Heavy Water Enhances IgE-Mediated Histamine Release from Human Leukocytes: Evidence for Microtubule Involvement[1] (36647)

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The IgE-mediated release of histamine from the isolated leukocytes of allergic individuals is a relevant *in vitro* model for allergic reactions: it can be initiated only with the allergen(s) to which the individual is sensitive and the response of the cells correlates with the level of clinical symptoms to a high degree (1, 2). The mechanism of this reaction has, therefore, been the subject of considerable study. Recent experiments have demonstrated that cyclic AMP is of importance in controlling the release reaction. Agents which raise cyclic AMP levels, for example, isoproterenol, theophylline, certain prostaglandins and histamine itself all inhibit the release process (3–5). In attempting to focus on this process more sharply the reaction sequence has been divided into two stages. The first stage is a calcium-independent activation process which involves the interaction of antigen and specific cell bound IgE antibody while the second stage is an energy-requiring secretory process initiated by the addition of calcium after the cells have been washed free of antigen (6). The agents which increase cyclic AMP levels act largely, if not solely, in the first stage of the reaction (5). Little is presently known about the mechanism of the second stage except that it can be inhibited by metabolic antagonists such as 2-deoxyglucose.

Colchicine, a drug that disrupts microtubules, has been shown to inhibit the overall release of histamine from leukocytes (7). Inasmuch as microtubules might well be involved in the second stage of the release reaction, we were interested in studying other agents known to interact with these cell structures. One such agent is deuterium oxide (D_2O) which appears to stabilize and favor the formation of microtubules in several systems (8, 9). In the present study D_2O greatly enhanced antigen-induced histamine release. It acted only in the second stage of the release reaction and its effect was directly antagonistic to that of colchicine. These observations strengthen the hypothesis that microtubules are involved in the allergenically induced secretion of histamine from leukocytes. Moreover, D_2O is the most potent enhancer of the leukocyte histamine release process yet described.

Materials and Methods. Leukocytes from allergic individuals were prepared by dextran sedimentation of whole blood as previously described (10). The cells were resuspended at a dilution of approximately 10^7/ml. In experiments in which the whole release reaction was studied, cells were incubated with the purified protein antigens of ragweed (E) or grass (Group I)[3] at 37° in a medium consisting of (m*M*): NaCl, 120; KCl, 5; $CaCl_2$, 0.6; $MgCl_2$, 1; tris(hydroxymethyl) aminomethane (Tris), 25; and human serum albumin, 0.1%; adjusted to pH 7.4. Experiments involving colchicine were also carried out using a phosphate buffer since it has been reported that Tris can interfere with

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the action of colchicine (11). Under our conditions results using these two buffers were essentially the same. Histamine released into the supernatant fluid was measured by a micro modification of the fluorometric technique of Shore, Burkhalter and Cohen (12, 13). D_2O, obtained from Bio-Rad Laboratories, replaced a portion of the H_2O in the incubation medium as indicated. The cells were always preincubated with colchicine at 4° for 45 min and the drug was removed by washing the cells twice before the addition of antigen. In experiments designed to study the two stages of the release process cells were exposed to antigen for 2 min in medium free of calcium and magnesium (6). They were then washed and resuspended without antigen in the complete medium described above.

Results and Discussion. Replacing a portion of the H_2O in the medium with D_2O resulted in marked potentiation of histamine release (Fig. 1). Similar enhancement was observed in each of 10 experiments carried out with the cells of different donors. As shown, D_2O does not significantly alter the time course of release but rather increases the rate. In experiments (not illustrated) in which cells were preincubated with D_2O before the addition of antigen there was no additional enhancement of release indicating that D_2O acts without an appreciable time lag. Its effects are also readily reversible. When cells were pretreated with D_2O, washed, and resuspended with antigen in normal medium there was no potentiation of release. D_2O at the highest concentration studied (80%) released no histamine in the absence of antigen. This result is different from that seen with rat mast cells (14) where D_2O above a concentration of 35% acts as a releasing agent. In the present system preliminary evidence suggests that D_2O enhances the release process at all levels of antigen including levels too low to act alone and in antigen excess. When D_2O was studied in the two stage process described above, it was found to be wholly ineffective in the first or activation stage while its presence in the second or release stage led to the same enhancement of release observed when the complete process was studied.

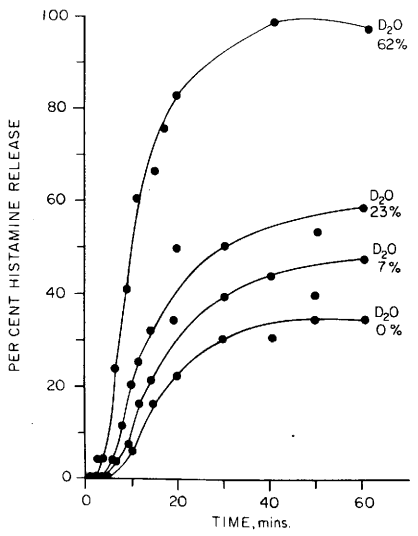

FIG. 1. Time course of histamine release in the presence and absence of D_2O.

Since the action of D_2O is postulated to be due to its effect on microtubules, it should counteract the effects of colchicine, an agent which is known to act by disaggregating microtubules. To study this, cells were incubated with or without colchicine, washed twice, and resuspended with antigen in a H_2O or D_2O medium. Colchicine was removed prior to the final incubation since its effects persist for some time (14, 15) and because it interferes with the assay of histamine. Over a wide range of concentrations D_2O and colchicine had antagonistic actions: D_2O could reverse the inhibition caused by colchicine and similarly colchicine could block the stimulating effects of D_2O. Which agent dominated in its activity depended upon their relative concentrations and it was possible to adjust these so that they neutralized each other completely and the resulting histamine release was the same as control. The results shown in Fig. 2 are typical; essentially the same results were obtained in 3 similar experiments with different cell donors.

These observations considerably strengthen the suggestion of Levy and Carlton (7) that microtubules are involved in the allergic release of histamine from basophils. Not only do agents which lead to the disaggregation

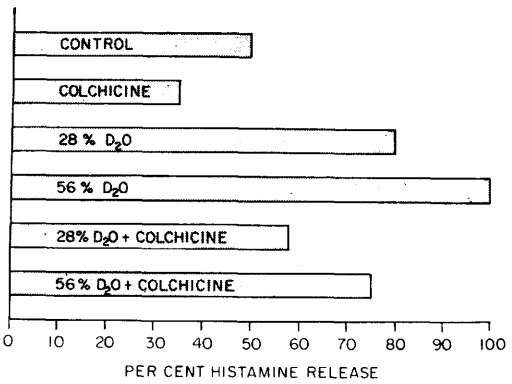

FIG. 2. The effects of D_2O and colchicine on histamine release. Cells were incubated with and without colchicine (3×10^{-4} *M*) at 4° for 45 min. They were washed twice, resuspended in media containing antigen and 0, 28 or 56% D_2O and incubated at 37° for an additional 45 min period.

of microtubules block the release response but an agent which facilitates the formation of the functional aggregated microtubule promotes the response. The present data add to our understanding of the allergic process. The process may now be viewed as consisting of a first stage which involves an antigen-induced activation controlled by the intracellular level of cyclic AMP which can itself be regulated by a variety of pharmacologic agents and a second stage requiring calcium and energy which involves the actual secretion of histamine from the granules which contain it. It now seems reasonable to suppose that some step prior to the actual release of histamine requires an intact and functioning microtubule system. An understanding of these stages and the control systems operative within them should open new paths to the pharmacologic control of allergic disorders.

Summary. Deuterium oxide, an agent known to stabilize microtubules, greatly enhances antigen-induced histamine release from human leukocytes. Concentrations as low as 7% are effective and enhancement occurs over a wide range of antigen concentrations. The effects of D_2O are partially reversed by colchicine, a drug known to disrupt microtubules. These findings strengthen the view that microtubules are involved in the secretory mechanism by which the mediators of the allergic response leave the IgE containing target cells.

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