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Lectin-induced histamine secretion from isolated rat and guinea pig mast cells

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Release of histamine from the mast cell is triggered by an increased level of free calcium in the cell cytosol. This ion may be derived from intra- or extracellular sources according to the nature of the secretory stimulus [1–5]. The lectin Concanavalin A from jack bean (*Canavalia ensiformis*) simulates the anaphylactic reaction by binding to the carbohydrate moieties of reaginic antibody attached to the surface of the target cells [6] and is an effective releaser of histamine from the peritoneal mast cells and basophil leucocytes of a variety of species [8–16]. The detailed response of rat peritoneal mast cells to the secretagogue has, however, been the subject of numerous conflicting reports in the literature. A number of authors have claimed that the lectin is ineffective unless the cells are obtained from actively sensitized animals [9] or the incubation medium is supplemented with exogenous phosphatidyl serine (PS) [2, 13, 16]. This lipid potentiates the histamine release evoked by a variety of agents [17, 18]. Other workers have obtained pronounced responses from unsensitized cells in the absence of PS [10–12, 15]. Some authors have reported the effect of Concanavalin A to be totally dependent on extracellular calcium [8, 12] whereas others have observed marked releases in the absence of the cation [10, 14].

The effect of other lectins has been less widely studied, but it is again a source of disagreement. Bach and Brashler [15] reported that the lectins from wheat germ (*Triticum vulgaris*), castor bean (*Ricinus communis*) and soybean (*Glycine max*) all produced dose-dependent histamine release from rat peritoneal cells. Wheat germ lectin was recently reported to be active in both the presence and absence of extracellular calcium [16]. However, other workers found the secretagogue to be totally ineffective under both conditions [11]. In view of this confusion, we have examined the effects of a number of lectins on rat peritoneal cells. Moreover, since mast cells from different tissues and species show marked variations in functional properties [19–21] we have also tested these compounds on mesenteric mast cells from the rat and guinea pig.

Mixed peritoneal cells were recovered from male and female Lister hooded rats (150–250 g) by lavage with modified Tyrode solution as previously described [1]. The buffer had the composition (mM): NaCl 137, glucose 5.6, KCl 2.7, $CaCl_2$ 1 and N-2-hydroxyethyl piperazine-N′-2 ethane sulphonic acid (HEPES) 10. The pH was adjusted to 7.4 before use. Isolated rat (Lister hooded, 150–250 g) and guinea pig (Dunkin Hartley, *ca.* 400 g) mesenteric mast cells were obtained by dissociation of the tissue with the enzyme collagenase as recently reported [19]. Peritoneal and mesenteric mast cells were thereafter processed in the same way. In some control experiments, rat peritoneal cells were treated with collagenase under the conditions employed in the dissociation procedure.

In simple release experiments, cells were washed and suspended in HEPES-Tyrode from which calcium had been omitted. Aliquots (0.5 ml) were then added to tubes containing an equal volume of Tyrode appropriately modified according to the experiment. This solution either contained calcium ions (to a final concentration of 1 mM), no calcium or EDTA (to a final concentration of 0.1 mM). The suspension was allowed to equilibrate (37°, 5 min) in a metabolic shaker with gentle mechanical agitation and a solution of lectin added in a minimum volume. PS (15 μg/ml) was included in the medium as indicated. Secretion was allowed to proceed for a further 10 min and then assessed as before [1]. All values are corrected for the spontaneous release (*ca.* 5 per cent) occurring in the absence of inducer and are given as means ± S.E. Statistical analysis of results was made by use of the Student's *t*-test for independent samples, with values of $P < 0.05$ being considered significant.

Concanavalin A was purchased from the Sigma London Chemical Co. Ltd. and the lectins from soybean and wheat germ were obtained from Pharmacia G.B. Ltd. The lectin from lentil (*Lens culinaris*) was isolated by affinity chromatography on Sephadex according to the procedure of Howard *et al.* [22]. PS was obtained as a solution in chloroform: methanol from Lipid Products Ltd. The solvent was evaporated in a stream of dry nitrogen and the lipid homogenized in buffer.

Concanavalin A (0.1–100 μg/ml) produced a dose-dependent release of histamine from rat peritoneal mast cells in the presence of calcium ions (Table 1). The release was significantly potentiated by PS at all the concentrations tested. Secretion evoked by the lectins from wheat germ and the lentil (which, to our knowledge has not previously been tested) was strongly dependent on added PS, with the cells releasing up to 60–70 per cent of their total histamine under these conditions. Rat mesenteric cells also responded to Concanavalin A and the lentil lectin. The response to Concanavalin A was not markedly different from that shown by the peritoneal cells in the absence of PS, but the lipid produced little enhancement of the release induced by either lectin from the tissue cells. This is in keeping with our previous results on antigen-induced secretion [19] and supports suggestions that the effect of PS is specific for the rat peritoneal cell [17]. In the absence of PS, the mesenteric cells were significantly more responsive than the peritoneal cells to maximal concentrations of the lentil lectin. Rat mesenteric cells showed very limited reactivity toward the wheat germ lectin and the guinea pig cells were generally unresponsive to all three secretagogues. The lectin from soybean (at concentrations up to 100 μg/ml, with or without PS) did not produce a significant release of histamine from any of the cells examined. Treatment of free rat peritoneal cells with collagenase under the conditions required to effect dissociation of the mesenteric tissue did not significantly alter their reactivity under any of the conditions tested.

Table 1. Lectin-induced histamine release from various mast cells in the presence (+) and absence (−) of PS

Lectin (μg/ml)	Histamine release (per cent) from Rat peritoneal cells (−) PS	(+) PS	Rat mesenteric cells (−) PS	(+) PS	Guinea pig mesenteric cells (−) PS	(+) PS
Concanavalin A	$n = 6$		$n = 4$		$n = 7$	
100	29.1 ± 4.7	71.2 ± 3.4	23.7 ± 3.3	24.9 ± 5.7*	–	13.7 ± 2.5*
10	30.8 ± 4.4	71.9 ± 2.5	18.0 ± 6.5	29.4 ± 3.9*	–	13.2 ± 2.2*
1	17.6 ± 2.5	57.0 ± 4.5	7.2 ± 2.3*	17.6 ± 6.0*	–	8.0 ± 2.6*
0.1	1.1 ± 0.4	8.8 ± 2.1	0.7 ± 0.5	2.7 ± 1.3	–	–
Wheat germ	$n = 3$		$n = 3$		$n = 4$	
100	3.0 ± 0.4	69.4 ± 1.1	4.1 ± 1.2	14.7 ± 4.5*	3.3 ± 1.7	2.3 ± 1.0*
10	6.3 ± 3.2	62.6 ± 2.6	6.0 ± 1.2	13.1 ± 2.5*	4.6 ± 2.1	2.1 ± 1.0*
1	2.4 ± 1.0	41.1 ± 5.3	5.1 ± 1.3	6.9 ± 1.9*	–	–
0.1	1.4 ± 0.7	6.6 ± 3.6	1.7 ± 1.4	1.6 ± 1.6	–	–
Lentil	$n = 4$		$n = 3$		$n = 4$	
100	8.8 ± 2.5	64.4 ± 1.5	20.9 ± 1.0*	27.7 ± 1.7*	4.4 ± 2.2	5.2 ± 2.8*
10	12.0 ± 4.3	64.7 ± 1.4	20.3 ± 4.9	24.9 ± 4.2*	4.7 ± 2.3	6.6 ± 2.5*
1	2.8 ± 1.5	26.5 ± 8.6	7.5 ± 5.2	11.9 ± 5.5	4.8 ± 1.4	2.5 ± 1.3
0.1	0.9 ± 0.4	9.7 ± 3.9	2.1 ± 1.1	2.9 ± 1.6	4.7 ± 1.6	2.1 ± 1.0

Cells were preincubated (37°, 5 min) in buffer containing calcium ions (1 mM) and then challenged with the stated concentration of lectin in the presence and absence of PS (15 μg/ml). Secretion was allowed to proceed for a further 10 min. Values are means ± S.E. for the number (n) of experiments noted. *Denotes values which are significantly ($P < 0.05$) different from the corresponding releases (± PS, as appropriate) from rat peritoneal cells.

Concanavalin A also produced a marked release of histamine from rat peritoneal mast cells in the absence of added calcium (Table 2). This release was significantly enhanced in the absence of PS by brief pretreatment with EDTA (37°, 5 min, 0.1 mM). As previously discussed [3, 4], this treatment may remove calcium from regulatory sites in the membrane, thus facilitating the release of more internal stores of the ion. PS significantly potentiated the release only in the presence of exogenous calcium, consistent with the view that the lipid may promote the influx of the cation from the extracellular environment [3, 4]. Maximum secretion was observed at a calcium concentration of 1 mM whereas supraoptimal concentrations (10 mM) significantly inhibited the release, particularly in the absence of PS. High concentrations of the cation may saturate the proposed regulatory sites in the membrane, thus restricting translocation of the ion [3, 4]. In contrast, the secretion induced by the lectins from wheat germ and the lentil was almost totally dependent on extracellular calcium. PS again had a striking enhancing effect only under these conditions, but the inhibitory effect of excess calcium was not observed with the wheat germ lectin. Soybean lectin (at concentrations up to 100 μg/ml) was again completely inactive under all the conditions tested.

The present study then extends our previous observations [19, 20] that there are marked functional differences between rat peritoneal, rat mesenteric and guinea pig mesenteric mast cells. The tissue cells may be isolated without morphological damage, exhibit a low spontaneous release of histamine and are fully responsive to immunological challenge [19]. However, they show marked variation in response to chemical histamine liberators [20]. This diversity clearly extends to lectin-induced secretion of the amine. These results cannot be attributed to artefacts in the isolation procedure since rat and guinea pig tissue cells were obtained in identical fashion and treatment of rat peritoneal cells with collagenase under the same conditions did not alter their reactivity. Previous studies have shown that similar incubation of peritoneal cells does not impair their ability to respond to other chemical histamine releasers or to immunological challenge [20, 23]. As formerly discussed [20], we then believe that our results reflect intrinsic differences in the cells examined.

The reasons for the conflicting reports in the literature concerning the effects of the test lectins on rat peritoneal mast cells remain obscure. These discrepancies may reflect the strains of animals used, their state of natural sensitization [9, 10], or apparently minor variations in experimental protocol. Further work will be required to resolve these possibilities.

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Table 2. Lectin-induced histamine release from rat peritoneal mast cells in various media

Lectin	Histamine release (per cent) in media containing							
	No calcium		EDTA (0.1 mM)		Calcium (1 mM)		Calcium (10 mM)	
	(−)PS	(+)PS	(−)PS	(+)PS	(−)PS	(+)PS	(−)PS	(+)PS
Concanavalin A (n = 3–5)	23.3 ± 2.2	31.7 ± 5.7	40.4 ± 2.8	41.6 ± 5.8	34.3 ± 3.2	73.9 ± 1.8	11.9 ± 1.2	62.6 ± 1.0
Wheat germ (n = 4–5)	2.7 ± 1.0	6.4 ± 1.1	3.2 ± 1.7	8.4 ± 2.5	17.4 ± 3.4	64.3 ± 10.0	22.5 ± 5.9	60.0 ± 8.7
Lentil (n = 4–5)	5.8 ± 3.6	9.2 ± 1.7	7.0 ± 2.7	7.5 ± 2.1	19.2 ± 4.4	62.6 ± 5.6	6.5 ± 2.4	53.8 ± 6.5

Cells were preincubated (37°, 5 min) in the media shown and then challenged with Concanavalin A (10 μg/ml), wheat germ lectin (100 μg/ml) or lentil lectin (10 μg/ml). Secretion was allowed to proceed for a further 10 min. Values are means ± S.E. for the number (n) of experiments noted.

REFERENCES

1. G. Atkinson, M. Ennis and F. L. Pearce, *Br. J. Pharmac.* **65**, 395 (1979).
2. J. H. Baxter and R. Adamik, *Biochem. Pharmac.* **27**, 497 (1978).
3. F. L. Pearce, M. Ennis, A. Truneh and J. R. White, *Agents Actions*, **11**, 51 (1981).
4. M. Ennis, A. Truneh, J. R. White and F. L. Pearce, *Int. Archs Allergy appl. Immun.* **62**, 467 (1980).
5. W. W. Douglas and Y. Ueda, *J. Physiol, Lond.* **234**, 97P (1973).
6. A. M. Magro, *Nature, Lond.* **249**, 572 (1974).
7. W. A. Hook, S. F. Dougherty and J. J. Oppenheim, *Infect. Immunity* **9**, 903 (1974).
8. P. A. Siraganian and R. P. Siraganian, *J. Immun.* **112**, 2117 (1974).
9. R. Keller, *Clin. exp. Immun.* **13**, 139 (1973).
10. A. J. Shores and J. L. Mongar, *Agents Actions* **10**, 131 (1980).
11. D. Lawson, C. Fewtrell and M. C. Raff, *J. Cell Biol.* **79**, 394 (1978).
12. K. Sugiyama, J. Sasaki and H. Yamasaki, *Jap. J. Pharmac.* **25**, 485 (1975).
13. T. J. Sullivan, W. C. Greene and C. W. Parker, *J. Immun.* **115**, 278 (1975).
14. T. J. Sullivan and C. W. Parker, *Am. J. Path.* **85**, 437 (1976).
15. M. K. Bach and J. R. Brashler, *Immunology* **29**, 371 (1975).
16. J. B. Lansman and D. E. Cochrane, *Biochem. Pharmac.* **29**, 455 (1980).
17. J. L. Mongar and P. Svec, *Br. J. Pharmac.* **46**, 741 (1972).
18. N. Grosman and B. Diamant, *Agents Actions* **5/4**, 296 (1975).
19. F. L. Pearce and M. Ennis, *Agents Actions* **10**, 124 (1980).
20. M. Ennis and F. L. Pearce, *Eur. J. Pharmac.* **66**, 339 (1980).
21. A. D. Befus, F. L. Pearce, J. Gauldie, P. Horsewood, R. L. Goodacre, F. Cole, R. V. Heatley and J. Bienenstock, in *The Mast Cell: its Role in Health and Disease* (Eds. J. Pepys and A. M. Edwards), p. 702. Pitman Medical, Tunbridge Wells (1979).
22. I. K. Howard, H. J. Sage and M. D. Stein, *J. biol. Chem.* **246**, 1590 (1971).
23. M. K. Bach, J. R. Brashler, K. J. Bloch and K. F. Austen, in *Biochemistry of the Acute Allergic Reactions* (Eds. K. F. Austen and E. L. Becker), p.65. Blackwell, Oxford (1971).