation of B lymphocytes and T helper cells, it can be concluded that the decrease in mitotic activity of the total lymphocyte pool under the influence of histamine was due to inhibition of proliferation of the T suppressor lymphocytes themselves by histamine. The other substances tested had no independent action on proliferative activity either of the total lymphocyte pool or of its individual fractions (Table 1). However, carnosine and, to an even greater degree, the H-2 histamine blocker metiamide, in a dose of 10^{-4} M, was able to inhibit the above-mentioned effect of histamine (Table 2). The H-1 histamine antagonist mepyramine had virtually no effect on histamine-dependent immunosuppression.

The results are evidence that histamine has an inhibitory action on antigen-induced proliferation of T suppressor lymphocytes through H-2 histamine receptors, for this effect was considerably inhibited by the H-2 histamine blockers metiamide, but not by the H-1 histamine blocker mepyramine. In turn, the ability of carnosine, to exert an action similar to that of metiamide, indicates that this compound belongs to the group of H-2 histamine antagonists. The fact that carnosine possesses such properties largely accounts for the nature of its antiallergic action [1, 2, 6], and also for the good prospects for its further study with a view to possible use in the treatment of allergic diseases.

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