INHIBITION BY TRITON X-100 OF HISTAMINE LIBERATION FROM MAST CELLS INDUCED BY SUBSTANCE 48/80

I. S. Gushchin and I. L. Deryugin

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Triton X-100, in concentrations below those liberating histamine, produced dose-dependent inhibition of histamine liberation from rat mast cells induced by substance 48/80. Triton X-100 (0.02 ml/liter) exhausted the ATP reserves in the mast cells and completely inhibited histamine liberation induced by substance 48/80, and exhaustion of the ATP content in the mast cells was abolished by glucose (10 mM). It was concluded that inhibition by Triton X-100 of histamine liberation induced by substance 48/80 depends on the inhibition of energy production.

KEY WORDS: mast cells; liberation of histamine; ATP; Triton X-100; substance 48/80.

The writers showed previously that various antihistamine compounds cause liberation of histamine from mast cells by a cytotoxic mechanism [3]. In doses below those liberating histamine, antihistamine agents inhibited liberation of histamine induced by noncytotoxic (selective) agents: substance 48/80, MCD-peptide, and specific antigen [4]. The inhibitory action of the antihistamines was linked with their cytotoxic histamine-liberating activity and was partially due to their action on the energy-dependent stage of induced histamine secretion. These facts suggest that this type of inhibitory action is a common property of widely different compounds with cytotoxic histamine-liberating activity on mast cells.

To test this hypothesis appropriate experiments were carried out with a known cytotoxic agent, Triton-X-100, the mechanism of whose histamine-liberating action has been well studied.

EXPERIMENTAL METHOD

Male albino rats weighing 300-350 g were used. The method of isolation of mast cells, the principles of setting up the experiments, the composition of the solutions used, and the method of spectrofluorometric determination of histamine were all described previously [2, 6]. The ATP content in the mast cells was determined by a bioluminescence method based on oxidation of reduced luciferin in the presence of luciferase and ATP [8, 9],* Triton X-100, in the final concentrations used, did not disturb the reactions for the determination of histamine and ATP.

EXPERIMENTAL RESULTS

The histamine-liberating action of Triton X-100 on isolated mast cells is illustrated in Fig. 1A. Within the limits of the concentration tested, Triton X-100 induced dose-dependent liberation of histamine up to the extent of total exhaustion of its reserves in the mast cells; this is a characteristic feature of cytotoxic (unselective) histamine-liberating agents [3].

In concentrations below those liberating histamine, Triton X-100 produced distinct dose-dependent inhibition of histamine liberation induced by a noncytotoxic (selective) agent, namely substance 48/80 (Fig. 1B).

On the addition of glucose (10 mM) to the medium, as Fig. 1 shows, the inhibitory action of Triton X-100 was substantially reduced.

Histamine liberation induced by selective agents is known to be an active process, dependent on the consumption of energy supplied by aerobic or glycolytic accumulation of ATP in the mast cells [1, 5, 7]. Since

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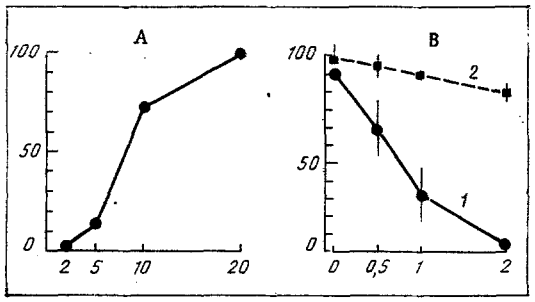

Fig. 1.

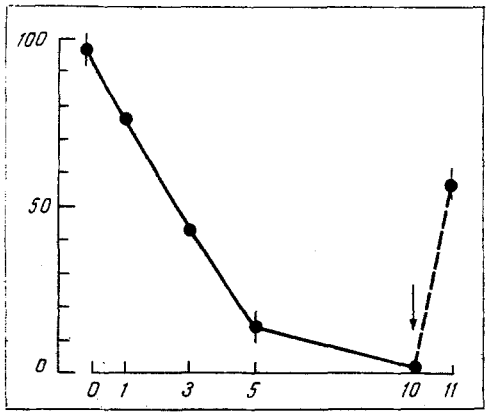

Fig. 2.

Fig. 1. Histamine liberation induced by Triton X-100 (A) and inhibition of histamine liberation induced by substance 48/80 (B). A) Cells incubated without or in presence of Triton X-100 in 200 μl buffer at 38°C for 5 min. Reaction stopped by addition of 2 ml cold buffer. Spontaneous histamine liberation 1.09%. Maximal histamine liberation 98.9%; B) cells preincubated without or in presence of Triton X-100 in 200 μl buffer at 38°C for 10 min. Substance 48/80 (0.5 μg/ml) then added to sample and incubation continued for 5 min. Reaction stopped by addition of 2 ml of cold buffer. Spontaneous histamine liberation 3.2 ± 1.0%. Histamine liberation in control (during action of substance 48/80 without Triton X-100 and glucose) 50.6 ± 6.1%. Curves: 1) without glucose; 2) with glucose (10 mM). Abscissa, concentration of Triton X-100 (in ml/liter $\cdot 10^{-2}$); ordinate, liberation of histamine in % of maximal (A) and in % of control (B).

Fig. 2. Inhibition by Triton X-100 of histamine liberation induced by substance 48/80 as a function of time. Cells preincubated without or with Triton X-100 (0.02 ml/liter) in 200 μl buffer at 38°C for different time intervals. Substance 48/80 (0.5 μg/ml) then added to it and incubation continued for 5 min. Reaction stopped by addition of 2 ml of cold buffer. Abscissa, duration of preincubation in presence of Triton X-100 (in min); ordinate, histamine liberation in % of control (in presence of substance 48/80 without Triton X-100). Each point on curve corresponds to its own control. Spontaneous histamine liberation 4.2 ± 0.64%. Histamine liberation in control 56.3 ± 1.4%. Arrow indicates washing to remove Triton X-100.

glucose, which causes accumulation of ATP in the cells by the glycolytic metabolic pathway, reduced the inhibitory action of Triton X-100, this suggests that this action of Triton X-100 is effected through inhibition of the energy-dependent stage of histamine liberation induced by substance 48/80 on account of depression of the oxidative pathway of ATP accumulation.

This suggestion also was confirmed by the study of the time-dependence of the inhibitory action of Triton-X-100. As Fig. 2 shows, inhibition of histamine liberation induced by substance 48/80 developed rather slowly under the influence of Triton X-100, to reach a maximum by the 10th min. On the other hand, the ability of the cells to liberate histamine during the action of substance 48/80 was quickly restored after washing to remove Triton X-100. This character of the curve corresponded to inhibition of a cofactor with high turnover rate [6]. The most likely candidate for this cofactor of the reaction of histamine liberation is ATP [5, 6]. Direct determination of the ATP content in the mast cells confirmed this suggestion. The initial ATP level in the mast cells was 1.5 μmole/10^9 cells, in agreement with results obtained by a different method [6]. Triton X-100, in a concentration of 0.02 ml/liter, led to virtually complete exhaustion of the ATP reserves in the mast cells and to blocking of histamine liberation induced by substance 48/80. In the presence of glucose (10 mM) the ATP content in the cells and their ability to liberate histamines under the influence of substance 48/80 were restored (Fig. 3).

The results thus confirmed the view that cytotoxic agents, in doses below those releasing histamine, can inhibit histamine secretion from mast cells. This conclusion is in agreement with earlier observations indicating that inhibition of histamine secretion by antihistamine substances is connected with the cytotoxic properties of the compound and not with their antihistamine activity [4], so that it is irrational to use this property of antihistamines for the deliberate search for new antiallergic agents [4].

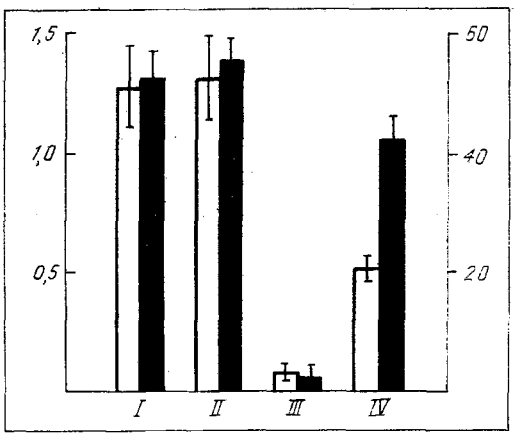

Fig. 3. Action of Triton X-100 on ATP content in mast cells and on histamine liberation induced by substance 48/80. Cells preincubated without or in presence of Triton X-100 (0.02 ml/liter) for 10 min at 38°C in 1 ml buffer. Next, 50 μl of cell suspension was taken for determination of histamine liberation and remaining cells were used for determination of ATP content. Ordinate: left, ATP content (in μmoles/10^9 mast cells); right, histamine liberation (in % of total content in batch of cells). I) Control (in absence of glucose and Triton X-100); II) in presence of glucose (10 mM); III) in presence of Triton X-100 (0.02 ml/liter); IV) in presence of Triton X-100 (0.02 ml/liter) + glucose (10 mM). Unshaded columns: ATP content; shaded columns: histamine liberation induced by substance 48/80 (0.5 μg/ml).]

The inhibitory action was shown to be due to inhibition of the energy-dependent stage of histamine secretion induced by substance 48/80, for Triton X-100 exhausted the reserves of ATP in the cells, whereas glucose, utilized in the glycolytic pathway of ATP accumulation, restored the ATP concentration and the ability of the cells to secrete histamine during the action of substance 48/80.

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