Secretion in Mast Cells Induced by Calcium Entrapped Within Phospholipid Vesicles

Abstract. *Purified mast cells secreted histamine when fused to phospholipid vesicles containing calcium but not magnesium or potassium. Microscopic observation revealed highly localized exocytotic responses involving punctate extrusion of individual granules. Calcium delivered from the vesicles to the cytoplasm is apparently a sufficient stimulus to initiate exocytosis. The results support the calcium hypothesis of stimulus-secretion coupling.*

Calcium is now known to be intimately involved in regulating secretory activity in many exocrine, endocrine, and other cells (*1*), and there is much evidence that it may act as a mediator in stimulus-secretion coupling to initiate a common secretory response, exocytosis (*2*). Here we provide fresh evidence that calcium has the essential attribute of such a mediator, namely, the capacity to induce exocytosis. In our experiments we used a novel way of delivering calcium by incorporating it into phospholipid vesicles.

Phospholipid vesicles (liposomes) have proved to be suitable carriers for many biologically active molecules, and their ability to fuse with and become incorporated into the plasma membrane, thereby unloading their contents into the cytoplasm, has been made use of for a variety of purposes (*3*). We find that calcium delivered by such vesicles stimulates extrusion of histamine-containing granules from mast cells. Mast cells, which can be readily harvested and purified, have been used increasingly as a model secretory system because their secretory activity conforms to the general pattern of calcium and energy-requiring exocytosis and because their secretory granules are sufficiently large to be observed by light microscopy (*4*).

Rat peritoneal mast cells were collected, purified (90 percent), and suspended in Hepes-buffered Locke's solution (*5*). Phospholipid vesicles were prepared from phosphatidyl choline, phosphatidyl serine, and cholesterol (in the molar ratio 9:1:1) in the presence of isotonic KCl with or without various amounts of calcium or magnesium and were sonicated to clarity, a process resulting almost exclusively in unilamellar vesicles (*6*). These vesicles (and their entrapped contents) were separated from the free solutes by passage through a Sephadex G-50 column (*7*) eluted with divalent cation-free Locke's solution containing 0.1 m*M* [ethylenebis(oxyethylenenitrilo)]-tetraacetic acid (EGTA). Mast cells in divalent cation-free Locke's solution (without EGTA) were incubated at 37°C in a solution containing the various preparations of vesicles. Histamine was assayed fluorometrically (*8*) and extruded (exocytosed) granules were identified by

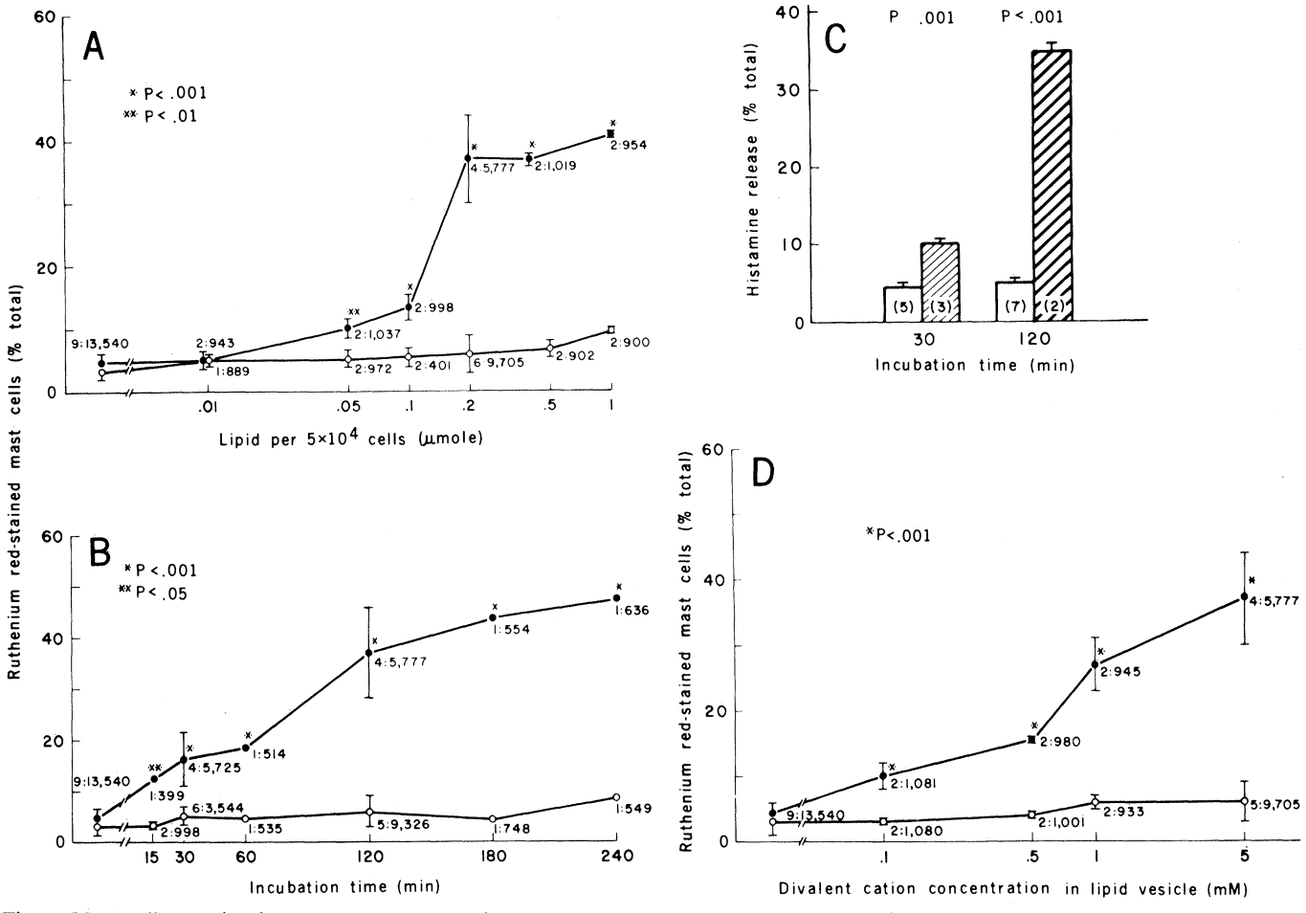

Fig. 1. Mast cell secretion in response to phospholipid vesicles as measured by ruthenium red staining or histamine release. (A) Effect of incubating (120 minutes at 37°C) mast cells (5×10^4) with increasing number of vesicles [1 μmole ≃ 10^{14} vesicles (*7*)] prepared in isotonic KCl (150 m*M*) to contain either calcium (5 m*M*), ●; or magnesium (5 m*M*), ○. (B and C) Effect of increasing the time of incubation with a constant number of vesicles (200 nmole of lipid per 5×10^4 mast cells) containing calcium (5 m*M*), ● and hatched columns; or magnesium (5 m*M*), ○ and open columns. (D) Effect of varying the amount of divalent cations in a constant number of vesicles (200 nmole of lipid per 5×10^4 mast cells) during a 120-minute incubation at 37°C; calcium, ●; or magnesium, ○. The numbers beside the points in (A), (B), and (D) indicate the number of experiments, followed by, after the colon, the number of cells counted. In (C) the numbers in parentheses indicate the number of experiments.

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selective staining with ruthenium red. This dye is confined largely to the extracellular environment and has a high affinity for the contents of the mast cell granules. Together these properties result in preferential staining of extruded granules and, indeed, allow visualization of single exocytotic events (*9*) as well as quantitation of the secretory response of the cell population (*10*).

Mast cells incubated with increasing numbers of lipid vesicles containing calcium showed a progressive secretory response as judged by the ruthenium red staining procedure (*10*), with about 35 percent of the cells responding by 120 minutes (Fig. 1, A and B). Measurements of histamine release, the common index of secretory activity in mast cells, yielded comparable results (Fig. 1C). No such stimulant effects were observed with vesicles containing magnesium in place of calcium or isotonic KCl alone (lower curves in Fig. 1, A, B, and D).

In the experiments described so far the phospholipid vesicles containing calcium and the other ions were added to mast cells suspended in calcium-free Locke's solution. This was done to exclude the possibility of secretion arising from some effect of the vesicles to promote influx of calcium from the bathing medium. Some calcium might have found its way into the medium from ruptured vesicles, but this is unlikely, since experiments with the calcium-sensitive dye Arsenazo III provided no evidence of leakage (*11*). Furthermore, phospholipid vesicles containing isotonic KCl with or without magnesium had no effect on secretion even when incubated with mast cells suspended in calcium-containing Locke's solution, whereas, under the same conditions, vesicles containing calcium again elicited secretion. This response to the calcium-containing vesicles was, indeed, somewhat enhanced (results not shown), possible because calcium in the medium facilitated vesicle-to-cell fusion (*12*).

The effect of calcium was concentration-dependent, rising from 10 percent responding cells with 0.1 m*M* calcium to 16 percent with 0.5 m*M*, 27 percent with 1.0 m*M*, and 35 percent with 5.0 m*M* (Fig. 1D). Again, the responses with each of these concentrations were significantly different from those obtained with magnesium in the same concentrations. Light microscopic observation of responding cells, stained for extruded granules with ruthenium red, indicated that the extent of the secretory response to calcium-containing vesicles often varied among mast cells (Fig. 2A) but was seldom as massive as that seen with other secretagogues. Invariably, secretion

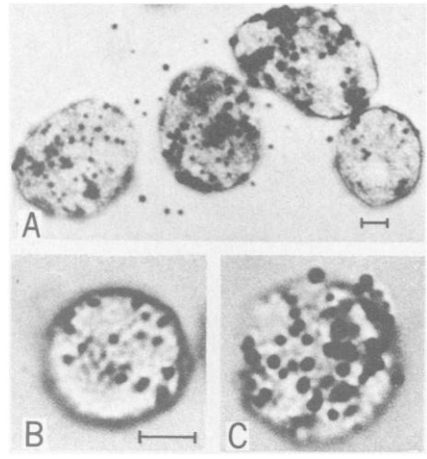

Fig. 2. Bright-field micrographs of mast cells stained with ruthenium red to show granules extruded in response to phospholipid vesicles containing calcium. Extruded granules take up the dye and appear black. Mast cells (4×10^5) were incubated for 30 minutes at 37°C with vesicles (200 nmole of lipid) prepared in isotonic KCl (150 m*M*) to contain calcium (5 m*M*). (A) A low-power view of a field of cells showing a spectrum of secretory responses. (B and C) Views of single cells at higher magnification to illustrate extrusion of individual granules. Scales, 3 μm [lower scale applies to both (B) and (C)].

would begin as highly localized exocytotic events in which the involvement of individual granules was readily apparent (Fig. 2, B and C).

The method we have used to prepare the phospholipid vesicles yields unilamellar, fluid, and negatively charged vesicles of the sort incorporated into cells by fusion of their lipid envelope with the plasma membrane (*12*). Consequently, we suppose that vesicle calcium is released intracellularly close to the cytoplasmic face of the plasma membrane and that the local elevation of calcium ions produced thereby accounts for the punctate secretory responses. Spread of calcium, we assume, would be hindered by local sequestering, as in other secretory cells after microinjection of calcium into the cytoplasm (*13*). The punctate exocytotic responses to the calcium-containing phospholipid vesicles we observed here are reminiscent of the localized extrusion of mast cell granules achieved by micropipetting familiar mast cell secretagogues such as compound 48/80 and antigen (in sensitized cells) onto the cell surface at discrete points (*14*).

Previous indications that calcium can elicit secretion have been provided by experiments in which calcium entry was apparently facilitated by rendering the plasma membrane leaky by decalcification (*15*) or exposure to the calcium ionophore A23187 (*16*), or in which calcium was injected by micropipettes into the cytoplasm (*17*). The use of phospholipid vesicles provides an additional way of introducing calcium into the cell that yields fresh testimony that calcium is a sufficient stimulus to initiate exocytosis, a property essential to its postulated role in the calcium hypothesis of stimulus-secretion coupling. In addition, this method has for the first time allowed the visualization of highly localized secretory responses involving exocytosis of individual granules as a result, apparently, of the introduction of calcium at discrete points at the cell surface.

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1B) corresponds with fusion of roughly 10^5 vesicles per cell. The use of the same two metabolic inhibitors mentioned above reduces the secretory response by more than 60 percent, thus indicating energy dependence. These points will be discussed in greater detail elsewhere.

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Writing, Dictating, and Speaking Letters

Abstract. *It is commonly assumed that dictation requires a long time to learn, but authors eventually dictate much faster than they write. Performance results now show that novice dictators can learn in a few hours to dictate with the speed and quality with which they write. However, they do not* think *they perform this well. Dictators with years of experience are from 0 to 25 percent faster than novices, depending upon the complexity of the letters. Planning time is about two-thirds of composition time, regardless of the method of composition.*

The composition of letters, memos, essays, and technical reports is widespread, time-consuming, and often difficult (*1*). Although most people write their compositions by hand, alternatives such as dictating and typewriting are used by some. Differences in the process of composition and in its resulting quality and speed made by these different methods have been speculative. We now summarize key findings from ongoing research that provide some understanding of these issues (*2*). Our experimental approach is to vary the tasks assigned to authors and the methods they are to use and to videotape them while they compose. The assigned tasks were varied by requiring each participant to compose 16 different letters. The methods they used were to write, dictate, or speak letters or to compose in "invisible writing." For invisible writing, participants wrote with a wooden stylus on paper with carbon paper underneath.

Dictating is potentially five times faster than writing, on the basis of estimates of maximum writing and speaking rates when composition is not required (*3*). Dictating may also be qualitatively superior: potentially faster transfer of ideas from limited capacity working memory to a permanent record may reduce forgetting attributable to interference or decay.

Speaking may be more "natural" than dictating because authors assume that a recipient will listen to what they say rather than read it. This allows a phraseology appropriate for listening but not necessarily for reading. Speaking may also be more natural because authors do not give typing instructions, which is a potentially disruptive secondary task.

Participants were generally college graduates, 25 to 45 years old. Eight had never dictated before. On a single day of training, they (i) learned the basic rules of dictation and how to use a dictation machine (IBM Executary) (45 minutes) and then (ii) dictated and subsequently proof-edited 16 fairly simple business letters (4 to 5 hours). They returned the next day for the experiments reported here. Eight other participants were experienced dictators, business executives who had dictated regularly for years and preferred dictating to writing. They did not go through the training day.

Each participant composed eight "routine" business letters, two each by writing, invisible writing, dictating, and speaking. These were replies to information requests. Each then composed eight more "complex," one-page letters, two each with each method. Topics included the author's feelings on capital punishment, the U.S. Bicentennial, and a letter of recommendation. The orders of the four composition methods and the eight specific letter-assignments, and the combinations of letter-assignments and methods, were counterbalanced across participants within a group with a modified 8 by 8 Greco-Latin square design.

Composition times were recorded from a participant's receipt of a letter-assignment until he or she indicated completion by stopping a clock. The videotapes were used to analyze composition times into three subtimes: pausing; generating (actual writing, dictating, or speaking); and reviewing. Written, dictated, and invisibly written letters were typed by a secretary and returned after 1 hour for participants to proof-edit. There was only one proof-editing cycle. The quality of the retyped letters was rated afterward by several independent judges on various attributes, for example, syntax and substance. Judges listened to and rated spoken letters on the same attributes (*2*).

Participants' experience, the type of letter (routine or complex), methods, and the combination of letter-assignments and methods were factors in the 2-by-2 by 4-by-2 analysis of variance for each measure, with the last three factors as the within-subjects sources of variance. Separate analyses of variance were carried out for each measure shown in Table 1.

Means for composition time and its component generation and pause times were longer for complex letters than for routine letters [F (1, 14) = 99.28, 70.84, 25.07, respectively; all $P < .001$]. In general, this was true in all methods and for both groups. Composition time depended upon method [F (3, 42) = 26.95; $P < .001$]. Speaking (6.5 minutes) was faster than dictating (7.7 minutes), and both were faster than writing (9.4 minutes) and invisible writing (8.9 minutes); Duncan's multiple range test, $P < .01$. The main reason for this was that participants' generation times were faster in dictation (3.7 minutes) and speech (3.1 minutes) than in writing (7.0 minutes) and invisible writing (6.6 minutes); Duncan's multiple range test, $P < .01$. On the other hand, pause times were longer in dictation (3.0 minutes) and speaking (2.9 minutes) than in writing (2.4 minutes) and invisible writing (2.3 minutes); Duncan's multiple range test, $P < .01$. These longer pause times in dictation and speech were caused entirely by the novice dictators [experience-by-method interaction, F (3, 42) = 9.10; $P < .001$]. Review times were brief in all methods (Table 1). They are not reported for writing because the videotapes rarely showed with certainty whether participants were reviewing. Reading time is included in pause times for all methods. Novice dictators wrote a little faster and dictated a little slower than the experienced dictators [experience-by-method interaction, F (3, 42) = 2.88; $P < .05$].

The speed advantage of speaking over dictating (6.5 versus 7.7 minutes; $P < .01$) may have arisen because composing an oral letter to be read rather than to be heard may require extra time. Alternatively, a person may just talk more slowly when the listener must type what is said.

Quality of letters, on the average, was about the same for both groups, all methods, and both types of letters. For example, on letters composed by novices,

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