INHIBITION OF NONCYTOTOXIC HISTAMINE LIBERATION FROM ISOLATED MAST CELLS BY ANTIHISTAMINES IN RATS

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Phenothiazine (chlorpromazine and promethazine) and antihistamine quinuclidine derivatives [Phencarol, quinuclidyl-d-di-(o-tolyl)carbinol, quinuclidyl-3-di-(o-methoxyphenyl)carbinol hydrochloride (QMCH)], in doses below those liberating histamine, inhibited histamine liberation from the mast cells of rats induced by compound 48/80. QMCH inhibited histamine liberation induced by selective liberators (compound 48/80, MCD-peptide, and specific antigen), but potentiated histamine liberation induced by nonselective liberators (Triton X-100 and chlorpromazine). The inhibitory action of QMCH on histamine liberation induced by compound 48/80 increased for 1 min and was reversible. The inhibitory effect of all the compounds tested was reduced to some extent in the presence of glucose.

KEY WORDS: antihistamines; inhibitors of histamine liberation; histamine liberators; mast cells.

The writers showed previously that antihistamines (including the phenothiazine derivatives promethazine and phenethazine, and the new Soviet antihistamines phencarol and its homologs), by blocking H_1-receptors, liberate histamine from mast cells by a cytotoxic method [3]. Some antihistamines also reduce the anaphylactic liberation of histamine from mast cells and basophils [11, 12]. In the opinion of some investigators, the presence of these properties can be used for the selection of new antiallergic drugs [11].

This paper gives data on the mechanism of inhibition of the noncytotoxic liberation of histamine from mast cells by phencarol and other related Soviet antihistamines [5-7], by comparison with data obtained previously for histamine-liberating properties of these compounds [3].

EXPERIMENTAL METHOD

Female Wistar rats weighing 200-300 g were used. The method of isolating the mast cells, the principles of the experiments, the compositions of the solutions used, and the method of spectrofluorometric determination of histamine and of sensitization of the rats were all described previously [2]. The following compounds were tested: phencarol (CI), quinuclidyl-3-di-(o-tolyl)carbinol (CII), and quinuclidyl-3-di-(o-methoxyphenyl)carbinol hydrochloride (CIII). Phenothiazine derivatives also were tested for comparison: phenethazine (CIV) and chlorpromazine (CV). In the final concentrations used, the test drugs did not disturb the reaction of histamine determination. Spontaneous liberation of histamine varied in different experiments from 0.5 to 5%.

EXPERIMENTAL RESULTS AND DISCUSSION

In concentrations immediately below those which began to liberate histamine, the test drugs inhibited histamine liberation with mast cells induced by the noncytotoxic (selective) histamine liberator — compound 48/80 (Fig. 1). The lower the concentrations in which the histamine-liberating action of the drugs was manifested (Fig. 1: 3), the lower the concentrations respectively in which they inhibited histamine liberation induced by compound 48/80.

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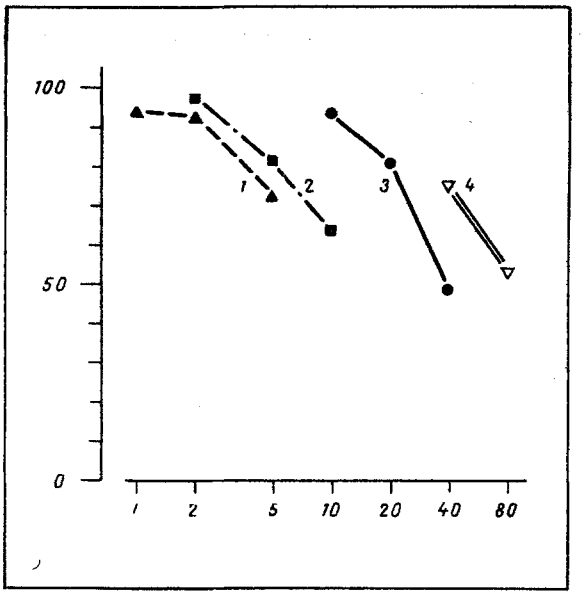

Fig. 1. Inhibition of histamine liberation induced by compound 48/80 (1 μg/ml) by compounds CIV (1), CII (2), CIII (3), and CI (4). Abscissa, concentration of inhibitors ($\times 10^{-5}$ M); ordinate, histamine liberation (in % of control — without inhibitors). Histamine liberation in control 69.9 ± 3.5%.

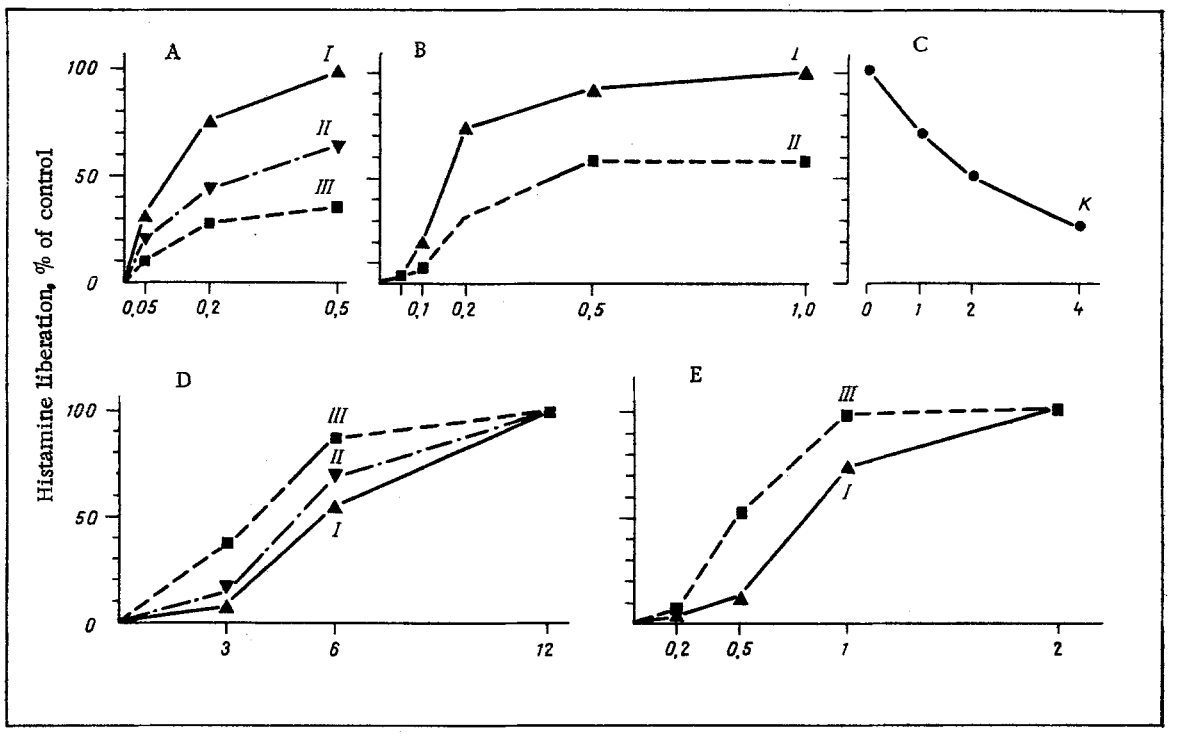

Fig. 2. Action of CIII on histamine liberation induced by compound 48/80 (A), MCD-peptide (B), antigen (C), chlorpromazine (D), and Triton X-100 (E). Abscissa, concentration of histamine liberators (μg/ml for A and B; $\times 10^{-5}$ M for D; $\times 10^{-4}$ liter/liter for E); for C, concentration of CIII ($\times 10^{-4}$ M); ordinate, histamine liberation (in % of maximal in control, without CIII). Histamine liberation in control: for A) 36.4%, for B) 45.4%, for C) 19.01%, for D) 97.0%, for E) 100.0%. For A, B, D, and E: I) control; II) in presence of CIII in concentration of $2 \cdot 10^{-4}$ M; III) in concentration of $4 \cdot 10^{-4}$ M. For C: K — concentration of antigen (horse serum) $5 \cdot 10^{-3}$ liter/liter.

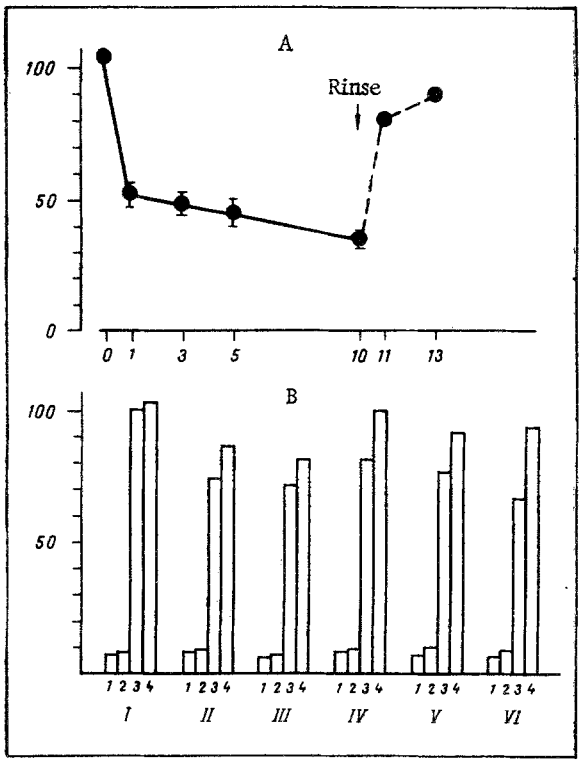

Fig. 3. Temporal characteristics of inhibitory action of CIII (A) and effect of glucose (B) on inhibition of histamine liberation induced by compound 48/80. Abscissa, for A: time (in min); ordinate, histamine liberation (in % of control — in the absence of inhibitors). Histamine liberation in control (compound 48/80 0.5 μg/ml) 49.37 ± 1.29% (for A) and (compound 48/80 1.0 μg/ml) 63.7 ± 1.69% (for B). For B: spontaneous histamine liberation without (1) and in presence of 10 mM glucose (2); histamine liberation in response to compound 48/80 without (3) and in presence of 10 mM glucose (4). I) Control, II) CIV (10^{-4} M), III) CV ($2 \cdot 10^{-5}$ M), IV) CII ($5 \cdot 10^{-5}$ M), V) CI ($5 \cdot 10^{-4}$ M), VI) CIII ($4 \cdot 10^{-4}$ M).

For a more detailed analysis compound CIII, which produced the most marked inhibition of histamine liberation within the range of concentrations tested (Fig. 1), was chosen. Depending on the dose, CIII inhibited histamine liberation induced not only by compound 48/80 (Fig. 2A), but also by other selective liberators — MCD-peptide and specific antigen (Fig. 2B, graph C). Analysis of the data in Fig. 2A by plotting on double reciprocal coordinates showed that the inhibition thus revealed was not competitive.

On the other hand, CIII, in doses not large enough to liberate histamine, clearly potentiated histamine liberation induced by nonselective (cytotoxic) liberators — Triton X-100 and CV (Fig. 2D, E).

The temporal characteristics of the inhibitory action of CIII on noncytotoxic histamine liberation corresponded to a comparatively rapid rise of inhibition (Fig. 3A). As the figure shows, the effect was reversible and, after washing the cells to remove CIII, their sensitivity to the action of compound 48-80 was restored.

The inhibitory action both of the phenothiazine derivatives (CIV, CV) and of the quinuclidyl-diaryl carbinol derivatives CI, CII, CIII was reduced in the presence of glucose (10 mM), although it was not completely prevented (Fig. 3B). It will be clear from Fig. 3 that the phenothiazine derivative CV, which has extremely low antihistamine activity but, like the other compounds tested, has a histamine-liberating action of cytotoxic type [3, 10], also exhibited an inhibitory effect.

Compound 48/80, MCD-peptide, and specific antigen are known to be selective histamine liberators and to have an action with a similar mechanism on mast cells, causing active energy-dependent liberation (secretion) of histamine [1, 4, 8].

On the other hand, the antihistamines tested and compound CV are nonselective liberators, liberating histamine by a cytotoxic method [3].

It can be concluded from these results that the inhibition of the noncytotoxic liberation of histamine described above may be connected with the cytotoxic histamine-liberating action of the substances used. Evidence in support of this conclusion is as follows: first, the relationship between the doses in which these compounds inhibit histamine liberation and liberate histamine themselves. All the compounds exhibited an inhibitory action in concentrations immediately below those causing histamine liberation. Second, in concentrations used to inhibit noncytotoxic histamine liberation these compounds enhanced the cytotoxic liberation of histamine. In addition, Triton X-100, in concentrations (5×10^{-6}–2×10^{-5} liter/liter) immediately below histamine-liberating concentrations, also inhibited histamine liberation induced by compound 48/80.

The decrease in the inhibition of noncytotoxic histamine liberation under the influence of glucose indicates that the inhibitory effect is due to the influence of the drug on the energy-dependent stage of histamine secretion [1, 2, 9, 13]. This influence can be explained either by inhibition of ATP accumulation in the cell, prevented by glucose as the source of the glycolytic pathway for a supply of energy to meet its expenditure [9], or by the leakage of ATP from the cell at a time of increased permeability of the cell membranes. Two circumstances contradict the first hypothesis. First, the temporal characteristics of the inhibitory action reflect a rapid rise in the effect. Second, addition of glucose to the medium simply reduced, but did not completely prevent the comparatively weak inhibitory action of the compounds, as would be expected if these substances inhibited the respiratory pathway of ATP accumulation [2, 9]. The more likely explanation is thus that the substances tested, in concentrations just below those liberating histamine, cause an increase in permeability of the cell membranes sufficient to allow leakage of ATP from the cell, but insufficient for histamine liberation. This hypothesis is confirmed by evidence showing that CV in fact induces ATP leakage, which is a manifestation of the cytotoxic action of the compound [13]. The results described above, in harmony with the view just expressed, do not substantiate the view that this property of the antihistamines can be utilized for the goal-directed search for new pharmacological agents with antiallergic activity.

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