DETERMINATION OF ANTIHISTAMINE PROPERTIES OF SOME HISTIDINE DERIVATIVES IN EXPERIMENTAL T AND B ROSETTE FORMATION BY GUINEA PIG LYMPHOCYTES

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The ability of carnosine, a natural histidine-containing dipeptide, to inhibit allergic reactions of immediate type *in vivo* has now been established in rabbits [11], guinea pigs [2, 3], and rats [5]. Several theories have been put forward to explain the mechanism of these effects of carnosine, but so far none has found direct experimental confirmation [3].

The aim of the investigations described below was to use T and B rosette-formation tests to determine the ability of carnosine, imidazole lactate, imidazole pyruvate, and N-acetylhistidine to inhibit effects of histamine on blood and splenic lymphocytes of ovalbumin-sensitized guinea pigs, and also to compare the action of these compounds with that of known H-1 and H-2 histamine blockers mepyramine and metiamide. The main task was thus to test the hypothesis that carnosine can block histamine receptors on lymphocytes, and a secondary task was to look for substances with a similar mechanism of action among a group of histidine derivatives.

EXPERIMENTAL METHOD

Histamine dihydrochloride, containing 60.5% of histamine base, was used in the experiments. Carnosine, imidazole lactate, imidazole pyruvate, and N-acetylhistidine were tested as histamine antagonists. The effect of these compounds was compared with the action of classical antihistamine preparations: the H-1 blocker mepyramine and the H-2 blocker metiamide.

Tests were carried out on 45 albino guinea pigs weighing 250-300 g. The animals were sensitized by a single intraperitoneal injection of 50 μg ovalbumin [1]. The animals were used in the experiments on the 10th day of sensitization, for that is the time when they begin to show significant changes in the relative proportions of different types of immunocompetent cells [4, 7]. Blood lymphocytes were obtained from guinea pigs by the method in [9] in the modification [12]. Splenic lymphocytes were obtained by the method in [8]. In the control group, each sample containing 2×10^5 lymphocytes in 0.1 ml of Hanks' solution was incubated at 37°C for 1 h with 1 mM histidine, carnosine, imidazole lactate, imidazole pyruvate, N-acetylhistidine, mepyramine, and metiamide. In the experimental group the samples were incubated for 1 h under similar conditions with the same compounds in a concentration of 10^{-4} M, and then for a further hour with 1 mM histamine. Later, the number of T and B lymphocytes was determined in both groups respectively by methods in [14] and [13] in the modification [4]. In control tests the independent action of each one of the test substances was studied on T and B rosette formation by lymphocytes from the blood and spleen of sensitized guinea pigs, and the ability of histidine derivatives to prevent the T rosette-inhibiting effect of histamine was evaluated and their action was compared with that of classical H-1 and H-2 histamine blockers experimentally.

T and B rosette-forming cells were counted in a Goryaev's chamber; a lymphocyte with three fixed erythrocytes or more was taken as a rosette. In each test at least 200 lymphocytes were counted.

The numerical data were subjected to statical analysis by Student's t test [6].

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TABLE 1. Effect of Histamine, Carnosine, Imidazole Lactate, Imidazole Pyruvate, N-Acetylhistidine, Mepyramine, and Metiamide on T and B Rosette Formation by Lymphocytes from Blood and Spleen of Sensitized Guinea Pigs (%, M ± m; n = 14)

Substance	T_{ros}				B_{ros}			
	blood	P	spleen	P	blood	P	spleen	P
Control	33,0±1,5	—	11,1±1,5	—	15,6±1,4	—	24,0±1,4	—
Histamine	12,1±1,2	<0,001	3,8±1,2	<0,001	13,5±1,3	>0,05	24,4±1,3	>0,05
Carnosine	32,3±1,4	>0,05	11,3±1,3	>0,05	14,8±1,4	>0,05	22,6±1,7	>0,05
Imidazole lactate	32,5±1,4	>0,05	10,8±1,1	>0,05	14,1±1,4	>0,05	22,2±1,5	>0,05
Imidazole pyruvate	32,2±1,3	>0,05	10,7±1,1	<0,05	14,2±1,3	>0,05	24,3±1,4	>0,05
N-acetyl-histidine	32,6±1,4	>0,05	11,6±1,4	>0,05	13,6±1,2	>,005	23,2±1,4	>0,05
Mepyramine	33,9±1,3	>0,05	11,3±1,2	<0,05	13,9±1,4	>0,05	23,2±1,4	>0,05
Metiamide	32,4±1,3	>0,05	10,6±1,1	>0,05	14,4±1,1	>0,05	23,1±1,3	>0,05

Legend. All substances tested were used in a concentration of 1 mM.

TABLE 2. Determination of Ability of Carnosine, Imidazole Lactate, Imidazole Pyruvate, N-Acetylhistidine, Mepyramine, and Metiamide to Compete with Histamine During Action on T Rosette-Forming Cells in Blood and Spleen of Sensitized Guinea Pigs (%, M ± m; n = 14)

Substance	% Inhibition of T rosette formation			
	blood	P	spleen	P
Control (histamine)	62,6±4,7	—	40,0±11,3	—
Histamine + carnosine	25,8±4,7	<0,001	6,9±10,9	<0,05
Histamine + imidazole lactate	63,4±4,4	>0,05	41,6±10,8	>0,05
Histamine + imidazole pyruvate	60,2±4,1	>0,05	44,8±10,9	>0,05
Histamine + N-acetyl-histidine	59,6±3,8	>0,05	36,4±11,8	>0,05
Histamine + mepyramine	59,8±3,8	>0,05	40,7±12,0	>0,05
Histamine + metiamide	15,4±4,1	<0,001	2,2±12,4	<0,05

Legend. Concentration of histamine 1 mM, of remaining substances 0.1 mM.

EXPERIMENTAL RESULTS

A study of the action of histamines on T rosette formation by lymphocytes from guinea pig blood and spleen showed that it reduces the number of T rosettes formed in blood from 33.0 ± 1.5 to 12.1 ± 1.2% ($P < 0.001$) and in the spleen from 11.1 ± 1.5 to 3.8 ± 1.2% ($P < 0.001$). Histamine had virtually no effect on B rosette formation (Table 1).

This T rosette-forming effect of histamine was considerably inhibited by its H-2 antagonist metiamide in blood from 62.6 ± 4.7 to 15.4 ± 4.1% ($P < 0.001$) and in the spleen from 40.0 ± 11.3 to 2.2 ± 12.4% ($P < 0.05$), and it was virtually independent of the action of the H-1 histamine blocker mepyramine (Table 2).

The histidine derviatives were unable by themselves to influence T and B rosette formation by blood and splenic lymphocytes (Table 1). Determination of the ability of these compounds to block inhibition of T rosette formation by histamine showed that carnosine possesses this kind of action and reduced histamine-dependent inhibition of T rosette formation in blood from 62.6 ± 4.7 to 25.8 ± 4.7% ($P < 0.05$) and in the spleen from 40.0 ± 11.3 to 6.9 ± 10.9% ($P < 0.05$). Imidazole lactate, imidazole pyruvate, and N-acetylhistidine had no effect on the T rosette-inhibiting action of histamine (Table 2).

It follows from these results that histamine inhibits the reaction of T rosette formation by lymphocytes in the blood and spleen of sensitizined guinea pigs to a considerable degree, evidence of the high sensitivity of T lymphocytes to it under the conditions of

developing sensitization to protein allergens. Histamine exerts its action on lymphocytes through H-2 histamine receptors, since its H-2 antagonist metiamide, but not the H-1 antagonist mepyramine, was able to suppress histamine-dependent inhibition of T rosette formation.

The metiamide-like action of carnosine, causing a marked reduction in the T rosette-inhibiting effect of histamine, is evidence of the high affinity of this compound for H-2 histamine receptors on T lymphocytes, and, by its mechanism of action, it can be included in the group of H-2 histamine antagonists, The ability of carnosine to compete with histamine for H-2 histamine receptors on immunocompetent cells may be one explanation of the nature of its antiallergic effect, and it justifies the further study of this compound as promising.

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