EFFECT OF COLCHICINE ON RAT MAST CELLS

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ABSTRACT

In the mast cell, a well-developed array of microtubules is centered around the centrioles. Complete loss of microtubules is observed when mast cells are treated with $10^{-5}$ M colchicine for 4 h at 37°C. The loss of ultrastructurally evident microtubules is associated with a marked change in the shape of mast cells from spheroids to highly irregular, frequently elongated forms with eccentric nuclei. In colchicine-treated cells the association of nucleus, Golgi apparatus, and centrioles is also lost.

Mast cells exposed to $10^{-5}$ M colchicine for 4 h at 37°C retain 80% of their capacity to release histamine when stimulated by polymyxin B. Exocytosis is evident in stimulated cells pretreated with colchicine and lacking identifiable microtubules. When the conditions of exposure of mast cells to colchicine are varied with respect to the concentration of colchicine, the length of exposure, and the temperature of exposure, dissociation between deformation of cell shape and inhibition of histamine secretion is observed. These observations indicate that microtubules are not essential for mast cell histamine release and bring into question the assumption that the inhibitory effect of colchicine on mast cell secretion depends on interference with microtubule integrity.

Lacy and co-workers in 1968 (15) observed that colchicine inhibited insulin secretion and proposed that microtubules were involved in cell secretion. Since then, a substantial number of reports have corroborated the inhibition of secretion by colchicine and other agents which depolymerize microtubules (3, 5, 6, 10, 14, 18, 21–23, 33, 35, 38, 43). Supportive evidence has in some cases been provided by experiments in which cells are exposed to D$_2$O, a microtubule-stabilizing agent (22, 33). However, not all experimental results have been consistent with a universal microtubular role in secretion (2, 4, 12, 20, 24, 36, 37, 41).

The mast cell is a charter member of the group of cells whose secretory activity has been reported to be inhibited by colchicine and vinblastine and potentiated by heavy water (7). The concentrations of spindle poisons required to diminish mast cell secretion 50% or more have been notably high (7, 26, 40), and the state of mast cell microtubules after treatment with the agents has not been studied. We have undertaken a correlative study of the effect of colchicine on mast cell ultrastructure and histamine secretion as part of a study of the role of microtubules in the mast cell secretory process.

MATERIALS AND METHODS

Animals used in this study were male CF rats, 300–500 g, obtained from Charles River Inc. as specific pathogen-free. Other than isolation from other rat colonies, no special precautions were taken to maintain the pathogen-free state. Cells were obtained from the peritoneal cavities of rats as previously described (17). Peritoneal washes from several animals were pooled and aliquots
prepared containing $2 \times 10^5$ mast cells/ml. Mast cells constituted between 2% and 5% of the total cells. Incubations were routinely performed in balanced salt solution (BSS) ($Na_2HPO_4$, 4.0 mM; $KH_2PO_4$, 2.7 mM; NaCl, 150 mM; KCl, 2.7 mM; CaCl$_2$, 0.9 mM; pH 7.2), containing 1 part in 200 of 35% bovine serum albumin (Pathocyte 4, Miles Laboratories, Inc., Kankakee, Ill.). A stock solution of 0.1 M colchicine (Aldrich Chemical Co., Milwaukee, Wis.) was prepared daily in BSS. In all experiments cells were routinely incubated in BSS under the same conditions with and without colchicine. Degranulation was induced by the addition of 2 $\mu$g/ml polymyxin B sulfate (Sigma Chemical Co., St. Louis, Mo.) for 5 min (16). To avoid the interference of colchicine with the histamine assay (19), cells were usually washed free of colchicine before adding polymyxin B. The wash and brief period of incubation in the absence of colchicine did not change either the number of cells exhibiting modified forms or the inhibitory effects of colchicine on histamine release at $10^{-8}$ M or $10^{-9}$ M colchicine.

Cells were routinely fixed for electron microscopy by adding an equal volume of 4% glutaraldehyde in cacodylate buffer to the cells suspended in BSS. The cells were promptly centrifuged and resuspended in 2% glutaraldehyde in 0.1 M cacodylate buffer for 2 h. The preparation of cells for electron microscopy has been described in detail (16). Briefly, cells were fixed for 2 h, washed in buffer, and centrifuged, and the pelleted was embedded in agar. Small bits of agar were then embedded in Epon. Thin sections were stained with uranyl acetate and lead hydroxide and examined in a JEOL-100 electron microscope.

Changes in cell shape produced by treatment with colchicine were also studied by light microscopy of cell suspensions. Cells suspended in BSS were fixed by adding an equal volume of absolute methanol and mixing thoroughly for 1 min. Another volume of 0.05% toluidine blue in 70% ethanol adjusted to pH 2.5 with glacial acetic acid was then added. Staining time in this solution was not critical. Differential counts of normal and abnormal mast cell forms and degranulated mast cells were performed on wet mounts of the stained suspensions at a magnification of 400. Mast cells were identified as abnormal when the nucleus was located at the periphery of an elongated cell, so that at least one-third of the nucleus was free of overlying granules, or if marked constrictions and lobulations were evident in the overall shape of the cell. Loss of the normally smooth periphery of a mast cell with assumption of a mulberry-like appearance was taken as evidence of degranulation (39).

Since the effects of colchicine treatment on mast cell form were obvious in electron micrographs, it was difficult to evaluate microtubule status in an unbiased fashion. An attempt was made to obtain an impartial analysis. 36 micrographs of control cells and nine of colchicine-treated cells ($10^{-6}$ M for 4 h at 37°C) in which a centriole or obvious satellite bodies were present were selected for analysis. The only other criteria for selection were magnification greater than 20,000 and reasonable focus. The micrographs were thoroughly shuffled, and two experienced electron microscopists unassociated with the study were asked to identify those micrographs in which microtubules were present in mast cells and those in which no microtubules were evident. The probability that there was no difference between the two groups of micrographs with respect to microtubules was evaluated by the chi-square test.

Histamine was determined in supernates and cell pellets by a modification of the o-phthalaldehyde fluorometric method (13). Supernates and PCA extracts of cell pellets were assayed directly. The mean total histamine in $10^6$ mast cells was 21 $\mu$g with a standard deviation for the mean of $\pm 7$ $\mu$g.

RESULTS

Normal Mast Cells: Light and Electron Microscopy

The normal rat mast cell in suspension is spheroidal or slightly ovoid with a more or less centrally located nucleus and numerous stubby ridges distributed irregularly over the cell surface. Specific granules surrounded by perigranule membranes fill much of the cytoplasmic volume. The Golgi apparatus and paired centrioles are typically located near one another, close to the nucleus and frequently in a concavity of a nucleus (Figs. 1-3, 6). Small strands of endoplasmic reticulum, largely rough surfaced, are distributed through the intergranule cytoplasm. Microtubules are seen most frequently radiating from the periphery of a centriole where the microtubules are associated with the pericentriolar satellites (Figs. 2-4, 6). Microtubules variously take an outward course from the centrioles toward the plasma membrane (Figs. 3, 5, 6) or an inward course toward the nucleus (Figs. 1, 2, 6). Both in the perinuclear region and at the periphery of the cell, longitudinal and cross sections of tubules are evident. While microtubules approach the nuclear membrane through a range of angles from 90° to the very acute (Figs. 1, 2, 6), microtubules as they approach to the plasma membrane tend to assume a course parallel to the plasma membrane (Fig. 5). Microtubules do not extend into the surface ridges (Fig. 5). Microtubules can be seen very close to, perhaps attached to the nuclear envelope (Fig. 6). No micrographs of close approach of microtubules to the plasma membrane were obtained in this study.
Cell center of normal mast cell. Three stacks of Golgi membranes (G) and a centriole (C) are present. Numerous microtubules (arrows) are evident close to the nucleus (N). × 46,700.
**Effects of Colchicine on Mast Cells: Light and Electron Microscopy**

Mast cells treated in vitro for 4 h at 37°C with $10^{-5}$ M colchicine consistently lose their spherical form, become elongated, and assume quite bizarre forms with constrictions and irregular protuberances (Figs. 7 and 8). Associated with the change in shape, the nucleus shifts from the center to the periphery of the cell. By electron microscopy, the eccentrically displaced nucleus is seen typically to be covered over its outer edge by a thin rim of cytoplasm. The centrioles and Golgi apparatus lose their typical association with the nucleus and with one another (Figs. 9–13). Microtubules are nowhere evident in the colchicine-treated cells, specifically in the vicinity of the centrioles (Figs. 9–13). The surface ridges appear normal in size and number, neither retracting nor enlarging. Large irregular protrusions or pseudopods and marked constrictions in the cytoplasmic mass of the cells produce a variety of unusual cell forms (Fig. 14).

The reliability of our identification of the loss of microtubules from mast cells exposed for 4 h to $10^{-5}$ M colchicine was established by having two electron microscopists who were not involved in the experiments evaluate a randomized set of electron micrographs of treated and control cells in which a centriole or obvious satellite body was present (Table I).

**Histamine Secretion in Colchicine-Treated Mast Cells**

At $10^{-5}$ M colchicine for 4 h at 37°C, there is 20% ± 4% inhibition of secretion (Table II). Direct observation by light microscopy indicates that cells deformed by the action of $10^{-5}$ M colchicine are able to secrete their granules. 200 mast cells exposed to $10^{-5}$ M colchicine for 4 h at 37°C and then polymyxin B, 2 µg/ml for 5 min, were evaluated by light microscopy for colchicine deformation and degranulation induced by polymyxin B. 80% of mast cells exhibited morphologic changes attributable to the action of colchicine, and 89% of these colchicine-modified cells responded to polymyxin B with secretion; 85% of the control cells showed evidence of degranulation. Most mast cells in which no microtubules were identifiable by electron microscopy after incubation in $10^{-5}$ M colchicine for the same time were clearly capable of secretory activity (Figs. 15, 16) when exposed to polymyxin B.

In order to further determine the relationship between the induction of morphologic changes and the inhibition of histamine release, the influence on these two effects of varying the concentration of colchicine (Fig. 17), the time of exposure to colchicine (Fig. 18), and the temperature of exposure (Fig. 19) was examined. In each instance, substantial discrepancies between the effects of colchicine on the two variables were observed. The effect of $10^{-4}$ M colchicine on mast cell secretion was independent of the concentrations of polymyxin B between 0.25 µg/ml and 5 µg/ml (Table III).

**DISCUSSION**

The study of the effect of colchicine and related substances on mast cells was initiated by Padawer (27, 31). He described changes in peritoneal mast cells after subcutaneous administration of colchicine ($1.6 \times 10^{-7}$ mol/100 g body wt) identical to those we see with colchicine in an in vitro system. Padawer's studies antedated both the description of microtubules in interphase cells and the evidence that colchicine caused the breakdown of these microtubules as well as those of the mitotic spindle. An electron microscope study of the effect of colchicine on mast cells by Padawer (30) has been reported in an abstract, and our observations in vitro are entirely consistent with his description of the disappearance of microtubules after in vivo administration of colchicine.

Studies of the effects of colchicine, low temperature, and high pressure on a variety of cell types have supported the thesis of Porter (34) that microtubules serve as a cytoskeleton to maintain intermitotic cell shape. Typically, loss of microtubules is associated with the conversion of an asymmetric cell to a symmetric form (11, 25, 32, 34). The effect of colchicine on the mast cell provides an example of the converse situation in which a spheroidal cell becomes asymmetric. Padawer (28), studying the mast cell, and Bhisey and Freed (1), the macrophage with time-lapse cinematography, described the formation of large ameboid protrusions from the cells associated with mass movements of cytoplasmic contents. Similar shape changes have also been described in lymphocytes (42). Bhisey and Freed (1) proposed that the mass flow of cytoplasm is produced by cortical microfilament contraction in the absence of microtubules.
TABLE I

Evaluation of Microtubule Status in Colchicine-Treated and Untreated Mast Cells

| Observer | Absent microtubules | Probability of null hypothesis |
|----------|---------------------|-------------------------------|
|          | Colchicine treated  | Control | \( \chi^2 \) | <0.001 |
| I        | 9/9                 | 0/36    | 43             | <0.001 |
| II       | 9/9                 | 5/36    | 24             | <0.001 |

9 electron micrographs of mast cells exposed to \( 10^{-5} \) M colchicine for 4 h at 37°C and 36 micrographs of mast cells not exposed to colchicine were evaluated for the absence of microtubules as described in Materials and Methods.

Inhibition of histamine secretion from rat peritoneal mast cells by colchicine and the similarly acting agents, vinblastine and griseofulvin, was reported in 1968 by Gillespie et al. (7). They observed 50% inhibition of histamine release stimulated with 48/80 after exposure to colchicine at \( 5 \times 10^{-4} \) M for 3 h at 37°C. Somewhat more effective inhibition of secretion induced by polymyxin B was observed with the same concentration of colchicine. The other agents studied also inhibited histamine release in their experiments.

Inhibition of histamine release by colchicine and similarly acting agents has also been observed with human basophils (8, 9, 19).

TABLE II

Effect of Colchicine on Histamine Release and Mast Cell Form

|               | Histamine release | Altered cell form |
|---------------|-------------------|-------------------|
| Control       | 74 ± 3            | 0                 |
| Colchicine 10^{-5} M | 59 ± 4*         | 76.5 ± 5.3       |
| Colchicine 10^{-3} M | 15 ± 2‡          | 75.7 ± 6.7        |

Cells were incubated for 4 h in vitro at 37°C in BSS (control), \( 10^{-5} \) M colchicine in BSS, or \( 10^{-3} \) M colchicine in BSS. Mast cells were evaluated by light microscopy for alteration in form. Histamine release was determined after adding polymyxin B, 2 \( \mu \)g/ml for 10 min. The values for altered forms are means ± SE from four experiments in which at least 100 cells were counted in each experiment. The values for histamine release are the means ± SE of duplicate determinations in 12 experiments.

* Mean difference of paired values was 14.4 ± 3.0, with \( P = 0.0003 \) by the paired \( t \)-test.
‡ Mean difference of paired values was 58.7 ± 2.7, with \( P < 0.0001 \) by the paired \( t \)-test.

Figure 2 Normal mast cell. A microtubule (mt) radiates from a dense satellite body on the centriole (C). \( \times 27,800. \)

Figure 3 A centriole in a normal mast cell. The microtubules appear to insert into the satellite bodies (arrows). \( \times 48,000. \)

Figure 4 Microtubules are clearly associated with the dense amorphous material of the satellite bodies (arrows). \( \times 52,000. \)

Figure 5 Subplasmalemmal region of a normal mast cell. One microtubule (mt1) courses beneath the plasma membrane. Another microtubule (mt2) is located between two granules. A microridge (mr) is evident. \( \times 28,000. \)

Figure 6 Cell center of a normal mast cell containing a centriole (C) and Golgi stacks (G). Occasional microtubules appear to terminate at the outer nuclear membrane (arrow). A few cross sections of microtubules (circle) can also be seen. Microtubule termination at a satellite body (S) is evident. \( \times 22,500. \)
A mast cell treated with $10^{-7}$ M colchicine in vitro for 4 h. The nucleus (N) is displaced to one end of the cell. An array of saccules suggestive of the Golgi apparatus (G) is present at the opposite pole of the cell from the nucleus. $\times 16,500$. 
Figure 8  A mast cell treated with $10^{-8}$ M colchicine for 3 h in vitro at 37°C. Small lobes of the displaced nucleus covered by a thin layer of cytoplasm protrude from the main nuclear mass. $\times$ 18,000.
Figures 9-13  Mast cells treated with $10^{-5}$ M colchicine for 4 h at 37°C. In each instance, a centriole or portion of centriole is present, yet no microtubules are evident. The centrioles are clearly displaced from their usual position in the central region of the cell to a peripheral site. Fig. 9, $\times$ 28,000. Fig. 10, $\times$ 30,000. Fig. 11, $\times$ 33,000. Fig. 12: A pair of centrioles located beneath the cell membrane. Amorphous dense material obscures details of centriolar structure. $\times$ 26,000. Fig. 13, $\times$ 35,000.

In our experiments $10^{-5}$ M colchicine for 4 h at 37°C was effective in eliminating mast cell microtubules. By light microscope criteria, 80% of mast cells exhibited changes attributable to loss of microtubules; nuclear displacement to the periphery and/or loss of their spheroidal form. Under the same conditions of colchicine treatment we were unable to identify microtubules in electron micro-
Figure 14 Mast cell treated with $10^{-3}$ M colchicine in vitro for 1 h at 37°C. Elongation is particularly prominent in this cell. A small mass of cytoplasm containing the nucleus appears, in this section, to be connected to the bulk of the cell by only a thin isthmus of cytoplasm. $\times$ 12,000.

### Table III

*Effect of Polymyxin B Concentration on the Inhibitory Effect of Colchicine*

| Polymyxin B concentration | 2.0 $\mu$g/ml | 1.0 $\mu$g/ml | 0.5 $\mu$g/ml | 0.25 $\mu$g/ml |
|---------------------------|---------------|---------------|---------------|----------------|
| Histamine release         |               |               |               |                |
| Control                   | 85, 80        | 76, 70        | 62, 47        | 40, 27         |
| Colchicine, $10^{-5}$     | 70, 66        | 59, 62        | 48, 40        | 33, 22         |
| Inhibition                | 17, 16        | 23, 12        | 23, 14        | 19, 17         |

Control cells and cells treated with colchicine, $10^{-3}$ M for 4 h at 37°C, were exposed to varying concentrations of polymyxin B for 5 min. Percent histamine release values for two separate experiments are means of duplicate determinations corrected for spontaneous histamine release.

Graphs of mast cells, while in untreated mast cells prepared for examination at the same time, microtubules were found consistently in the region of the cell center. In order to answer the objection that a population of cells unaffected by colchicine was responsible for much of the persisting secretory activity, colchicine-treated cells were examined by light microscopy for degranulation; it was observed that colchicine-deformed cells had as high an incidence of degranulation as normal mast cells. In confirmation, electron micrographs showed secretion by colchicine-deformed, microtubule-free mast cells. The results demonstrating differences between the conditions required for inhibition of histamine release and those required for production of a change in mast cell shape support the proposal that inhibition of secretion by colchicine cannot be simply attributable to the depolymerization of microtubules. This argument would be stronger if quantitative measurements of...
FIGURE 15 A mast cell treated with $10^{-8}$ M colchicine for 4 h at 37°C, then treated with 2 μg/ml polymyxin B for 5 min. The characteristic pattern of degranulation is evident with a few completely discharged granules and several channels of varying size containing one or more granules in different stages of dissolution. × 14,000.

The state of microtubules were to confirm the morphologic evidence.

Dissociation of microtubule loss and inhibition of histamine release induced by colchicine might be the consequence of: (a) a facilitatory rather than an essential role of microtubules in mast cell secretion; (b) an effect of colchicine on a subpopulation of microtubules, membrane-associated for instance, that is relatively resistant to colchicine, contributes little to maintenance of cell form, and is difficult to identify by electron microscopy; or (c) an action of colchicine on a mast cell compo-
FIGURE 16 Mast cell treated with $10^{-3} \text{M}$ colchicine for 3 h at 37°C, then exposed to 2 $\mu$g/ml polymyxin B for 5 min. In spite of the colchicine-induced distortion of cell form, extensive granule secretion is evident. $\times 12,000$.

FIGURE 17 Effect of colchicine concentration on histamine release and mast cell form. Cells were incubated for 4 h at 37°C in varying concentrations of colchicine in BSS. At least 100 cells were evaluated for form at each concentration. Cells were exposed to polymyxin B 2 $\mu$g/ml for 5 min. Values for percent histamine release are the means of duplicate determinations.

FIGURE 18 Effect of time of exposure to colchicine on histamine release and mast cell form. Cells were incubated for varying times at 37°C in colchicine, $10^{-3} \text{M}$. At least 100 cells were evaluated for form in two separate experiments. Values for percent histamine release in the presence of 2 $\mu$g/ml polymyxin B for 5 min are means $\pm$ SE for nine experiments.
FIGURE 19 Effect of temperature on histamine release and cell form. Cells were incubated in 10⁻³ M colchicine for 4 h at varying temperatures. At least 100 cells were evaluated for form in two separate experiments. Values for percent histamine release in the presence of 2 μg/ml polymyxin B for 5 min are means ± SE for four separate experiments.

ment other than microtubules. Further studies will be required to distinguish among these possibilities.

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