HISTAMINE-LIBERATING ACTION OF ANTIHISTAMINES ON ISOLATED RAT MAST CELLS

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Of the new antihistamines (quinuclidine derivatives) tested, quinuclidyl-3-(o-tolyl) carbinol possessed histamine-liberating activity (HLA) on isolated rat mast cells. In concentrations tested (up to 0.4 mM) all phenothiazines (promethazine, phenethazine, chlorpromazine, methylene blue) possessed HLA. No connection was found between HLA and the antihistamine action of the compounds tested. Histamine liberation induced by antihistamines had a steep dose–response curve, took place at a low temperature, and was not inhibited under conditions inhibiting the energy-dependent stage of histamine liberation induced by compound 48/80. It is concluded that the antihistamines tested and found to possess HLA are nonselective liberators of histamine.

KEY WORDS: antihistamines; histamine liberation; mast cells.

There is reason to suppose that certain properties distinguishing the action of antihistamines are due to their ability to liberate histamine. In fact, this property has been found in certain antihistamines when tested on isolated tissues and heterogeneous cell systems [10, 11]. It is accordingly interesting to use a pure suspension of cells which are sources of histamine (mast cells) in order to study the following problems: 1) is the histamine-liberating action of antihistamines effected directly on the mast cells or through other (intermediate) cells; 2) does direct correlation exist between antihistamine and histamine-liberating activity (HLA); 3) are the compounds discovered histamine liberators with a selective or nonselective type of action [7].

This paper describes the results of a comparative study of original Soviet antihistamines (phencarol and its analogs), which belong to a new chemical class of antiallergic drugs – derivatives of quinuclidyl-diaryl-carbinols [4-6], and of known antihistamines as well as of compounds closely related to them in structure (the compounds tested are designated C I-XI).

EXPERIMENTAL METHOD

Female Wistar rats weighing 200-300 g were used. The method of isolation of the mast cells, the principles of design of the experiments, the compositions of the solutions used, and the method of spectrofluorometric determination of histamine were all described previously [2, 8]. The content of mast cells was 90-95%. In the experiments with methylene blue the mast cells ($4 \cdot 10^5$ cells per portion) were incubated in a small volume (100 μl) of buffer; the reaction was then stopped by transferring 5 μl of the cell suspension into 2 ml of cold buffer. In the final concentrations used, the test substances did not disturb the histamine determination reaction.

EXPERIMENTAL RESULTS AND DISCUSSION

Of the substances tested (Table 1) all phenothiazine derivatives (C I-IV) and C VII [quinuclidyl-3-(o-tolyl) carbinol hydrochloride] which, like C VIII-X, is a quinuclidine derivative, possessed HLA. It can be concluded from the results in Table 1 that HLA is not an essential property of antihistamines blocking H-I receptors competitively.

The HLA of phenethazine and promethazine is due to the phenothiazine ring, for HLA is also found in chlorpromazine, which confirms the existing data [9], and in methylene blue, which differs from the other phenothiazine derivatives tested in not having a side chain attached to the nitrogen atom of the phenothiazine ring.

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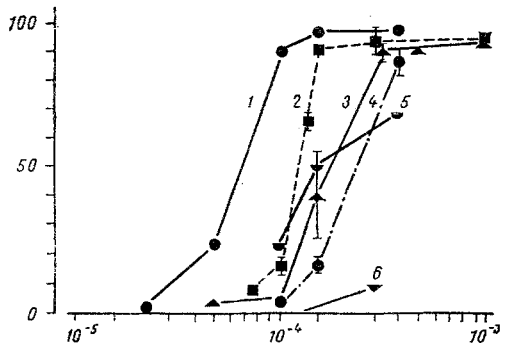
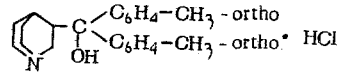
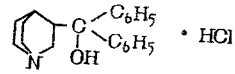
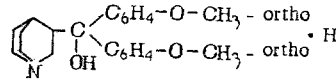
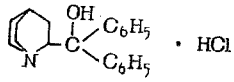
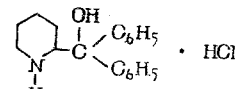


Fig. 1. Dose–response curves for chlorpromazine (1), C VII (2), promethazine (3), phenethazine (4), methylene blue (5), and C IX (6). Abscissa, concentration of substances, in M (logarithmic scale); ordinate, histamine liberation in % of total content in portion of cells. Incubation for 10 min at 38°C. Spontaneous histamine liberation 2.76 ± 0.45%. Graph plotted from values of M ± m.

TABLE 1. Comparison of Histamine-Liberating and Antihistamine Activity

Substance	Concentration, mM	HLA	Antihistamine activity
I. Chlorpromazine	0.025—0.4	Yes	0.5—0.7
II. Methylene blue	0.1—0.4	"	0
III. Phenethazine	0.1—0.4	"	1.2
IV. Promethazine	0.003—1.0	"	1.5
V. Pyrilamine maleate	0.1—0.4	No	1.5
VI. Diphenhydramine	0.001—0.4	"	1.0
VII. 	0.001—0.3	"	1.5†
VIII.  Phencarol	0.001—0.4	"	1.5
IX. 	0.001—0.3	"	1.5†
X. 	0.075—0.3	"	0.1
XI.  Piridrol	0.075—0.3	"	0

*Relative to activity of diphenhydramine, taken as 1.0 and obtained in experiments on intact animals.

†Compounds with prolonged antihistamine action.

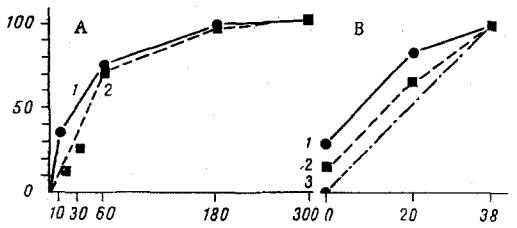


Fig. 2. Liberation of histamine induced by promethazine (1), C VII (2), and compound 48/80 (3) as a function of time (A) and temperature (B). Cells incubated in presence of substances at 38°C (for A) and for 10 min (for B). Promethazine 0.3 mM (A) and 0.4 mM (B); C VII 0.15 mM (A) and 0.4 mM (B); compound 48/80 0.1 μg/ml. Abscissa, time, sec (A) and temperature, °C (B); ordinate, histamine liberation in % of maximal. Maximal histamine liberation for promethazine 74.35% (A) and 94.86% (B); for C VII 90.51% (A) and 92.64% (B); for compound 48/80 57.97%. Spontaneous liberation: 2.35% (A) and 2.63% (B).

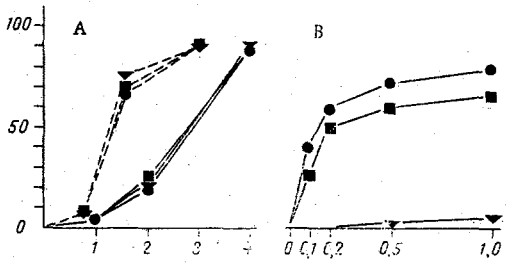


Fig. 3. Action of papaverine without (triangles) and in presence of (squares) glucose (10 mM) on histamine liberation induced by promethazine (continuous line, A), C VII (broken line, A), and compound 48/80 (B). Circles – control (in absence of papaverine).

HLA is evidently not connected with $c-N\begin{matrix}CH_3\\CH_3\end{matrix}$ groups, for other antihistamines (diphenhydramine, pyrilamine maleate), which have the same groups in their structure, do not possess such activity.

The HLA of C VII is evidently not connected with any special features of the quinuclidine ring, for neither phencarol (C VIII) nor C IX or X caused histamine liberation in the concentrations used. Finally, C XI, containing a piperidine ring as well as a quinuclidine ring, showed no HLA.

Chlorpromazine and C VII had the highest HLA (Fig. 1). Promethazine and phenethazine exhibited their maximal HLA in comparable concentrations. The dose–response curves were very steep, a characteristic feature of histamine liberators with a nonselective type of action.

The HLA of promethazine and C VII reached a maximum after 3 min (Fig. 2A). This is comparable with the time course of the HLA of chlorpromazine [9].

Manifestation of HLA even at a low temperature (0°C) was characteristic of the action of promethazine and C VII (Fig. 2B), which distinguishes them from compound 48/80 and the other selective histamine liberators [1, 7].

The writers showed previously that in medium without glucose and in the presence of papaverine, ATP reserves in the mast cells become exhausted, evidently on account of inhibition of cell respiration, with the consequent inhibition of the energy-dependent stage of histamine liberation induced by the selective liberators: compound 48/80 [1, 8], specific antigen [2], and MCD-peptide [3]. Addition of glucose to the medium abolishes this action of papaverine by restoring glycolytic metabolism and so promoting the accumulation of ATP [1-3, 8]. Under identical conditions it was shown (Fig. 3) that papaverine, although inhibiting histamine liberation induced by compound 48/80, did not affect histamine liberation induced by promethazine and C VII. Independence of histamine liberation of energy expenditure is known to be the mean feature of the action of nonselective liberators, including the phenothiazine derivative chlorpromazine [7, 9].

The results thus showed, first, that HLA of the antihistamines tested is manifested on account of their direct action on the mast cells—the sources of biologically active substances. Moreover, HLA is not an essential property of antihistamines, at least in the concentrations tested. Finally, it was shown in the case of promethazine and C VII that as regards the character of the dose–response curve, temperature dependence, and independence of energy-yielding processes of their HLA these substances belong to the category of liberators of nonselective type.

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