TEMPERATURE DEPENDENCE OF MAST CELL HISTAMINE SECRETION

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INTRODUCTION

A diverse set of direct experimental observations and theoretical considerations supports the existence of fluidity in cell membranes (1). Several membrane-dependent functions of *Escherichia coli* have been shown to exhibit biphasic Arrhenius plots with transition temperatures that are determined by the degree and configuration of the unsaturation of fatty acids incorporated into the membrane phospholipids (2, 3). Biphasic plots and even discontinuities in the plots have been demonstrated for several mitochondrial enzymes in their membrane-bound states (4, 5). It has been proposed that the transitions in activation energy for these reactions are related to a phase change in the organization of the membrane phospholipids (2–5).

In mammalian cells the mixing of surface antigens in newly formed heterokaryons (6) and the capping of surface antigens in the presence of specific antibodies (7) have been interpreted as evidence for lateral movement of proteins in a more or less fluid membrane. Both these phenomena have a temperature dependence that is consistent with a transition in membrane structure occurring in the vicinity of 15°C, but the available data do not permit distinction between phase transitions and high activation energies for lateral diffusion of membrane proteins (7).

Secretion by exocytosis would be expected to be dependent on the state of the cell membrane and thus substantially affected by any temperature-dependent phase change in the membrane. This reasoning led to study of the effect of temperature on the secretion of histamine by mast cells.

MATERIALS AND METHODS

All the experiments were performed using mixed peritoneal cells collected from adult male rats (Charles River CD, Charles River Breeding Laboratories, Inc., Wilmington, Mass.) and handled as previously described (8). Histamine release was induced with polymyxin B sulfate at a final concentration of 2 μg/ml. The only change made for these experiments was the addition of 4 vol of 0°C balanced salt solution (BSS) to the cell suspension to stop the secretion reaction rapidly. The resulting cold, diluted suspension of cells was immediately placed on ice and the cells were subsequently removed by centrifugation at 4°C. The efficacy of this strategem, previously used by Bloom et al. (9), was demonstrated by the absence of any release when polymyxin B sulfate was incorporated into the 0°C BSS added to cells at 27°C. The temperature of the cells was adjusted before the addition of polymyxin B by two alternative procedures: In one procedure, samples of cell suspensions were kept at 0°C and then equilibrated at the desired temperature for 5 min before adding polymyxin B; in the other procedure, samples of cell suspensions were placed at the lowest temperature in the experimental set, and then the temperature was raised stepwise. At each temperature a sample of cells was tested for histamine secretion. The temperature of a blank solution of BSS was monitored to assure equilibration. No significant differences were discernible between the results obtained with the two procedures. Histamine in the supernate and in the cell pellet was assayed by the *o*-phthalaldehyde method (8), and histamine secretion was calculated as percent of total histamine and micrograms histamine/10^6 mast cells/minute. Extent of release was measured at two time-intervals chosen for each temperature so that the values yielded a linear or close to linear rate of release (Fig. 1). At 27°C it was necessary to assume that histamine

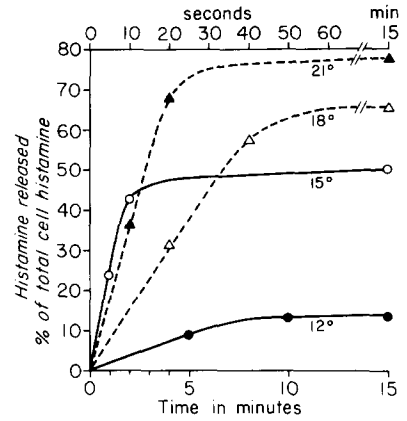

FIGURE 1 Rate of histamine release from mast cells in vitro. Each point represents the mean of determinations on two samples in a single experiment. The plots for 12°C and 15°C utilize the minute scale, while those for 18°C and 21°C utilize the second scale.

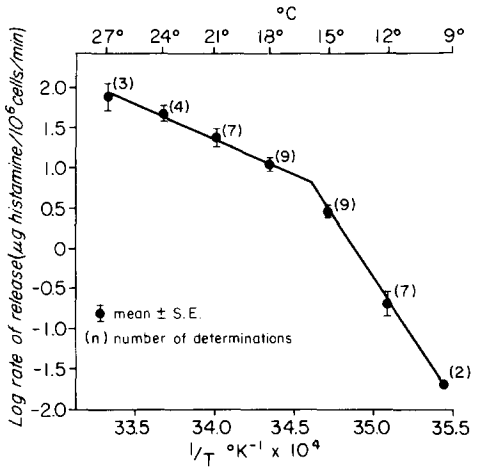

FIGURE 2 Arrhenius plot for histamine release. The rate of histamine release was determined in (*n*) experiments as the mean of values at two time-intervals on the linear portion of the rate curves at the different temperatures, with the exception of 27°C as described in the text. The means ± SE of the log of the rate of histamine release were calculated. The regression lines were calculated from all the determinations, not from the means. Plots for individual experiments exhibited a similar apparent break between 15°C and 18°C.

release at the earliest time accessible to study, 5 s, was a reasonable estimate of the initial rate of release, since linearity could not be demonstrated. The assumption seems justified by the fact that the values fall on the linear portion of the plot above 15°C. At 9°C the rate of release is low and the reaction proceeds to only a small extent.

RESULTS AND DISCUSSION

The temperature dependence of histamine secretion determined in these experiments resembles but is not completely in agreement with less complete data previously reported for other agents acting on mast cells (10, 11). The values obtained yield a good fit to two straight lines with an intersection near 16°C (Fig. 2). Such a biphasic Arrhenius plot is consistent with the proposal that the reactants in the rate-limiting step in histamine secretion undergo a phase transition. Phase transitions have been demonstrated to occur in bacterial membranes and to be correlated with biphasic Arrhenius plots for membrane functions (2, 3). The temperature dependence of lateral movement of membrane proteins suggests a phase change in membrane structure in the vicinity of 15°C (6, 7), although the data are open to other interpretations (7). Since histamine secretion from mast cells requires participation of the plasma membrane (12), a phase change in membrane structure would account for the biphasic plot. A phase change in membrane could influence secretion by affecting

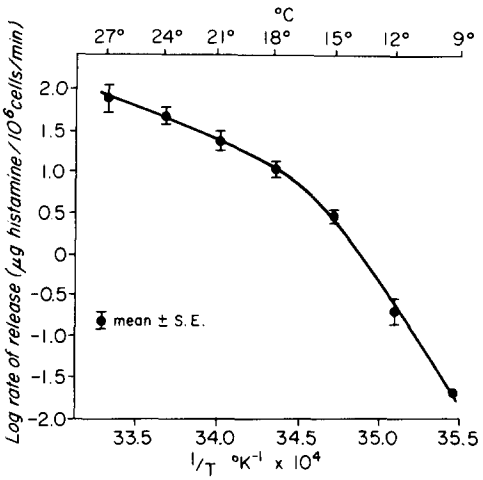

FIGURE 3 The equation of the curvilinear regression was determined by successive approximation to the determined points, varying the parameters A_1, A_2, E_1, and E_2 in the equation

$$\log v_o = \log (A_1 e^{-E_1/RT}) / (1 + A_1 e^{-E_1/RT} / A_2 e^{-E_2/RT})$$

(derived from reference 15).

the ability of membranes to fuse by modifying the activity of a membrane enzyme or by influencing the translational or rotational movement of a nonenzyme protein in the membrane.

Models for biphasic Arrhenius plots have been proposed (13–15) that do not require a phase transition. All of the alternative models yield curvilinear plots. This difference does not, however, permit assignment of the biphasic plot since it is possible to calculate a significant curvilinear regression for the data by adjusting the parameters of at least one of the models (15) (Fig. 3).

Direct study of the effect of temperature on the mast cell membrane is necessary to support the interpretation of the Arrhenius plot. The data at present are best interpreted as consistent with, but not proof of, a phase change in the mast cell membrane as a determinant of histamine secretion. It will be of interest to determine whether or not other exocytotic and endocytotic processes yield comparable biphasic Arrhenius plots.

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