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Temperature Dependence of Histamine Release from Rat Mast Cells by Dextran (38045)

JAMES H. BAXTER AND RONALD ADAMIK

Molecular Disease Branch, National Heart and Lung Institute, Bethesda, Maryland 20014

Dextran releases histamine from rat mast cells in a reaction that is species specific and noncytotoxic, and in many respects resembles anaphylactic histamine release (1, 2). Like anaphylactic release it requires Ca^{2+}, although the role of Ca^{2+} does not appear to be identical in the two reactions (3). Anaphylactic histamine release is maximal at 37° (4–6). Maximal release by dextran occurred at 25°, however, and this caused us to investigate further the effects of temperature on the dextran reaction.

Methods and Materials. Cells were obtained from large male Sprague-Dawley rats by washing out the peritoneal cavity with buffer solution. The buffer was Krebs-Ringer phosphate, pH 6.8, with 1.0 m*M* Ca^{2+}, no Mg^{2+}, and 1 mg/ml crystalline bovine serum albumin. Heparin 5 μg/ml was added for harvesting the cells. Only about 5% (by number) of the cells were mast cells, but the mast cells were larger than the others and contained virtually all of the histamine (2). After centrifugation at 25°, the cells were resuspended without fractionation in a fresh portion of buffer.

Aliquots of the cell suspension (each containing about 10^5 mast cells) were pipetted into siliconized tubes and incubated with dextran (Dextran 2000, Pharmacia Fine Chemicals, Piscataway, N. J., av mol wt 2×10^6) for 15 min at the desired temperature. Final volume in each tube was 3.5 ml, and final dextran concentration 1 mg/ml unless otherwise stated. Phosphatidyl serine 7 μg/ml was always added with the dextran to enhance the release of histamine (1, 2). Dextran was omitted from the control tubes. Phosphatidyl serine was without effect in the absence of dextran. Histamine release was completed well within the 15 min incubation period. Details of the procedures were as previously described (2, 3).

After incubation, the cell suspensions were chilled by adding 10 ml of ice-cold buffer and placing the tubes in ice. Cells and medium were separated after centrifugation at 4°. Histamine in medium and in cells was determined fluorometrically (7) as previously described (2). Similar total histamine values (in cells plus medium) were always obtained from replicate samples of cells, irrespective of temperature and duration of the preincubation to which they had been subjected, indicating that there was no appreciable net synthesis or degradation of histamine during the experimental period.

For investigating the rate of deterioration of the cells at 25° and at 37°, tubes of cells (prepared at 25°) were preincubated at 25° and at 37°, respectively, for either 5 min (sufficient to bring them to ambient temperature) or 35 min before dextran was added. Quantities of histamine released in various tubes were then compared.

To determine whether the lower release at 37° than at 25° was reversible or irreversible, tubes of cells (prepared at 25°) were preincubated at 25° and 37°, respectively, for various periods. Before addition of dextran, some of the 37°-tubes were changed back to 25°. Release from cells in the changed tubes (at 25°) was compared with that from the cells left at 25° and 37°, respectively. Cells were also preincubated

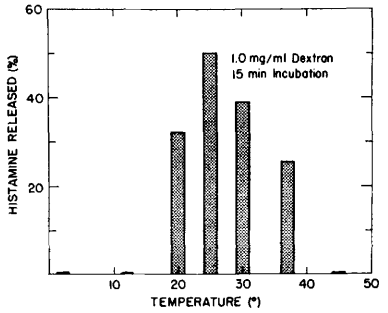

FIG. 1. Effect of incubation temperature on the quantity of histamine released from rat mast cells by dextran. The 20°, 25° and 37° values are mean results from 4 experiments. Release at 25° was significantly greater than that at 20° or 37° ($P <$ 0.005, calculated for paired data).

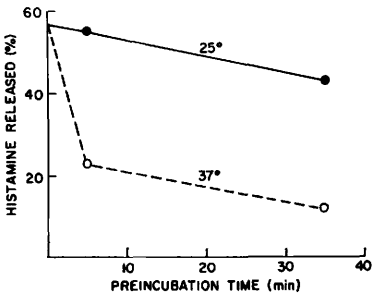

FIG. 2. Effect of preincubating cells at 25° and 37°. Histamine released by dextran from cells prepared at 25° and preincubated at 25° or 37° for 5 min or 35 min before dextran was added is shown. Values are mean results from 4 experiments. The respective differences in release at the two temperatures were highly significant ($P <$ 0.001, based on paired data), but the absolute decreases in release between 5 and 35 min at the two temperatures were not significantly different ($P > 0.9$).

at 0° and changed back to 25° for comparison of their release with that of cells preincubated at 25°.

Results. The quantity of histamine released from the mast cells at different temperatures by dextran in the usual concentration (1 mg/ml) is shown in Fig. 1. Maximal release always occurred at 25°, and this result was not changed by diluting the usual cell suspension 10-fold before the addition of dextran. Release at 37° was about half that at 25°. Below 20° release fell off rapidly, and virtually failed to occur below 12°. Cells were irreversibly inactivated within 5 min at 45°.

Effect of preincubation at 25° and 37°. We suspected that the lower release at 37° than at 25° might have resulted from more rapid deterioration of the cells at the higher temperature before dextran was added. Five minutes after the cells had been changed from 25° to 37°, however, their ability to release histamine in response to dextran addition was already markedly reduced compared with that from similar cells left at 25° (Fig. 2). Continuing the preincubations at 25° and 37°, respectively, for an additional 30 min, caused approximately the same additional decrease in release by dextran at the two temperatures. This indicates that the cell condition responsible for the lower release at 37° developed promptly when the cells were changed to the higher temperature, and did not progress as preincubation was continued.

Reversibility of temperature effects. Cells preincubated at 37° but changed back to 25° before dextran was added, released considerably more histamine than the cells left at 37°, and at least as much as the cells preincubated at 25° (Fig. 3). It is also shown in Fig. 3 that cells preincubated at 0° for 15–20 min and then changed to 25°, also released as much histamine as the 25°-preincubated cells. Cells preincubated for a longer time (1 hr) at 37° or at 0° and then changed back to 25° (not shown), also released as much histamine as did cells similarly preincubated at 25°. Thus, the inhibitory effect of the 37° temperature was completely reversible by changing the temperature back to 25°, as was the inhibitory effect of the 0° temperature. It should be noted, however, that the effect of heating the cells at 45°, which was referred to earlier, was not reversible.

Influence of dextran concentration on temperature dependence of release. Since about two times more histamine was released at 25° than at 37° by 1 mg/ml of dextran, we had assumed that this same release ratio would be obtained with other concentrations of dextran. This assumption proved to be faulty, however, as shown by studies of the effect of dextran concentration on the

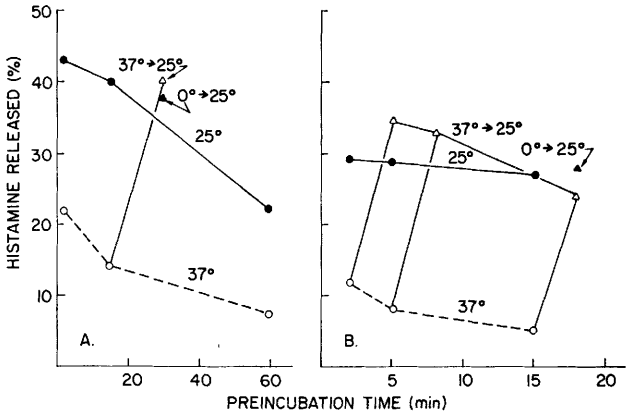

FIG. 3. Histamine released by dextran from cells prepared at 25° and preincubated for various periods of time at 25° (●), 37° (○) or 0°. The 0° tubes (▲) and some of the 37° tubes (△) were changed back to 25° before dextran was added.

quantity of histamine released at different temperatures (Fig. 4). A change in temperature from 25° to 37° caused a large shift in the dextran dose-response relationship, such that 100-fold more dextran (on average) was required at 37° in order to obtain the same degree of release. This temperature effect was reversible, as already described. It can be seen that the ratio of release at 25° to that at 37° varied marked as dextran concentration was changed. Thus, a decrease in dextran from 10 mg/ml to 0.1 mg/ml, for example, caused a much sharper decrease in release at 37° than at 25°. Release at 20° was influenced by dextran concentration in about the same manner as that at 25°, but was lower. The significance of the apparent reversals in the release curves at very high dextran concentrations is not known. We determined that dextran T-70 (Pharmacia Fine Chemicals, av mol wt 7×10^4) gave results qualitatively similar to those obtained with the dextran preparation used routinely, of higher molecular weight.

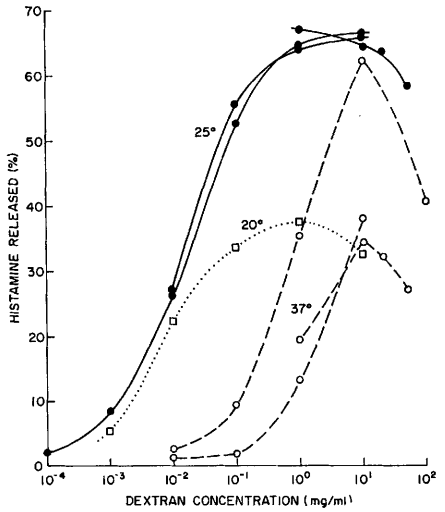

FIG. 4. Relationship between dextran concentration and histamine release at 25° (—●—), 37° (—○—), and 20° (··□··). The lines connect data obtained at a given temperature with a single preparation of cells.

Discussion. The effects of temperature on histamine release by dextran apparently have not been described in detail, although Dias da Silva and Lemos Fernandes (8) found much greater release at 37° than at 20°. Release in the present experiments was maximal at 25°. The lesser degree of release which we obtained at 37° was not due to irreversible deterioration of the cells at the higher temperature, but rather to a reversible difference in response of the cells to dextran at 37°.

When the temperautre was changed from 25° to 37°, dextran concentration had to be increased about two orders of magnitude in order to maintain the same degree of histamine release. This indicates that dextran was less effective in acting on the cells at 37°, due to decreased binding to the receptors or to decreased cell sensitivity at the higher temperature.

We have not studied directly the effect of temperature on dextran binding by mast cells, since it has not been possible to separate the mast cells from the other peritoneal cells without seriously diminishing their responsiveness to dextran (2).

Generalizations concerning relative amounts of histamine released at different temperatures could not be made from results obtained with a single concentration of the releasing agent. It would therefore be of interest to know whether the relative amounts of histamine released by specific antigen at different temperatures might also vary with changes in antigen concentration.

In the present study, as has been customary in studies of histamine release, total quantity of histamine released, rather than rate of release, was measured. In recent studies (unpublished observations) we have found that increasing the temperature increases the (early) rate of release, but decreases the duration of release. It appears to be the interaction of these two antagonistic effects (on total release) that explains the maximal release at 25°.

Summary. Maximal release of histamine from rat mast cells by dextran occurred at 25°. If the cell temperautre was changed from 25° to 37°, beginning promptly thereafter dextran released considerably less histamine. This effect was fully reversible, however, by restoring the temperature to 25° before dextran was added. Studies employing a wide range of dextran concentrations showed that changing the temperature from 25° to 37° systematically increased, by about two orders of magnitude, the dextran concentration required to produce the same degree of release as obtained at 25°. The amount of histamine released at 37° relative to that released at 25° varied markedly as dextran concentration was changed.

Note added in proof. We have now carried out experiments with cells obtained from rats immunized with egg albumin and pertussis vaccine. When such cells were challenged with the antigen (egg albumin) in concentrations ranging from 10^{-6} to 10^{-1} mg/ml at 25° and at 37°, results similar to those shown in Fig. 4 for dextran were obtained. With the higher antigen concentrations, however, release at 37° often exceeded that at 25°. We believe that the quantity of histamine released by dextran or by antigen is dependent on the rate of release and the rate of cell desensitization, both of which are influenced by the environmental conditions (Baxter, J. H. and Adamik, R. Fed. Proc. 33, 761, 1974).

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