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Wheat germ agglutinin stimulates exocytotic histamine secretion from rat mast cells in the absence of extracellular calcium

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Secretagogues are generally thought to initiate secretion by mobilizing calcium from extracellular and/or cellular pools, as a consequence of their binding at the cell surface [1, 2]. How various secretagogues mobilize calcium, particularly cellular calcium, is unclear, in part, because the cell membrane constituents to which secretagogues bind are unknown. Lectins, because of their ability to bind specifically with particular sugars, have become valuable tools for studying the role of the cell surface in many cellular functions [3–5]. Studies of secretion have primarily used concanavalin A (Con A), the lectin isolated from the jack bean which binds glucose and mannose residues. Con A evokes the release of ADP and serotonin from human platelets [6], causes specific granule discharge from human polymorphonuclear leukocytes (neutrophils) [7], and its addition to suspensions of human basophils [8–10] or hamster mast cells [11], results in the prompt release of histamine. However, in the non-sensitized rat mast cell, a cell that has been used extensively in recent years to study secretory events [12, 13], Con A by itself is not an effective stimulus for secretion [8, 14–16] even though it has been shown to bind to the cell surface [14,15]. Only in the presence of phosphatidyl serine [14,15], a potentiator of antigen [17, 18] mediated histamine release, or when mast cells obtained from rats infected with *Nippostrongylus braziliensis* [16] are used, is a substantial secretory response to Con A achieved. Thus, for non-sensitized mast cells, the binding of glucose or mannose residues by Con A at the cell surface does not seem to be sufficient to trigger the release of histamine. Wheat germ agglutinin (WGA), the lectin isolated from *Triticum vulgaris*, binds specifically to *N*-acetylglucosamine (NAG) and *N*-acetylneuraminic acid

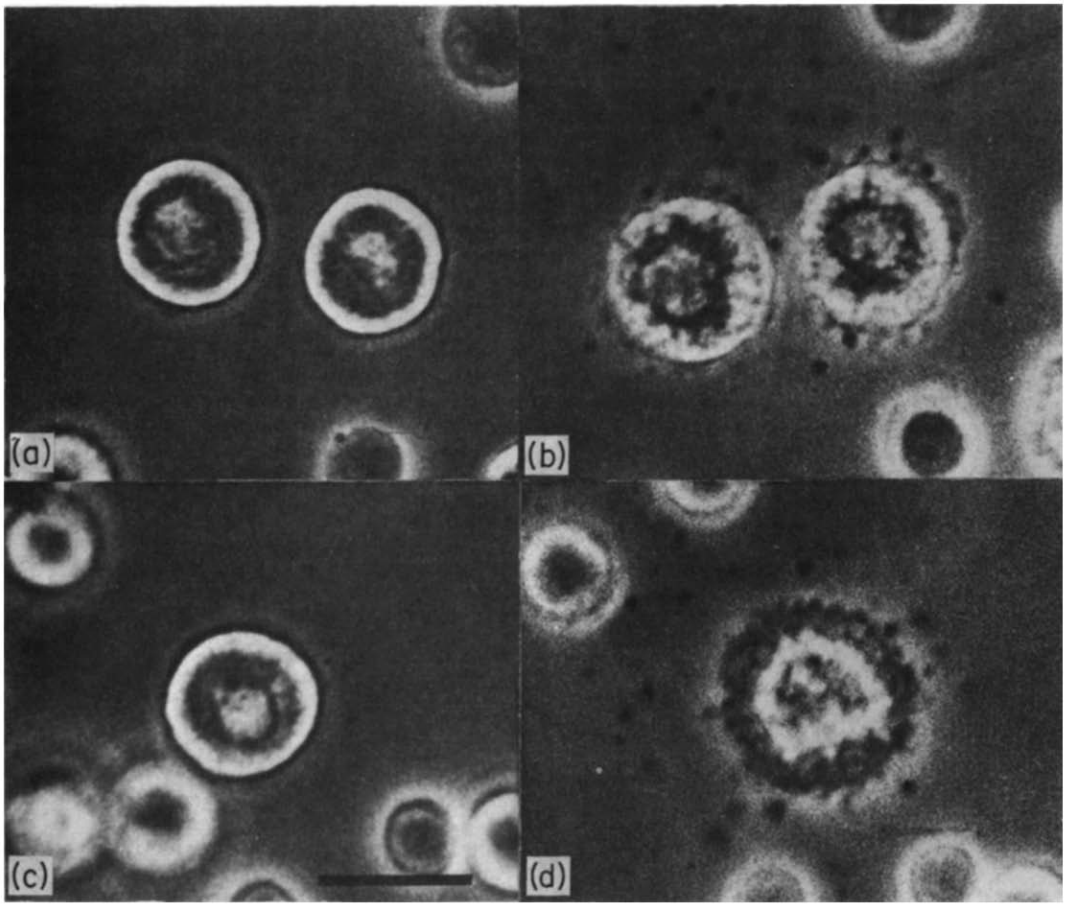

Fig. 1. Mast cell responses to wheat germ agglutinin in the presence or absence of calcium. This figure shows peritoneal cells viewed through an inverted microscope by phase contrast. The large, highly refractile cells are mast cells. The horizontal bar represents 10 μm. (a) Mast cells in Ca-free Locke containing 2 mM EGTA. (b) The same cells 1 min after the addition of WGA (5×10^{-4} g/ml) showing the classical degranulating response. Note the presence of many extruded granules. (c) A mast cell that has been incubated in 2 mM EGTA for 3 hr, resuspended in 0.4 mM EGTA, and then exposed to WGA (5×10^{-4} g/ml) for 5 min showing the absence of degranulation (compare with a and contrast with b). (d) The same cell after the addition of Ca (2 mM). Note the typical degranulating response.

(NANA) groups [19, 20], both common constituents of cell surfaces. We report here that WGA stimulates a calcium and energy-dependent histamine secretion and granule extrusion from non-sensitized rat mast cells and that this secretory response occurs in the absence of phosphatidyl serine and in the absence of extracellular calcium.

Peritoneal mast cells were obtained from male rats (200–350 g), as described previously [21], and incubated in Ca-Locke [150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 10 mM Hepes (*N*-2-hydroxethyl piperazine-N'-2-ethanesulfonic acid)] for 10 min. The cells were then washed twice with Ca-free Locke [$CaCl_2$ was omitted and 2 mM EGTA (ethylene glycol-bis-(β-aminoethyl ether) *N,N'*-tetra acetic acid) added] and resuspended in Ca-free Locke. All solutions were adjusted to pH 7.2 and contained glucose (5.6 mM) and bovine serum albumin (BSA, 1 mg/ml) which control experiments showed did not affect the secretory response to WGA. WGA (Sigma Chemical Co., St. Louis, MO) was dissolved as a stock solution in Ca-free Locke and kept frozen until use. For light microscopy, suspensions of mast cells were pipetted into Sykes–Moore culture chambers, and the fields were observed and photographed as described previously [21].

Isolated rat mast cells bathed in Ca-free Locke solution containing EGTA and viewed by phase-contrast microscopy are typically spherical, highly refractile, with regular cellular outlines (Fig. 1a), and are indistinguishable from mast cells bathed in Ca-containing Locke [21–23]. Within a few seconds of the addition of WGA, such cells begin to extrude granules and undergo the classic degranulation response (Fig. 1b) which has been shown by electron microscopy to result from exocytosis [24–29]. Accompanying this granule extrusion is the release of histamine (Fig. 2). At a concentration of 2.5×10^{-5} g/ml, WGA caused the release of some 10 ± 1.7 per cent of the histamine, and this response increased as the concentration of WGA was raised (Fig. 2). When cell suspensions were purified to contain more than 90 per cent mast cells by differential centrifugation through BSA[31], histamine release and granule extrusion in response to WGA were the same as with the mixed peritoneal cell washings. In contrast, Con A (1.0–100 μg/ml), in the presence or absence of glucose and with

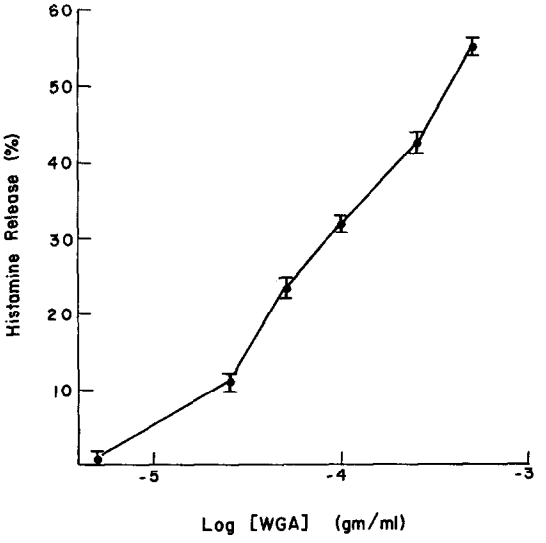

Fig. 2. Histamine release from rat mast cells in response to wheat germ agglutinin. Suspensions of peritoneal cells were incubated in Ca-Locke at 37° for 10 min with varying concentrations of WGA. Histamine was assayed by the fluorometric method of Kremzner and Wilson [30], and histamine secretion is expressed as a percentage of total cell histamine. Each point represents the mean ± S.E. of three experiments.

normal or reduced BSA (0.1 mg/ml), failed to elicit a secretory response in mast cells obtained from the same group of rats. Histamine release in response to Con A was never greater than 7.5 ± 1.2 per cent which is not significantly different from basal release (4.8 ± 1.0 per cent). This agrees with earlier work by Sullivan *et al.* [14]. These variations in the concentrations of glucose and BSA served as controls since Con A binds to glucose and mannose residues [3–5].

It is well known that calcium ions are required for secretion [1, 2]. When mast cells were incubated in Ca-free Locke containing EGTA (2.0 mM), and WGA (5×10^{-5} g/ml) was subsequently added, granules were extruded and histamine was released (23.1 ± 2 per cent, N = 3). This secretory response is similar to that obtained from mast cells incubated in calcium containing Locke (Fig. 2). The question arose as to whether the secretory response elicited by WGA in media free of calcium was an exception to the general requirement for calcium in secretion [1, 2]. Earlier experiments with mast cells using media with reduced sodium [32] or the secretagogue, compound 48/80 [21, 27, 33, 34], in order to elicit histamine release in the absence of extracellular calcium have shown that treatment of mast cells with 2 mM EDTA or EGTA for 3 hr at 37° can abolish the secretory response. The reintroduction of calcium restored the response [21, 32]. In the present experiments, treatment of the mast cells with chelating agent for 3 hr at 37° abolished the histamine released in response to WGA (Fig. 1c and Fig. 3A). The subsequent reintroduction of calcium, but not magnesium, restored the response (Fig. 1d and Fig. 3A) and, if magnesium was included with the reintroduced calcium, histamine release was prevented (Fig. 3A). The reintroduction of calcium in the absence of WGA produced no increase in histamine secretion.

Metabolic energy, in addition to calcium, is required for secretion [1, 2, 35]. In the present experiments, when mast cells were incubated for 10 min in glucose-free, Ca-Locke containing deoxyglucose (5×10^{-4} M) and antimycin A (2×10^{-6} M) before the addition of WGA (5×10^{-5} g/ml), little histamine secretion occurred (4.1 ± 1.0 per cent, N = 3) as compared to that released from cells whose energy supply was intact (25.3 ± 2.4 per cent, N = 3).

In order to determine if the secretory response produced by WGA was due to the binding of NAG or NANA residues, these sugars were added to solutions containing WGA (5×10^{-5} g/ml). These combined solutions were then added to mast cells in Ca-Locke and the cells were incubated for 10 min. The sugars, NAG or NANA, inhibited the secretory response produced by WGA in a concentration-dependent fashion (Fig. 3B), NANA being more effective in producing inhibition than NAG. This inhibition of the secretory response to WGA by these sugars was specific, for the addition of other sugars in similar amounts was without effect, and cells exposed to NAG or NANA in the absence of WGA still responded to the addition of compound 48/80 with a typical secretory response. Whether WGA initiates its secretory effect by binding to NAG or NANA or both is at the present time unknown. We have found, however, that incubation of mast cells with the enzyme neuraminidase (Sigma, 50 m-units/10^6 cells for 1 hr at 37°), a procedure that removes some 50 per cent of the neuraminic acid from isolated chromaffin cell granules [36], significantly reduces (by 19.8 ± 0.2 per cent) the histamine released in response to WGA (5×10^{-5} g/ml) but has no effect on that released in response to compound 48/80 (1 μg/ml) or the ionophore A23187 (0.5 μg/ml).

The secretory response to WGA that we have observed is dependent on a source of calcium (Fig. 3) and metabolic energy and the granule extrusion seen via phase contrast microscopy (Fig. 1, panels b and d) is indistinguishable from that produced by such diverse secretagogues as antigen [29], compound 48/80 [21, 24–28], the ionophore

A23187 [21, 37], or "Na-lack" [32]—a response that has been shown to be the result of exocytosis [24–28, 32]. Our experiments suggest that this exocytotic release of histamine in response to WGA is most likely initiated by the binding of this lectin to NANA and/or NAG residues at the cell surface.

Two significant points are raised by our experiments and deserve further comment: (1) the clear difference between the abilities of WGA and Con A to elicit a secretory response from non-sensitized mast cells, and (2) the different calcium requirements shown by these two lectins when they do initiate secretion.

A clue to explaining these differences is the observation that Con A stimulates histamine secretion from sensitized human basophils by binding to IgE molecules at the cell surface [9, 10]. In the sensitized rat mast cell, antigen binding to cell surface IgE molecules elicits histamine secretion presumably by increasing the calcium permeability of the cell membrane [38, 39]. In this cell, Con A probably initiates secretion by a similar mechanism. The inability of Con A to stimulate histamine secretion from non-sensitized mast cells may reflect the inability of this lectin to sufficiently alter the calcium permeability of the cell membrane due to the lack of suitably bound IgE molecules.

Our results show that WGA *can* elicit secretion from such non-sensitized mast cells; therefore, it probably acts by a different mechanism—one that may not involve binding of cell surface IgE molecules. This conjecture is supported by the observations that WGA and Con A show different calcium requirements for eliciting secretion (see Fig. 3 and Sullivan *et al.* [14]). The secretory response initiated by WGA occurs in the *absence* of extracellular calcium while that produced by Con A does not [14, 15]. In the present experiments, prior treatment of the mast cells with a chelating agent abolished the response to WGA, and the subsequent reintroduction of calcium (but not magnesium) restored it. Earlier studies [21, 23, 27, 32] have suggested that the source of calcium removed by such chelation is cellular in location, perhaps the plasma membrane. WGA may, therefore, initiate secretion in the non-sensitized mast cell by displacing membrane calcium as a consequence of its binding with particular groups at the cell surface. Such displacement of membrane calcium has also been suggested to occur with stimulation of mast cells by compound 48/80 [21, 27] and "Na-lack" [32]. The release of calcium from the same membrane pool by structurally related but different stimuli has recently been directly demonstrated in chlorotetracycline-loaded neutrophils stimulated by synthetic or native chemotactic factors [40]. The inability of Con A to elicit secretion from non-sensitized mast cells, even though it has been shown to bind [14,15], suggests that its binding is either unable to displace membrane calcium or that additional perturbations required for secretion are produced by the binding of WGA and secretagogues like 48/80 but not by the binding of Con A.

Very recently, while this manuscript was in preparation, Lawson *et al.* [41] reported that soluble WGA, coupled to fluorescein or ferritin, bound to both sensitized and non-sensitized mast cells but in concentrations up to 100 μg/ml failed to induce significant histamine secretion from sensitized mast cells. These authors did find, however, that

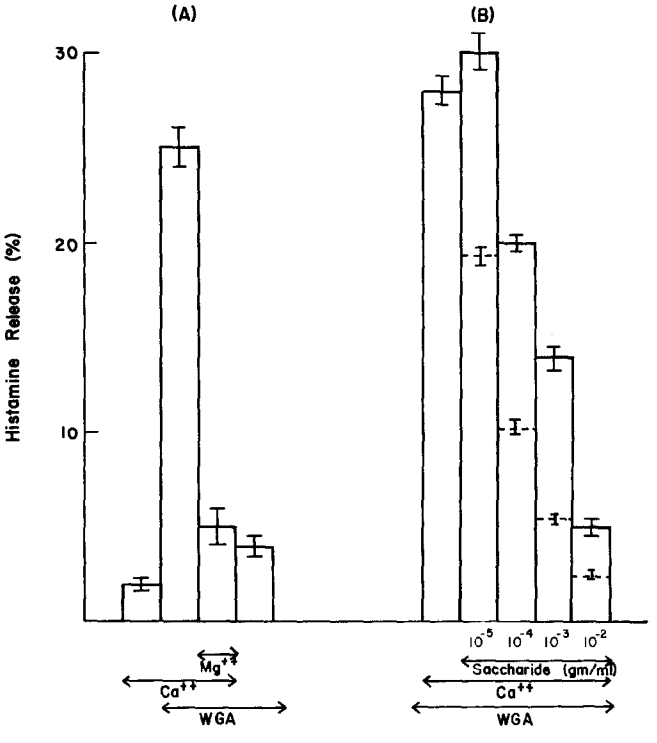

Fig. 3. Histamine release from rat mast cells in response to WGA (5×10^{-5} g/ml) showing its dependency on calcium and its inhibition by the sugars, *N*-acetylglucosamine (NAG) or *N*-acetylneuraminic acid (NANA). Each point is the mean ± S.E. of three experiments. (A) Cells incubated in 2 mM EGTA for 3 hr at 37° and resuspended in either Ca-Locke, Ca-Locke containing 10 mM magnesium, or Ca-free Locke containing 0.4 mM EGTA. Note the drastic reduction in WGA-evoked histamine release when calcium was absent or present with magnesium. (B) Mast cells in Ca-Locke and stimulated with WGA released some 27 ± 1.8 per cent of their histamine (first column, from the left). As the concentration of the inhibitory saccharide was increased from 10^{-5} to 10^{-2} g/ml, the amount of histamine released in response to WGA declined. The solid horizontal line in each column represents histamine release in the presence of *N*-acetylglucosamine and the dashed line the response in the presence of *N*-acetylneuraminic acid.

when sensitized mast cells were incubated with WGA bound to Sepharose 6 B beads, some 30 per cent of the cells showed a generalized pattern of degranulation in the presence *or* absence of calcium.

In summary, we have found that the lectin wheat germ agglutinin (WGA) stimulates granule extrusion and histamine release from isolated rat mast cells. This secretory response occurs in the absence of extracellular calcium but is prevented by pretreating the mast cells with a chelating agent and restored by the reintroduction of calcium (but not magnesium) to the bathing medium. This secretory response to WGA is also prevented by energy deprivation or by the sugars, NANA or NAG, to which WGA specifically binds. Unlike the lectin, Con A, WGA is able to elicit this secretory response from non-sensitized mast cells in the absence of the cofactor phosphatidyl serine. It is suggested that WGA initiates secretion by mobilizing a "cellular" or bound source of calcium and thus resembles the mast cell secretagogue, 48/80 [27].

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Induction of δ-aminolevulinic acid synthetase in chick embryo kidney

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δ-Aminolevulinic acid (ALA) synthetase is the rate-limiting enzyme of porphyrin and heme synthesis. Induction of this enzyme by porphyrinogenic agents is most marked in the liver. The response of this organ to various chemicals is the basis of *in vivo* or *in vitro* systems for assessing the induction potential of these agents [1–3]. Heme synthesis and turnover in kidney have rates and magnitudes comparable to those of liver in some species [4]. However, ALA synthetase induction in kidney has not been studied extensively. Barnes *et al.* [5] described the induction of ALA synthetase activity in kidney mitochondria in rats treated with allylisopropylacetamide (AIA). Schwartz *et al.* [6] were unable to induce ALA synthetase in Syrian hamster kidney with 3,5-dicarbethoxy-1,4-dihydrocollidine (DDC). We previously reported significant induction of ALA synthetase in the kidneys of 1-day-old chicks treated