Histamine Release from Mast Cells: Role of Microtubules

The release of histamine and other pharmacological mediators is a prominent feature of acute inflammatory processes including immediate type anaphylactic reactions. Histamine is located in mast cells in cytoplasmic granules[1] and its release may be accomplished by two distinct mechanisms[2]: a selective discharge of amine-containing granules through a process of exocytosis, and a non-selective release of amine and other cellular components as a result of lysis of cell membrane. The selective release of histamine is supposed[3] to be consequent to a conformational change in a plasma membrane constituent which may be triggered either by positively charged molecules, such as compound 48/80[4] and polimorphonuclear lysosomal polypeptides[5], or following the interaction of antigen with cell-bound IgE antibody[6]. In both cases, the reaction is dependent upon the intracellular level of cyclic AMP[7], thus suggesting that activation of a secretory step modulated by cAMP is involved. In many cell types, including mast cells, cyclic nucleotides modify processes thought to require an intact microtubule function[8]; in fact, microtubule proteins have been found to be substrates for specific cAMP-activated protein kinases[9]. Therefore, it has been suggested[10] that exocytosis of pharmacologically active substances depends upon integrity of microtubules.

To test this hypothesis, we have examined the effects of drugs, such as colchicine and vinblastine which directly affect microtubules integrity[11–13], on the release of histamine from mast cells induced by compound 48/80 and by a purified Ascaris degranulator[14].

Material and methods. Preparation of mast cells. Male Wistar rats (200–300 g) were anesthetized with ether and decapitated. 10 ml of room-temperature Tyrode solution containing 10 U/ml of heparine U.S.P. were injected into the peritoneal cavity, the abdomen was massaged gently for 60 sec and, through a midline incision, the resulting cell suspension was aspirated into a siliconized Pasteur pipette. The cells were washed twice by centrifugation at 500 *g* for 10 min in siliconized tubes, resuspended at a concentration of 5×10^5 mast cells per ml and divided into 0.25 ml aliquots for experimental procedures.

Ion-exchange chromatography. Diethylaminoethyl-cellulose (DEAE-SH cellulose, 0.85 meq/g) was activated according to KING[15] and equilibrated with 5 m*M* phosphate buffer pH 7.9. Samples to be chromatographed were equilibrated with the starting buffer and fractionated with 9 g DEAE-cellulose in a column of 2.2×18 cm at 4 °C. Stepwise elution was performed using buffers of increasing molarity. Fractions of 6 ml were collected and protein was detected by measure of its extinction at 280 nm.

Polyacrylamide disc electrophoresis. The procedure described by DAVIES[16], with slight modifications, was performed. Electrophoresis was carried out in a 7% acrylamide gel (Cyanogum 41, BDH Chem.) at a constant current of 2.5 mA/tube. Protein bands were localized by fixing gel in trichloroacetic acid (TCA) 12.5% and staining with Coumassis bleu 0.05% in TCA.

Ascaris fluid. Ascaris fluid was obtained from *P. equorum* helminths of both sexes by cutting the caudal part. The fluid was collected dropwise and centrifuged twice (10 min at 500 *g* and 30 min at 60,000 *g*). Sediments were discarded and clear supernatants were dialyzed against buffered saline pH 7.3 and stored.

Isolation of Ascaris degranulator. Ascaris degranulator was purified from the pseudoglobulin fraction of the celomatic fluid by salting out with ammonium sulphate at 50% saturation. The precipitate, solubilized in and dialyzed against 5 m*M* phosphate buffer pH 7.9, was applied to a DEAE-cellulose column equilibrated with the same

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Table I. Effect of colchicine on histamine release from rat peritoneal mast cells induced by compound 48/80, Ascaris degranulator and decylamine [a]

Colchicine concentration (*M*)	Histamine release (%)		
	48/80	Ascaris	Decylamine
None	75 ± 4.5	88 ± 5.0	85 ± 5.0
5×10^{-6}	72 ± 6.0	84 ± 5.8	82 ± 4.8
5×10^{-5}	60 ± 3.2 [b]	70 ± 2.8 [b]	80 ± 5.2
5×10^{-4}	53 ± 2.7 [b]	62 ± 3.1 [b]	89 ± 3.6

[a] Cells were incubated with or without colchicine at the indicated concentrations for 60 min before exposure to compound 48/80 (0.2 μg/ml), Ascaris degranulator (1 mg in proteins/ml) or decylamine (60 μg/ml). Mast cell concentration = 1.25×10^5. [b] Difference from appropriate controls (no colchicine) significant at $P < 0.05$.

Table II. Effect of vinblastine on histamine release from rat peritoneal mast cells induced by compound 48/80, Ascaris degranulator and decylamine [a]

Vinblastine concentration (*M*)	Histamine release (%)		
	48/80	Ascaris	Decylamine
None	82 ± 4.0	89 ± 3.6	83 ± 5.0
5×10^{-6}	75 ± 3.5	83 ± 4.0	79 ± 2.8
5×10^{-5}	53 ± 2.9 [b]	62 ± 1.8 [b]	85 ± 7.2
5×10^{-4}	37 ± 2.8 [b]	47 ± 2.0 [b]	80 ± 4.8

[a] Cells were incubated with or without vinblastine at the indicated concentrations for 60 min before exposure to compound 48/80 (0.2 μg/ml), Ascaris degranulator (1 mg in proteins/ml) or decylamine (60 μg/ml). Mast cell concentration = 1.25×10^5. [b] Difference from appropriate controls (no vinblastine) significant at $P < 0.01$.

buffer. A protein peak, which proved to contain mast cell-degranulating factors, was eluted at this molarity. Proteins comprizing the ascending part of the peak were pooled, concentrated by ultrafiltration and rechromatographed as before. The protein fraction, obtained after rechromatography, appeared to fulfill criteria of purity when tested on acrylamide gel electrophoresis.

Histamine assay. Mast cell suspensions were incubated with the specified concentrations of colchicine or vinblastine in a final volume of 1 ml for 60 min before exposure to compound 48/80 at 0.2 µg per ml or Ascaris degranulator at 1 mg in proteins per ml. After a further 10 min, the reaction was stopped by centrifugation (500 *g* for 10 min) and aliquots of the supernatants and boiled cell debris were analyzed for histamine content by a fluorometric method[17].

Drugs. Colchicine (Sigma), vinblastine sulfate (Eli Lilly) and compound 48/80 (Burroughs Wellcome) were dissolved in Tyrode solution immediately before use.

Results and discussion. The results in Table I and II show that preincubation of mast cells with colchicine or vinblastine for a suitable time interval inhibited the selective histamine release induced by compound 48/80 and by Ascaris degranulator. By contrast, both drugs did not affect the non-selective histamine release caused by decylamine. Compound 48/80 and Ascaris degranulator both release histamine from mast cells within seconds by an energy-dependent process, whereas decylamine is a surface active agent which disrupts mast cells by a non-enzymatic mechanism not involving the biochemical steps required for granule exocytosis[18].

The mechanism of histamine release induced by compound 48/80 has been investigated in detail[19,20]. Morphological evidence suggests that the first event which follows 48/80 interaction with cell membrane is a pore formation by fusion of the cell membrane with the perigranular membrane of adjacent granules. Exposure of histamine-containing granules to the extracellular fluid then results and histamine is released by a simple cation exchange between granule amine and extracellular sodium. On the basis of this mechanism, it seems justified to equate histamine release with degranulation and to conclude that colchicine and vinblastine affect the degranulation process, probably by interfering with microtubule function. This suggestion is supported by the electron microscopic demonstration of microtubules in mast cells[21] and by the findings[13,22] that colchicine and vinblastine bind to microtubule subunits which are in equilibrium with formed microtubules thereby preventing their formation.

Microtubules, in connection with microfilaments, may provide a framework which helps to support peripheral cytoplasmic structures and guide the movements of amine-containing granules towards the plasma membrane or, if PADAWER's suggestion[23] that granules are stored in folds external to the cell prove to be correct, microtubules may favour the opening of the fold and extrusion of the granule.

Riassunto. Il rilascio selettivo di istamina da mastociti peritoneali di ratto è significativamente ridotto in seguito a preincubazione delle cellule con colchicina o vinblastina. Poichè entrambi i farmaci agiscono alterando il grado di organizzazione degli elementi strutturali del citoplasma, primariamente i microtubuli, viene suggerito che tali elementi esplicano un ruolo preminente nel processo di exocitosi delle amine farmacologicamente attive.

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A Simple Procedure for the Preparation of Ehrlich Ascites Cells for the Induction of Immunity

Although Ehrlich ascites tumour (EAT) cells have been used extensively for biochemical and immunological studies, little is known regarding the nature of the antigens associated with the cell which are responsible for immunizing mice against a lethal challenge with living cells. As a result of immunizing mice with EAT cell heterokaryons, CHEN and WATKINS[1] concluded that the antigens responsible for protecting immunized mice from a subsequent lethal challenge with living EAT cells were not of the class of strong H-2 histocompatibility antigens. These authors attributed the protection to weak antigens of an unknown nature.

A variety of methods have been used to produce devitalized ascites cells suitable for immunizing mice[2-7]. These methods suffer from one or more of the following disadvantages. 1. The antigenic components might be damaged by the procedure e.g. X-irradiation or chemical treatment resulting in the denaturation of proteins; 2. the preparative procedures are involved and 3. the recovery of antigenic material is low. This report describes a simple, mild method for the production of devitalized EAT cells which will induce a high level of immunity in the host mice, with an excellent recovery of antigenic material.

EAT cells were grown routinely by serial passage in female CFLP mice (Carworth Europe) by the inoculation of 0.1 ml. of freshly drawn ascitic fluid (concentration 2×10^7 cells) i.p. The cells were harvested after 10 days. Hypotonic lysis of the cells was effected after washing the suspension twice in PBS (0.85% NaCl, 0.01 *M* phosphate buffer pH 7.4) and subsequently resuspending in 10 to 20 times the packed cell volume of 0.01 *M* phosphate buffer pH 7.4. After standing at 4 °C for 5 min the cells were sedimented by centrifugation at 1,000 *g* for 5 min at

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