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Histamine release from rat mast cells induced by protamine sulfate and polyethylene imine

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Abstract

The protamine sulfate-induced release of histamine from rat mast cells is a non-cytotoxic reaction, similar to the 48/80-induced secretion. Polyethylene imine was found to be a less potent releaser. It is a cytotoxic substance without specificity for mast cells and acts on membranes generally. Although the two agents are related concerning their molecular weight and polybasicity, their mode of action on mast cells is clearly different.

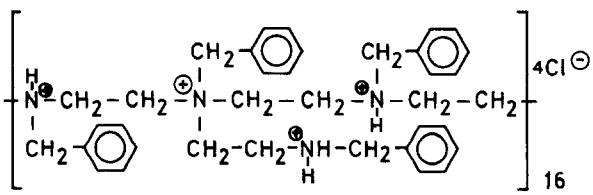

Figure 1
Benzyl-polyethylene imine.

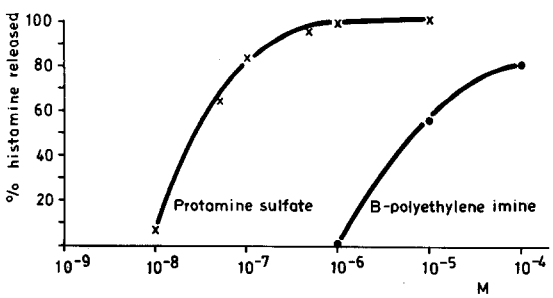

Figure 2
Histamine release from rat mast cells induced by protamine sulfate and benzyl-polyethylene imine, respectively, after incubation for 30 min at 37°C in Tyrode solution.

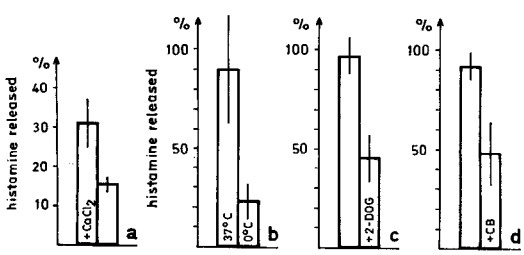

Figure 3
Histamine release from rat mast cell induced by protamine sulfate. (a) With (10^{-3} *M*) or without $CaCl_2$, (b) at 0°C and 37°C, (c) after a preincubation of 10 min with 0.1 *M* 2-deoxyglucose, (d) after a preincubation of 2 h with 3.6 μg/ml cytochalasin B, mean ± S.D.

Introduction

Protamine sulfate has been known for a long time as a histamine releaser [1, 2]. In a previous paper [3] we presented evidence that this substance, labeled with fluorescine isothiocyanate (FITC), stained the mast cell fraction of isolated rat peritoneal cells in a selective way. After a short time of incubation, the fluorescence was found inside the mast cells, labeling the granules. To obtain more information concerning the release mechanism triggered by polycations, we investigated the action of protamine sulfate in more detail and compared it with benzyl-polyethylene imine (B-PEI). This substance (Fig. 1) has a similar molecular weight and charge to protamine sulfate.

Materials and methods

The mast cells were isolated according to UVNÄS and THON [4] from female Wistar rats weighing 200 g. Further purification was achieved by the method of SULLIVAN et al. [5]. Histamine was measured by the fluorometric method of SHORE et al. [6], but omitting the extraction step. Protamine sulfate was purchased from the VEB Berlin-Chemie, B-PEI was synthesized as described earlier [7, 8]. A FITC-labeled conjugate was also prepared.

Results

Figure 2 shows the dose-response relationship of both the substances tested. B-PEI is clearly a less potent releaser.

Under the electron microscope, mast cells treated with protamine sulfate showed a loss of the electron density of their granules and the appearance of peripheral vacuolar structures containing altered granules. Evidence for a direct connection between the vacuoles and the extracellular fluid was gained by incubating mast cells in FITC-labeled human serum albumin (FITC-HSA) followed by the addition of unlabeled protamine. After the cells were washed, the fluorescence was again found inside the cells, demonstrating an influx of the FITC-HSA under the influence of protamine sulfate. Essentially the same results were published by RÖHLICH et al. [9] using compound 48/80 as releaser and lanthanum and hemoglobin as markers for the influx. The appearance of pores in the plasma membrane of mast cells was shown directly by several authors (e.g. Ref. [10]).

The protamine-induced release reaction is increased after addition of Ca^{2+} to the reaction mixture, reduced by lowering the temperature, preincubation of the cells with 2-deoxyglucose, or cytochalasin B (Fig. 3). Disodium chromoglycate also diminished the histamine release in a dose-dependent manner. Protamine sulfate did not induce a loss of lactate dehydrogenase from the mast cells. The release reaction is mediated by the plasma membrane, shown by the releasing activity of insolubilized protamine. Again, similar results were obtained with insolubilized compound 48/80 [11]. A full paper about the action of protamine sulfate on mast cells is in press [12].

Discussion

Apart from the lower releasing activity of B-PEI in comparison with protamine, this substance did not show any specificity for mast cells. The FITC-substituted derivative labeled all peritoneal cells and also lymphocytes from rats in a similar manner (membrane fluorescence), indicating a non-specific affinity for membranes. B-PEI also led to a lysis of red blood cells in the same range of order as it induced the histamine release.

We conclude that the protamine sulfate-induced release is a non-cytotoxic reaction, fulfilling some criteria of the 48/80-induced or anaphylactic histamine secretion [13]. On the other hand, benzyl-polyethylene imine, first tested for its action on mast cells, was found to be a less potent histamine releaser. It is a cytotoxic substance without specificity for mast cells and acts on membranes generally. Although the two agents are related concerning their molecular weight and polybasicity, their mode of action on mast cells is clearly different.

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Pulmonary function and histaminemia after polymyxin B inhalation

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Abstract

Bronchial challenge with polymyxin B caused a significant bronchoconstriction in 85% of atopic asthmatics. In all atopic asthmatics in whom inhalation of polymyxin B elicited bronchoconstriction, a significant, although not always parallel to the grade of bronchoconstriction, increase in histaminemia was observed. If bronchoconstriction was not elicited, the increase in histaminemia did not occur. It constitutes evidence that the bronchoconstricting effect of polymyxin B is exclusively due to degranulation of mast cells and that histamine released in the bronchi can increase the concentration of histamine in the blood.

Introduction

It is known that bronchoconstriction may occur in patients with chronic obstructive lung diseases (COLD) after inhalation of polymyxin B, which is known as a potent agent for degranulating mast cells [1]. The aim of this study was to evaluate the incidence of bronchoconstriction following polymyxin B application in a group of atopic asthmatics, and to measure the histamine concentration in blood before and after inhalation of polymyxin B.

Patients and methods

85 patients (46 men, 39 women) with atopic asthma and 20 healthy controls participated in the study. The asthmatic patients were aged from 17–49 (mean age 32), the controls from 19–28 (mean age 24). The patients did not show dyspnea immediately before starting the trial. They inhaled a 1 ml solution of polymyxin B sulfate in saline at gradually increasing concentrations of the antibiotic (0.5, 1.0, 2.5, 5.0 mg/ml). The control group inhaled 5 mg/ml and the next day 20 mg polymyxin B in 1 ml saline solution.

Bronchial obstruction was estimated based on the FEV_1 (forced expiratory volume) in the first second with the use of a Godart spirograph in all 85 patients, but only in 17 randomly selected patients was histamine measured in whole venous blood with the spectrofluorimetric method of