Histamine Release from Human Leukocytes: Studies with Deuterium Oxide, Colchicine, and Cytochalasin B

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ABSTRACT Agents known to interact with either microtubules or microfilaments influenced the antigen-induced release of histamine from the leukocytes of allergic individuals. Deuterium oxide (D_2O) which stabilizes microtubules and thereby favors their formation enhanced histamine release markedly. Concentrations as low as 5% increased antigen-induced release somewhat while concentrations as high as 75% had no effect on release in the absence of antigen. Enhancement occurred over a wide range of antigen concentrations and was also seen when release was initiated by antibody to IgE or IgG. When the release process was divided into two stages a D_2O activity could be demonstrated only in the second stage. However, when D_2O was present in the first stage together with agents which raise cyclic AMP levels and thereby inhibit release it partially reversed this inhibition. Colchicine, demecolcine, and vinblastine, compounds known to disaggregate microtubules, i.e., have an effect opposite to that of D_2O, inhibited the release of histamine and counteracted the effects of D_2O. The inhibitory action of colchicine was greater if cells were treated with colchicine before rather than after activation with antigen. Cytochalasin B, a compound which causes the disappearance of microfilaments, had variable effects on histamine release. The most frequently seen response was slight enhancement. Neither D_2O nor cytochalasin B altered cyclic AMP levels in leukocytes. These observations support and strengthen the view that an intact and functioning microtubule system is directly important for the secretion of histamine from leukocytes and suggest that microfilaments might have multiple indirect effects.

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INTRODUCTION

Antigen-mediated release of histamine from the leukocytes of allergic individuals is a good in vitro model for allergic reactions. The extent of release correlates well with clinical symptoms (1) and drugs, e.g., isoproterenol and theophylline, which alleviate the in vivo symptoms also inhibit the in vitro release process (2). At the same time the mechanism by which histamine is released from leukocytes is fundamentally similar to the release process in other tissues, with cell-bound antibody (IgE) acting as a receptor and antigen replacing a hormone or other releasing agent.

Colchicine, a drug known to disrupt microtubules, has been shown to inhibit the release of a variety of compounds (3–6) including histamine from leukocytes (7). This observation has been interpreted in all cases as indicating that microtubules play a role in the release process. Deuterium oxide (D_2O) which is known to stabilize microtubules (8, 9), i.e., act in a manner opposite to colchicine, has been studied in three release systems. In the rat peritoneal mast cell it potentiates the release of histamine by other releasing agents and also acts as a releasing agent itself (3); in the cat adrenal gland it potentiates the release of catecholamines by acetylcholine (6); and in the thyroid gland it inhibits release (10). Recently cytochalasin B, a drug which causes the disappearance of 40–70 A microfilaments from cells but has no effect on the morphology of microtubules (11, 12) has been shown to inhibit the release of thyroid hormone and growth hormone (13, 14) and potentiate the release of insulin (15).

The present study was designed to evaluate the role of microtubules and microfilaments in the release of histamine from leukocytes through the use of D_2O, colchicine, and cytochalasin B individually and in combination. It was found that D_2O potentiated the release process markedly while colchicine and other microtubule dis-

rupters inhibited release and counteracted the effect of D_2O. Cytochalasin B had variable effects depending on the individual cells used and the level of histamine release obtained. In the majority of cases it potentiated release somewhat although inhibition was occasionally observed. These results support the view that microtubules are directly important in the release process and suggest that microfilaments have multiple indirect effects.

METHODS

Leukocytes from allergic individuals were prepared by dextran sedimentation as previously described (16). They were resuspended at a dilution of approximately 10^7 cells/ml and incubated at 37°C in a medium consisting of (mM): NaCl, 120; KCl, 5; $CaCl_2$, 0.6; $MgCl_2$, 1; tris (hydroxymethyl) aminomethane, 25; human serum albumin, 0.1%, adjusted to pH 7.4. D_2O replaced H_2O in the medium as required. Experiments involving colchicine were also carried out using a phosphate buffer since tris has been reported to interfere with the action of colchicine (17). Under our conditions the effect of colchicine in the two buffers was the same. In experiments designed to study the two stages of the release process (18) cells were exposed to antigen for 2 min in medium free of calcium and magnesium, washed twice and resuspended in the absence of antigen in the complete medium described above. Histamine released from the cells into the supernatant fluid was measured by a micromodification of the fluorometric technique of Shore (19, 20). All experiments were carried out in duplicate and repeated using the cells of at least two and usually three to five different individuals. The average difference between duplicate measurements using this technique was less than 5%.

Cyclic AMP was measured by the protein-binding method of Brown, Albano, Ekins, and Sgherzi (21). This procedure is based on the competition between cyclic AMP in the sample and added radioactive cyclic AMP for a binding protein prepared from bovine adrenal cortex. Charcoal is used to separate free and bound nucleotide.

Ragweed antigen E was provided by Dr. T. P. King (22) and rye grass group I antigen by Dr. D. G. Marsh (23). D_2O was obtained from BioRad Laboratories (Richmond, Calif.), demecolcine (Colcemid) from Ciba Pharmaceutical Co. (Summit, N. J.), vinblastine sulfate (Velban) from Eli Lilly & Co. (Indianapolis, Ind.), and cytochalasin B from Imperial Chemical Industries (Alderly Park, England). This last drug was dissolved in dimethylsulfoxide (DMSO) (10 mg/ml) and diluted as required with incubation medium. DMSO controls were included in all experiments involving cytochalasin B.

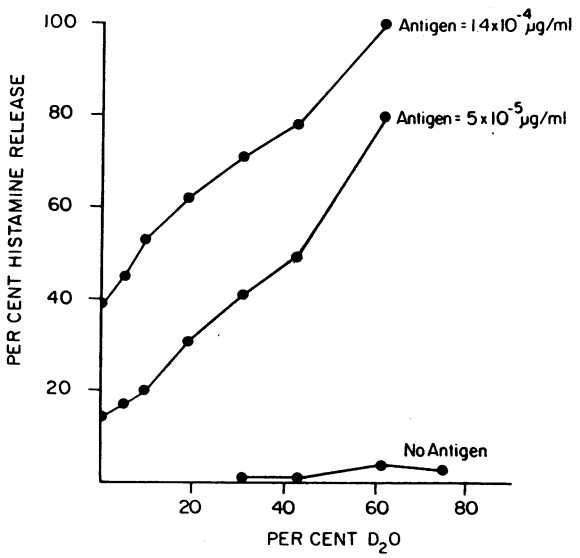

FIGURE 1 The effect of various concentrations of D_2O on histamine release. Ragweed antigen E and D_2O were added at 0 time and the cells incubated for 45 min.

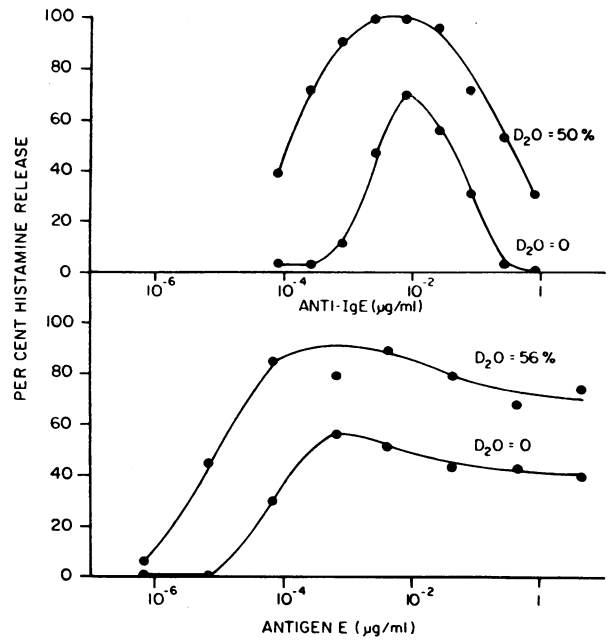

FIGURE 2 The effect of D_2O on histamine release induced by either antigen E or anti-IgE antibody. Releasing agent and D_2O were added at 0 time and the cells incubated for 45 min.

RESULTS

Effects of D_2O on histamine release. D_2O markedly enhanced the release of histamine from leukocytes under a variety of experimental conditions. D_2O at levels as low at 5% significantly increased release due to antigen while concentrations as high as 75% had no effect in the absence of antigen (Fig. 1). Enhancement occurred over a wide range of antigen concentrations including levels insufficient to cause release alone and levels high enough to inhibit the process (Fig. 2). Enhancement of anti-IgE mediated release (24) was similar in all respects to that noted with antigen (Fig. 2). Potentiation of release by D_2O was also seen using the cells of individuals who can maximally release only 10–20% of their total histamine and in situations where the releasing agent usually can induce only a limited response, i.e.,

antibodies to IgG (25) (Fig. 3). When the time course of release was studied, D_2O increased the rate of release rather than otherwise altering the time course (results not shown). Enhancement of histamine release by D_2O of the order of magnitude illustrated in Figs. 1–3 was seen in experiments with the cells of all individuals studied (Table I). The per cent enhancement is, of course, related to control release in that if release in the absence of D_2O is 50% enhancement cannot exceed 100% while if control release is 5% enhancement can be 2000%.

The release of histamine from leukocytes can be operationally divided into two stages, an antigen-dependent, calcium-independent first stage and an antigen-independent, calcium-dependent second stage (18). The effects of D_2O on the two stages of the release reaction were studied by carrying out either the first or the second incubation in medium containing D_2O. Enhancement occurred only when D_2O was present during the second stage (Table II).

Interaction between D_2O and colchicine, demecolcine, or vinblastine. Since the effects of D_2O on histamine release are presumably due to its action on microtubules, drugs such as colchicine, demecolcine (Colemid), and vinblastine which disaggregate microtubules should counteract the enhancement of release caused by D_2O. To study this interaction cells were incubated with or without colchicine, demecolcine, or vinblastine for 20 min at 4°C., washed and resuspended with antigen in either normal medium or medium in which a portion of the H_2O was replaced by D_2O. Colchicine, as reported previously (7) inhibited histamine release; inhibition was also observed after treatment of the cells with demecolcine or vinblastine (Fig. 4). In the presence of both D_2O and either colchicine, demecolcine, or vinblastine, release was always intermediate between that seen in the presence of either alone (Fig. 4). Similar results were obtained in four separate experiments involving D_2O and colchicine and in two experiments with D_2O and demecolcine or vinblastine.

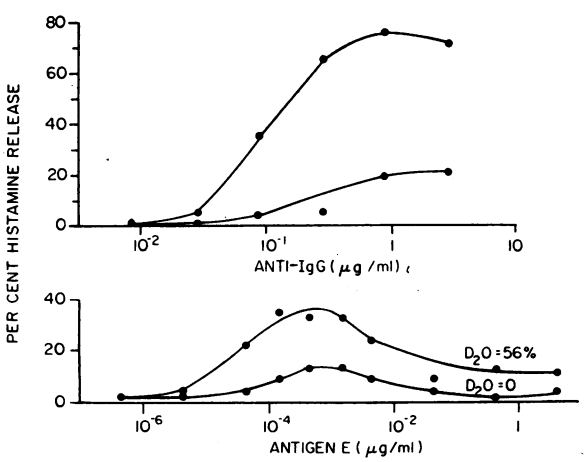

FIGURE 3 The effect of D_2O on histamine release in situations where maximum release is low. Ragweed antigen E or anti-IgG and D_2O were added at 0 time and the cells incubated for 45 min.

TABLE I

Effects of D_2O on Histamine Release from the Leukocytes of Various Individuals

Subject	Control histamine release	Potentiation by D_2O			
		D_2O: 7%	15%	31%	62%
	%	%	%	%	%
M. C.	38*			85	
M. C.	56			42	
P. A.	34	35			182
B. W.	14		100	321	414
B. W.	24		58	158	316
J. S.	4				1100
J. S.	34				162
C. S.	5				520
M. M.	13				154
K. B.	40				85
S. M.	38			68	118
D. L.	50			60	100

* Each number is the average of duplicate measurements in a single experiment. Duplicates did not vary by more than 5%.

Interaction between D_2O and isoproterenol or prostaglandin E_2. Compounds that raise cyclic AMP levels in leukocytes, e.g., isoproterenol and prostaglandins E_1 and E_2, are known to inhibit histamine release through an action confined largely if not completely to the first stage of the process (26). In contrast D_2O acts only in the second stage (Table II). The interraction between D_2O and cyclic AMP was studied in two different sets of experiments. In the first set either isoproterenol or prostaglandin E_2 was present during the first incubation with

TABLE II

The Effect of 62% D_2O on the First and Second Stages of Histamine Release

Exp.	Histamine release		
	Control (no D_2O)	D_2O present, 1st stage	D_2O present, 2nd stage
	%	%	%
1	32*	32	79
2	11	13	68
3	23	25	65

* Each number is the average of duplicate measurements in the same experiment. Duplicates did not vary by more than 5%.

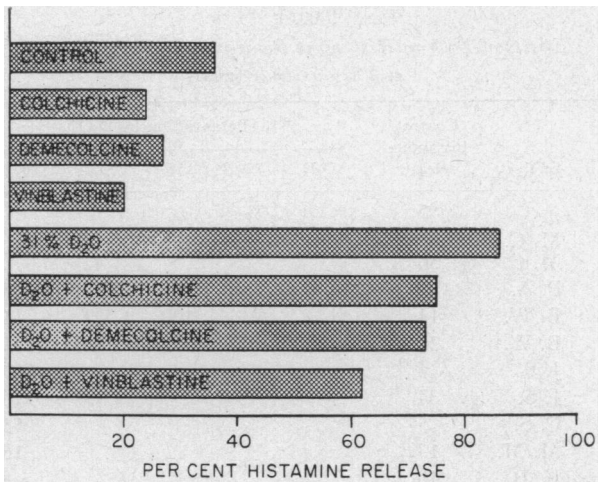

FIGURE 4 Interaction between D_2O and agents which disaggregate microtubules. Cells were preincubated at 4°C with colchicine, demecolcine, or vinblastine (3×10^{-4} M) for 20 min. They were then washed twice and resuspended with antigen in H_2O or D_2O medium for 45 min at 37°C.

antigen and D_2O was present during the second incubation. Under these conditions isoproterenol and PGE_2 inhibited release, D_2O enhanced it, and the combination of compounds led to intermediate levels of release. Fig. 5 illustrates results obatined using PGE_2 to raise cyclic AMP levels.

In the second experimental approach the interaction between D_2O and either isoproterenol or PGE_2 when both were present in the first stage of the release reaction was examined. Cells were first incubated in the

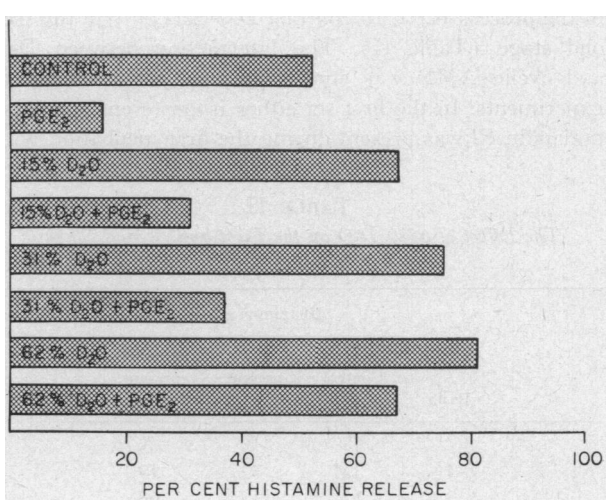

FIGURE 5 Interaction between D_2O and prostaglandin E_2. Cells were incubated with antigen and PGE_2 (1.4×10^{-6} M) for 2 min in the absence of calcium and magnesium, washed twice, and resuspended in H_2O or D_2O medium containing calcium and magnesium for 45 min.

TABLE III

Interaction in the First Stage of Histamine Release between D_2O and Agents which Raise Cyclic AMP Levels

Exp.	Addition (X)	Inhibition of histamine release*		
		X only	62% D_2O	X + 62% D_2O
		%	%	%
1	Isoproterenol 5×10^{-6} M	60	10	18
2	Isoproterenol 7×10^{-8} M	57	0	26
3	Prostaglandin E_2 2×10^{-6} M	90	5	51
4	Prostaglandin E_2 7×10^{-7} M	61	8	0

* Release in the absence of added drugs was 50–80%. The values reported are the average of duplicate determinations which did not vary by more than 5%.

presence of 62% D_2O for 10 min. Isoproterenol or PGE_2 and antigen were then added for 2 min after which the cells were washed free of all added compounds, resuspended in calcium containing medium and incubated for 45 min. Under these conditions D_2O partially reversed the inhibition caused by either isoproterenol or PGE_2 (Table III). Cyclic AMP was measured after the first incubation period in leukocytes incubated in parallel vessels. D_2O itself had no effect on cyclic AMP levels and also did not alter the response to either isoproterenol or PGE_2.

Effects of colchicine on histamine release before and after treatment of the cells with antigen. The possibility that "activation" of the cells by antigen in the first stage of the reaction involves a change in the state of the microtubule system was investigated by determining the inhibitory effects of colchicine before and after treatment of the cells with antigen. Cells were exposed separately to antigen for 2 min at 37° and to colchicine (3×10^{-4} M) for 15 min at 0° in a calcium and magnesium free incubation medium. The order of treatment was either antigen followed by colchicine or the reverse, colchicine followed by antigen. The cells were washed twice after each treatment and finally resuspended in calcium containing medium for 45 min at 37°. Histamine release from control cells, i.e., those not treated with colchicine, was unaffected by the difference in handling. The inhibitory action of colchicine was considerably less when the cells were treated with antigen before treatment with colchicine (Table IV).

Effects of cytochalasin B on histamine release. The effects of cytochalasin B on histamine release from the cells of several individuals were examined (Table V). The results varied considerably ranging from inhibition in two cases to moderate enhancement of release in others. Cytochalasin B (5 μg/ml) had no effect on release in the absence of antigen. It also had no effect on cyclic AMP levels in leukocytes after incubation for

TABLE IV

Effect of Colchicine on Histamine Release before and after Activation of Cells with Antigen

Exp.	Histamine release	Inhibition by 3×10^{-4} M colchicine: Before activation	Inhibition by 3×10^{-4} M colchicine: After activation
	%	%	%
1	89*	31±3‡	4±7
2	49	64±4	24±4
3	62	54+	28

* Average of duplicate measurements which differed by not more than 5%.

‡ Average of quadruplicate measurements ±SD.

from 2 to 45 min (eight time intervals studied). The effect of cytochalasin B on the time course of histamine release is shown in Fig. 6. The drug appears to decrease the initial time lag seen before release begins and also increase the rate of release slightly. DMSO at a level equal to that in the cytochalasin solution (0.05%) had no effect on the release process.

TABLE V

Effect of Cytochalasin B on Histamine Release from the Leukocytes of Various Individuals

Subject	Histamine release: Control	Histamine release: Cytochalasin B (5 μg/ml)	Per cent change
	%	%	
M. K.	18*	12	−33
E. E.	19	10	−47
E. E. (2nd exp.)	12	6	−50
E. E.	74	82	+11
D. L.	61	68	+12
K. B.	60	74	+23
M. S.	15	24	+60
M. S.	45	61	+36
L. W.	31	51	+64
M. C.	25	43	+72
L. M. L.	31	59	+90
L. M. L.	76	100	+31

* Each number is the average of duplicate measurements in the same experiment which differed by not more than 5%. Dimethylsulfoxide (DMSO) at a level equal to that in the cytochalasin solution (0.05%) had no effect on the release process.

DISCUSSION

The present study explores the effects of compounds known to interact with microtubules or microfilaments on the antigen-induced release of histamine from human leukocytes. The results obtained considerably strengthen the view of Levy and Carlton (7) that microtubules are involved in this process. These investigators found that colchicine, a drug which acts by binding to the subunits of microtubules and thereby leading to their disaggregation (27–29) inhibited histamine release. This action of colchicine was considerably enhanced by cold which is known to cause the reversible breakdown of microtubules into subunits (30, 31). In the present study we have found that two other drugs, demecolcine and vinblastine, which disrupt microtubules (32, 33) also inhibit the release of histamine from leukocytes. At the same time D_2O which is known to favor the formation of microtubules in other cells (8, 9) markedly potentiates antigen-induced histamine release. The enhancement of release caused by D_2O is greater by far than that seen with any other agent reported to date. The effects of D_2O, unlike those of colchicine, were found to be rapid in onset and readily reversed by removing the D_2O. This made it possible to determine at what stage in the release reaction a functioning microtubule system is required. When the release reaction was divided into two stages D_2O acted only in the second stage of the process suggesting that microtubules are necessary for the actual secretion of histamine rather than for the initial antigen-induced activation of the process. This finding fits well with the idea of others (3, 6, 7) that the microtubule system is involved in some way with the movement of granules toward the cell membrane.

The interaction seen between D_2O and isoproterenol or prostaglandin E_2 is of particular interest. These latter two compounds inhibit the release of histamine presumably by raising cyclic AMP levels through an action in the first (activation) stage of the release process (26). D_2O, when also present in the first stage, can partially prevent this inhibition. This result has important implications. First since D_2O alters an event taking place

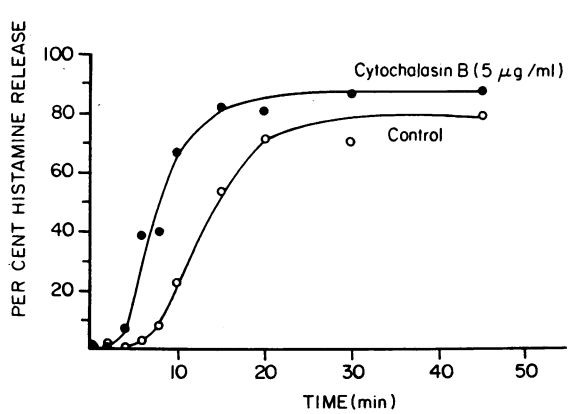

FIGURE 6 The effect of cytochalasin B on the time course of histamine release. Cytochalasin B and antigen were both added at 0 time.

in the first stage of the release reaction it suggests that this stage includes a change in the state of the microtubule system. Second it supports the idea that cyclic AMP inhibits release through an action on the microtubule system. Cyclic AMP has been shown to alter the equilibrium between formed microtubules and their subunits in tissue slice preparations (34). In the absence of calcium, the nucleotide promoted the disaggregation of microtubules into subunits, i.e. had an effect opposite to that of D_2O. The present finding that D_2O partially prevents the inhibition of release by cyclic AMP is consistent with these findings.

The observation that colchicine is a less effective inhibitor of release when the cells are exposed to antigen before rather than after colchicine treatment provides additional strong evidence that the first stage of the release process involves a change in the state of the microtubule system. The change appears to be in the direction of greater microtubule stability since it leads to increased resistance to cold and/or colchicine.

The effects of cytochalasin B on the release of histamine are of interest. This drug, a metabolite of the mold, *Helminthosporium dematoideum,* has been shown to cause the disappearance of 40–70 A microfilaments but has no apparent effect on microtubules in several cell types (11, 12). Its potential effects on metabolism have not been studied in any detail. Cytochalasin B inhibits several processes not known to involve microtubules, e.g. cytokinesis (11), cell motility (35, 36), the ingestion step in phagocytosis (36, 37), axonal elongation (38), and the dispersal of melanin granules in frog melanocytes (39, 40). At the same time it inhibits the release of thyroid hormone (13), growth hormone (14) and histamine from mast cells (41) but potentiates the release of insulin (15). The effects of cytochalasin B on histamine release observed in the present study were variable. The most frequently seen effect was enhancement of release although with the cells of two out of eight individuals significant inhibition of release was observed. These results can best be explained by either assuming that cytochalasin B has two or more independent actions or that its on known action, i.e. disruption of microfilaments can have two or more effects on the release process. The occurrence of microfilaments in basophils does not appear to have been described. In other cell types however microfilaments are frequently found in close association with the cell membrane (12, 42). If this is also the case in basophils loss of these microfilaments could well alter the configuration of the membrane which, in turn, could influence both the movement of compounds across the membrane and also the interaction between releasing agents and membrane receptors. The present results with cytochalasin B are most easily interpreted in this last framework.

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