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A comparison of histamine secretion from peritoneal mast cells of the rat and hamster

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1 Functional mast cells have been obtained by peritoneal lavage of the rat and hamster.

2 Both cell types released histamine on stimulation with appropriate dilutions of anti-rat IgE and anti-hamster serum. The maximum response evoked by each reagent was significantly greater for the hamster cells. The release was non-cytotoxic and was in each case blocked by the corresponding soluble antigen.

3 The rat and hamster cells responded to concanavalin A and the lectin from lentil. Phosphatidylserine (PS) potentiated the release only from the rat cells. In the absence of the lipid, the hamster cells were more reactive. The lectin from wheat germ, in the presence of PS, evoked histamine secretion only from the rat cells. Both populations were refractory to the lectin from soybean and to protein A.

4 Rat peritoneal cells were more responsive to the basic secretagogues compound 48/80 and peptide 401 (the MCD-peptide from bee venom). These differences were less marked in the case of polylysine and polyarginine.

5 The two cell populations responded to the calcium ionophores A23187, ionomycin and chlortetracycline. The hamster cells were significantly more sensitive to the former two liberators but markedly less reactive to chlortetracycline.

6 Adenosine 5′-triphosphate (ATP) and dextran were potent histamine liberators from the rat cells but were totally ineffective against the hamster.

7 Acetylcholine and carbamylcholine had no effect on either cell type.

8 These results are discussed in terms of the functional heterogeneity of mast cells from different sources.

Introduction

Mast cells are widely distributed in the animal body but are predominantly found in association with blood vessels in the subepithelial connective tissue of the bronchi, conjunctiva, gut, ear, nose, throat and skin. In addition, significant numbers of free mast cells are found in the peritoneal cavities of some rodents, principally the rat, mouse and hamster. Studies on rat peritoneal mast cells in particular have provided much important information concerning the mechanism of histamine secretion and have been widely regarded as providing model systems for the investigation of stimulus-response coupling (Foreman *et al.*, 1976; Pearce, 1982a). However, it has long been appreciated, and emphasized by more recent studies, that mast cells from different species and even from different tissues within a given animal are functionally heterogeneous (for reviews, see Pearce, 1982b; 1983). In particular, they may vary in their response both to histamine liberators and to anti-allergic drugs. These studies have been greatly facilitated by the development of enzymic dispersion techniques for the isolation of mast cells from intact tissues. However, such investigations require the use of extensive controls to exclude the possibility that the digestion treatments themselves do not induce functional changes in the target cells (Pearce *et al.*, 1982). For this reason, we wish here to compare the properties of mast cells obtained by simple peritoneal lavage of the rat and hamster. The latter cells have been surprisingly little examined.

Methods

Isolation of mast cells and determination of histamine secretion

Sprague–Dawley rats (150–250 g) and Syrian hamsters (130–180 g) were used throughout this study. Mixed peritoneal cells were recovered by direct lavage (Atkinson *et al.*, 1979) with modified Tyrode solution of the following composition (mM): NaCl 137, glucose 5.6, KCl 2.7, NaH_2PO_4 0.4, $CaCl_2$ 1 and N-2-hydroxyethyl piperazine-N'-2 ethane sulphonic acid (HEPES) 10. The pH of the solution was adjusted to 7.2 before use.

To examine the effects of histamine liberators, aliquots of cells (to a final volume of 1 ml) were allowed to equilibrate (5 min, 37°C) in a metabolic shaker with gentle mechanical agitation. A solution of the releasing agent was then added in a minimum volume, secretion allowed to proceed for a further 10 min and the reaction terminated by addition of ice-cold Tyrode solution (2 ml). Cells and supernatants were separated by centrifugation (2 min, 4°C, 150 *g*). The cell pellets were resuspended in Tyrode solution (3 ml) and allowed to stand in a boiling water bath (10 min) to release residual histamine. Individual supernatants were treated similarly. Histamine was then determined spectrofluorimetrically (Atkinson *et al.*, 1979) using a commercial automated apparatus (Technicon Autoanalyzer II). Histamine release was expressed as a percentage of the total cellular content of the amine and was calculated as the ratio (histamine in supernatant)/(histamine in supernatant + residual histamine in cells) (× 100). All values were corrected for the spontaneous release occurring in the absence of any inducer. In some experiments with inhibitors, results were conveniently expressed in terms of the percentage inhibition of the control release and thus given as the ratio (histamine release in absence of inhibitor-histamine release in presence of inhibitor)/(histamine release in absence of inhibitor) (× 100).

Characterization of mast cells

The number of mast cells in a given suspension was assessed by metachromatic staining with toluidine blue dye (0.005%,w/v) and counting in an improved Neubauer haemocytometer. The histamine content was then determined by comparison with known standards. Slides for microscopic examination were prepared according to the method of Mota & Vugman (1956). The size distribution of rat and hamster peritoneal mast cells was determined by the systematic measurement of the mean (average of maximum and minimum) diameters of a total of 700 cells from 7 animals using a calibrated eyepiece (15 × GK, Wild Heerbrug, Switzerland).

Histamine liberators and reagents

Acetylcholine chloride, adenosine 5'-triphosphate (ATP), antimycin A, carbamylcholine chloride, concanavalin A, chlortetracycline hydrochloride, 2-deoxyglucose, eserine, L-α-lysophosphatidyl-L-serine (lyso-PS), poly-L-arginine hydrochloride (mol. wt. 60,000) and poly-L-lysine hydrobromide (mol. wt. 64,000) were purchased from the Sigma London Chemical Co. Protein A and the lectins from soybean (*Glycine max*) and wheat germ (*Triticum vulgaris*) were obtained from Pharmacia G.B. Ltd. Rabbit antiserum to whole hamster serum proteins (anti-hamster serum, Nordic Immunological Laboratories), sheep antiserum to rat IgE (anti-rat IgE, Miles Laboratories), dextran (6%, w/v in saline, mol. wt. 110,000, Fisons), L-α-phosphatidyl-L-serine (PS, Lipid Products) and rat IgG (Miles Laboratories) were purchased as indicated. Compound 48/80 (Dr A.N. Payne, The Wellcome Research Laboratories), the ionophores A23187 (The Lilly Research Centre) and ionomycin (Dr S.J. Lucania, The Squibb Institute), the lectin from lentil (*Lens culinaris*, Dr M. Ennis, University College London), peptide 401 (the MCD-peptide from bee venom, Dr A.J. Garman, University College London) and soluble, myeloma rat IgE (IR 162, Dr H. Metzger, National Institutes of Health, Bethesda) were generous gifts from the sources specified.

Stock solutions of the ionophores A23187 (1 mM) and ionomycin (10 mM) were prepared in dimethyl sulphoxide and stored at −20°C until required. Aliquots were then diluted in buffer to the desired final concentration. Residual dimethyl sulphoxide (<0.1%, v/v) did not affect histamine secretion. Solutions of lyso-PS and PS were obtained or prepared in a mixture of chloroform and methanol (1:1 or 3:1, v/v). The solvent was evaporated in a stream of dry nitrogen and the lipid homogenized in buffer. All other reagents were dissolved directly in buffer.

To obtain hamster serum, animals were lightly anaesthetized with ether and bled from the neck. Clotting of the blood was initiated at 37°C (1 h) and completed at 4°C (overnight). The clot was then loosened, the serum recovered by centrifugation (10 min, 4°C, 2,000 *g*) and heat inactivated (30 min, 56°C).

Statistical analyses

All values are given as means ± s.e.mean for the number (*n*) of experiments noted. Unless otherwise stated, statistical evaluation of results was carried out using the Student's *t* test for the difference between two independent means. Values of $P < 0.05$ were considered significant.

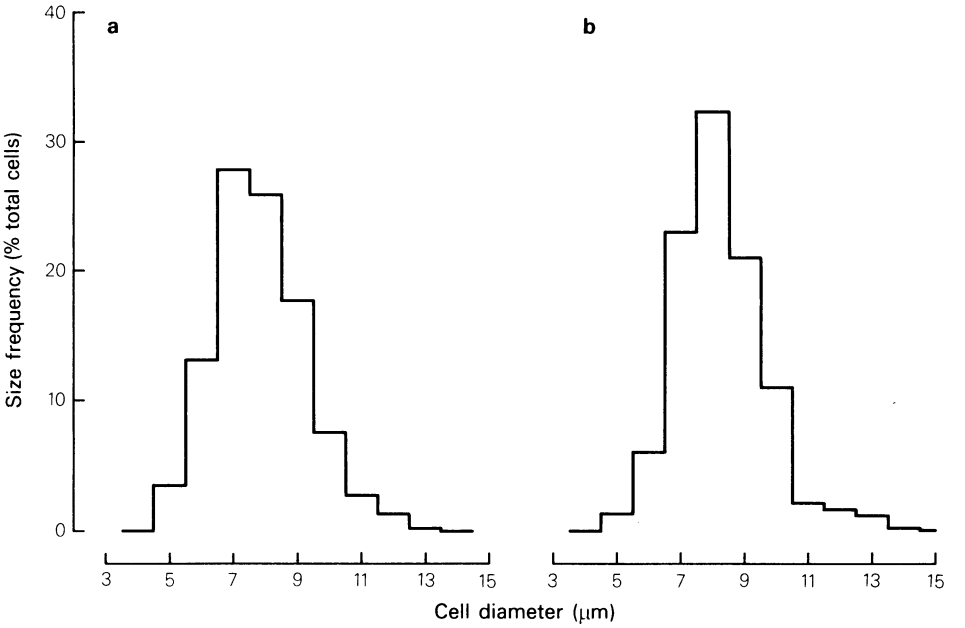

Figure 1 Distribution of size frequency for (a) hamster and (b) rat peritoneal mast cells. Values are expressed as a percentage of the total number of cells examined.

Results

Peritoneal lavage yielded approx. 5×10^5 and 10^6 mast cells per hamster and rat, respectively. The mast cells comprised approx. 5% of the total nucleated cells in both cases. The size-frequency distribution of the two populations was similar (Figure 1), as was the histamine content per cell (28.2 ± 2.0 pg, $n = 28$, hamster and 33.8 ± 1.9 pg, $n = 6$, rat). The spontaneous releases of the amine were also comparable ($6.8 \pm 0.4\%$, $n = 100$, hamster and 3.8 ± 0.3, $n = 100$, rat).

Treatment with anti-hamster serum produced a dose-dependent release of histamine from both hamster and rat peritoneal mast cells (Figure 2). The hamster cells were markedly more reactive than those from the rat, releasing considerably larger amounts of histamine and at much lower dilutions of antiserum. The two cell types also responded to anti-rat IgE. The effective concentrations of antiserum were now comparable in both cases but, despite the heterologous nature of the antibody, the maximum response obtained with the hamster cells was again significantly greater than with the rat (Figure 2). In each case, the release of histamine was totally abolished by preincubation (20 min) of the cells in a glucose-free medium with the combined metabolic inhibitors antimycin A (1 μM) and 2-deoxyglucose (5 mM) and was not significantly affected by heat-treatment (30 min, 56°C) of the antiserum.

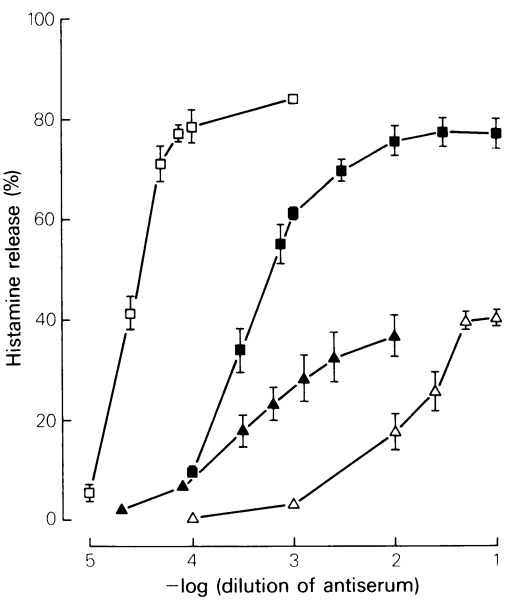

Figure 2 Log dose-response curves for the release of histamine from rat (△,▲) and hamster (□,■) peritoneal mast cells stimulated with anti-hamster serum (open symbols) and anti-rat IgE (closed symbols). The points are the means from 4 experiments and the vertical bars show s.e.mean.

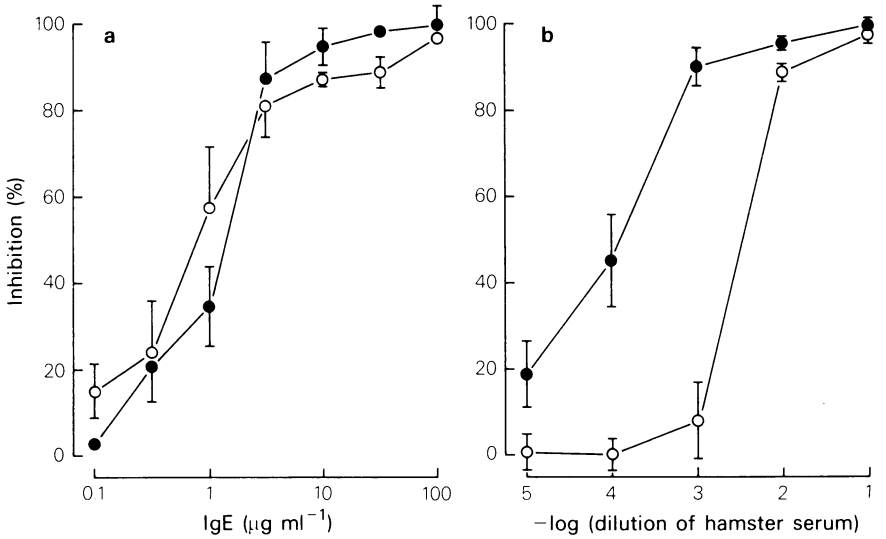

Figure 3 Inhibition by soluble antigen of immunologically induced histamine release from rat (○) and hamster (●) peritoneal mast cells. (a) Effect of myeloma rat IgE on secretion induced by anti-rat IgE (400-fold dilution). Control releases were 31.7 ± 4.8 (rat) and 63.5 ± 10.9 (hamster). (b) Effect of heat-inactivated hamster serum on secretion induced by anti-hamster serum (20-fold dilution, rat and 1000-fold dilution, hamster). Control releases were 34.8 ± 2.5 (rat) and 86.6 ± 1.0 (hamster). The displacement in the dose-inhibition curves reflects, at least in part, the disparate concentrations of anti-hamster serum required to induce maximal histamine release in the two species (see text). All points are the means from 4–5 experiments and vertical bars show s.e.mean.

Table 1 Histamine release from peritoneal mast cells treated with lectins in the presence and absence of phosphatidylserine (PS) (15 μg ml^{-1})

		Histamine release (%) from:			
	Concentration	*Rat mast cells*		*Hamster mast cells*	
Lectin	*(μg ml^{-1})*	*(−) PS*	*(+) PS*	*(−) PS*	*(+) PS*
Concanavalin A	100	29.0 ± 2.8*†	49.6 ± 3.5*†	68.4 ± 4.4†	70.3 ± 4.6†
	33	29.7 ± 4.2*†	63.7 ± 6.9*	72.0 ± 3.2†	71.6 ± 5.5
	10	32.8 ± 4.9*†	68.6 ± 3.6*	73.0 ± 3.0†	73.8 ± 3.2
	3.3	29.1 ± 4.9*†	69.7 ± 3.6*	72.3 ± 3.3†	72.4 ± 3.9
	1	13.9 ± 3.0*†	49.1 ± 3.2*	62.9 ± 5.0†	66.4 ± 4.9
	0.33	5.0 ± 1.0*†	20.8 ± 3.1*	37.2 ± 6.6†	49.6 ± 7.6
	0.1	1.5 ± 1.3	3.7 ± 0.5	8.5 ± 2.3	12.5 ± 1.1
Lentil	100	25.4 ± 4.3*†	53.6 ± 6.6*	74.7 ± 2.6†	73.1 ± 2.4
	33	25.2 ± 5.3*	59.3 ± 5.3*	—	—
	10	26.8 ± 6.1*†	60.2 ± 5.8*†	82.1 ± 1.9†	81.6 ± 2.0†
	3.3	11.8 ± 4.0*	42.8 ± 6.8*	—	—
	1	3.3 ± 1.5†	20.0 ± 8.8†	63.0 ± 8.4†	72.6 ± 6.2†
	0.33	0.7 ± 0.1†	9.4 ± 5.6†	40.3 ± 13.7†	48.6 ± 12.2†
	0.1	0.4 ± 0.3	2.8 ± 2.4	3.3 ± 1.8	6.8 ± 3.8
Wheat germ	100	1.1 ± 0.1*	56.9 ± 2.7*†	1.2 ± 1.4	0 ± 0.5†
	10	2.4 ± 0.5*	29.0 ± 5.6*†	1.7 ± 0.7	0.1 ± 0.3†
	1	0.6 ± 0.4	9.0 ± 3.5†	0.2 ± 0.2	−0.8 ± 0.9†

Values are given as means ± s.e.mean of 5 experiments.

*Denotes points at which PS has a significant ($P < 0.05$) potentiating effect on histamine release for a given cell type (t test for related measures).

†Denotes points at which the histamine secretion from the rat cells (± PS as appropriate) is significantly different ($P < 0.05$) from that of the hamster cells.

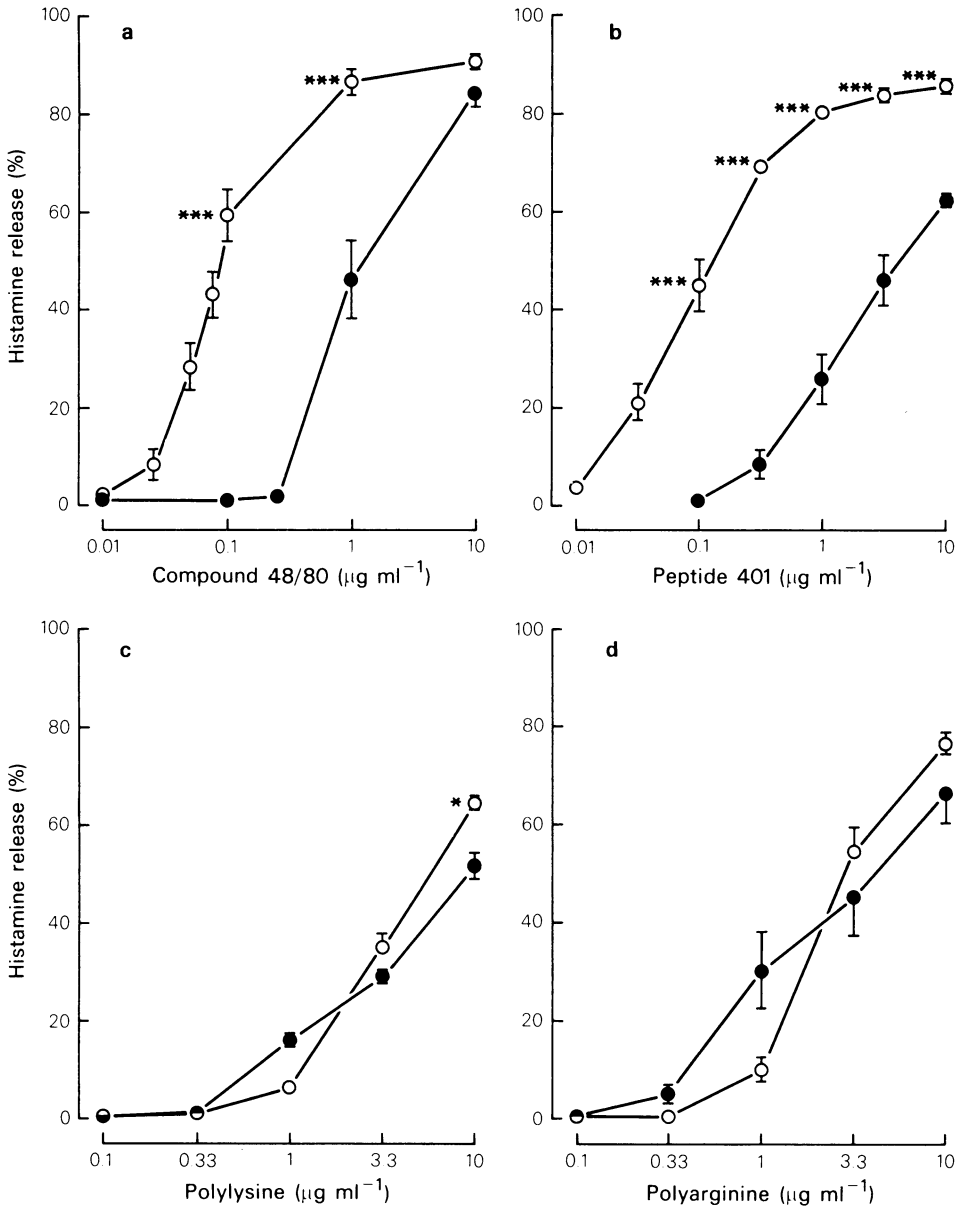

Figure 4 Log dose-response curves for the release of histamine from rat (○) and hamster (●) peritoneal mast cells stimulated with (a) compound 48/80, (b) peptide 401, (c) polylysine, and (d) polyarginine. The points are the means from 4 experiments and the vertical bars show s.e.mean. Asterisks denote points at which the histamine secretion from the rat cells is significantly different from that of the hamster cells (*$P<0.05$, **$P<0.01$ and ***$P<0.001$).

The effects on both cell types of anti-rat IgE and anti-hamster serum were blocked in comparable, dose-dependent fashion by preincubation (5 min, 37°C) of the antisera with soluble, myeloma rat IgE and heat-inactivated hamster serum, respectively (Figure 3). Further studies revealed an extensive cross-reactivity between the various preparations (data not shown). Thus, the response of the hamster cells to anti-rat IgE was also inhibited (to a maximum of 100%) by rat IgG (1–100 μg ml^{-1}) and by hamster serum (10 to 100-fold dilutions). However, the latter reagents had no effect on the reactivity of the rat cells to this antibody, presumably reflecting the greater affinity of anti-rat IgE for the cell fixed, homologous

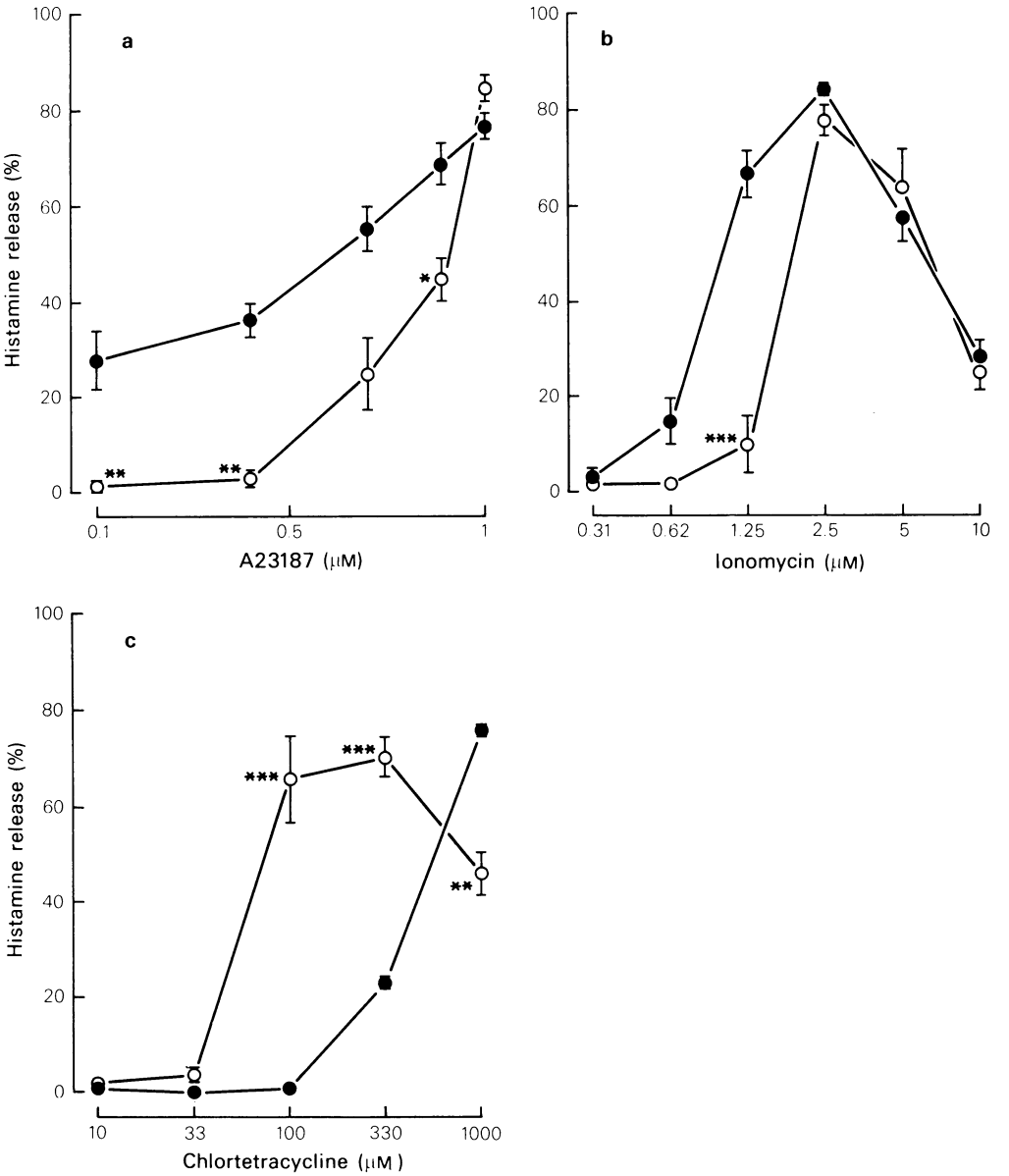

Figure 5 Log dose-response curves for the release of histamine from rat (○) and hamster (●) peritoneal mast cells stimulated with the calcium ionophores (a) A23187, (b) ionomycin, and (c) chlortetracycline. The points are the means from 4–5 experiments and the vertical bars show s.e.mean. Asterisks denote points at which the histamine secretion from the rat cells is significantly different from that of the hamster cells (*$P<0.05$, **$P<0.01$ and ***$P<0.001$).

IgE. The effect of anti-hamster serum on both cell types was comparably inhibited (to a maximum of 50% for the rat and 80% for the hamster) by rat IgG and rat IgE (both 1–100 $\mu g\,ml^{-1}$). The latter protein was noticeably less effective than against anti-rat IgE and at least ten-fold higher concentrations were required to produce comparable inhibition.

Concanavalin A and the lectin from lentil produced a dose-dependent release of histamine from both the rat and hamster cells (Table 1). In the absence of PS, the latter were markedly more reactive. However, the release from the rat cells was strikingly potentiated by PS whereas that from the hamster cells was not significantly affected by the

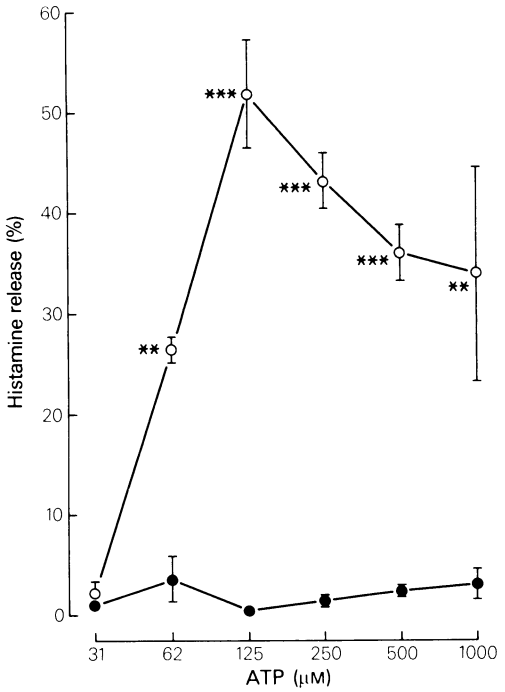

Figure 6 Log dose-response curves for the release of histamine from rat (○) and hamster (●) peritoneal mast cells stimulated with ATP. The points are the means from 4 experiments and the vertical bars show s.e.mean. Asterisks denote points at which the histamine secretion from the rat cells is significantly different from that of the hamster cells ($^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$).

lipid. Under these conditions, the reactivity of the rat cells approached more closely to that of the hamster. The response of the rat cells, but not that of the hamster, was suppressed at supramaximal concentrations of concanavalin A. In contrast, rat peritoneal cells responded to the lectin from wheat germ only in the presence of PS whereas the hamster cells were totally refractory to this agent. In all cases, lyso-PS potentiated histamine release from rat cells in a similar fashion to PS itself (data not shown). However, this compound (5–50 $\mu g\,ml^{-1}$) itself induced a release of histamine (to a maximum of approx. 60%) from the hamster cells (data not shown). The spontaneous release from the rat cells was not affected under these conditions and PS alone ($<15\,\mu g\,ml^{-1}$) had no effect in either case. Protein A and the lectin from soybean (both $<100\,\mu g\,ml^{-1}$) had no effect on either cell type in the presence or absence of PS (data not shown).

Rat peritoneal cells responded strongly to the basic secretagogues compound 48/80, peptide 401, polylysine and polyarginine (Figure 4). The hamster cells were significantly less responsive to the former two compounds but showed comparable reactivity towards the poly-amino acids.

The ionophores A23187, ionomycin and chlortetracycline induced a pronounced secretion of histamine from both hamster and rat cells (Figure 5). The hamster cells were significantly more responsive to the ionophore A23187 and to ionomycin but markedly less sensitive to chlortetracycline.

ATP evoked a characteristic release of histamine from rat mast cells (Figure 6). The dose-response curve was bell-shaped with some inhibition of the release at supramaximal concentrations of secretagogue. In sharp contrast, the hamster cells were totally unresponsive to the nucleotide.

Rat peritoneal cells released histamine ($19.6\pm2.8\%$, $n=4$) on treatment with dextran ($12\,mg\,ml^{-1}$) and the response was again enhanced (to $44.9\pm4.6\%$, $n=4$) by PS ($15\,\mu g\,ml^{-1}$). In contrast, the hamster cells were completely unresponsive to the action of this agent, both in the presence and absence of the lipid (releases $2.1\pm0.9\%$ and $1.8\pm1.1\%$, $n=6$, respectively).

Acetylcholine (0.1 pM–1 mM, decade steps in each experiment) produced no significant release of histamine from either rat or hamster cells in both the presence ($n=5$, both species) or absence ($n=5$, hamster; $n=15$, rat) of eserine (10 nM). Carbamylcholine (10 pM–10 μM, decade steps in each experiment) was similarly inactive ($n=5$, hamster; $n=15$, rat).

Discussion

The present study has shown that functional mast cells may be obtained by peritoneal lavage of the hamster, as well as from the rat, and has characterized the response of the cells to a variety of histamine liberators. The hamster cells responded strongly to challenge with anti-hamster serum and anti-rat IgE. These effects were manifest without prior immunization of the animals, indicating a high degree of natural sensitization to environmental antigens. The responses did not reflect complement-mediated cytolysis of the target cells as they were unaffected by heat-inactivation of the antisera and were blocked by metabolic inhibitors and by preincubation with the appropriate soluble antigens. Detailed analysis of the latter experiments revealed a complex pattern. Thus, the releases induced by both anti-hamster serum and anti-rat IgE were each inhibited by hamster serum and by purified rat IgG and IgE. These results indicate a high degree of cross-reactivity between and within the hamster and rat immunoglobulins but prevent determination of the nature of the antibody involved in the release process. Since both IgG or IgE

may sensitize the mast cell for mediator release (for references see Moodley & Mongar, 1981) either or both antibodies may be implicated in the present response. Consistently, rat peritoneal cells also reacted to both anti-hamster serum and to anti-rat IgE. The maximum response was in each case less than with the hamster, indicating the presence of smaller amounts of cell-fixed antibody.

In keeping with their high degree of reactivity towards immunological stimuli, hamster peritoneal cells were also extremely responsive to treatment with concanavalin A. A similar effect, albeit less marked, has been previously reported by Hook *et al.* (1974). The lectin is believed to cross-link cytotropic antibody by binding to glucose and mannose residues in the carbohydrate portion of the immunoglobulin. The cells also responded to the lectin from lentil, which has a similar specificity to concanavalin A but a lower binding affinity for the free sugars, but were refractory to the lectins from wheat germ (specific for N-acetylglucosamine) and soybean (N-acetylgalactosamine). In no case was the release significantly potentiated by PS. In sharp contrast, but in keeping with our previous results (Ennis *et al.*, 1981), the responses of the rat peritoneal cells to concanavalin A and the lentil lectin were significantly enhanced by PS and the effect of the wheat germ lectin was totally dependent on the lipid. In the absence of PS, the hamster cells were significantly more reactive towards the former lectins. Rat cells were again unresponsive to the soybean lectin and both cell types were refractory towards protein A. The latter binds to the F_c region of IgG antibody but its inability to induce histamine secretion may indicate that it is unable to produce the cross-linking of immunoglobulin molecules thought necessary to evoke exocytosis. The specific enhancement due to PS is noteworthy and the present data extend previous observations that the effect is confined largely to the peritoneal mast cells of the rat and mouse (Goth *et al.*, 1971; Mongar & Svec, 1972; Siraganian & Hazard, 1979; Ennis & Pearce, 1980; Ennis *et al.*, 1981; Magro, 1981; Ennis, 1982).

The hamster cells were markedly less responsive than those of the rat to the basic liberators, compound 48/80 and peptide 401. Keller (1968) has obtained similar results with the former secretagogue. This is in accord with the findings *in vivo* where compound 48/80 produces a severe or fatal anaphylactoid reaction in the rat but has a much less dramatic effect in the hamster (Parratt & West, 1957). In total, basic secretagogues exhibit a high degree of tissue and species specificity in their action (for references, see Pearce, 1982b; 1983). However, the differential reactivity of the cells towards polylysine and polyarginine was less marked. These agents appear to have a broader spectrum of activity than compound 48/80 or peptide 401 and, for example, induce histamine release from human basophil leucocytes which are refactory to the latter secretagogues (Foreman & Lichtenstein, 1980; Pearce, 1983).

The hamster cells released histamine on treatment with the calcium ionophores A23187, ionomycin and chlortetracycline. The ionophoretic properties of the latter antibiotic have recently been described in detail (White & Pearce, 1982). These results suggest that, as in other mast cells, an elevated level of the cation in the cytosol of the hamster cell is a necessary and sufficient trigger for secretion (Foreman *et al.*, 1976; Pearce, 1982a). The hamster cells were significantly more responsive than those of the rat to the former two ionophores but markedly less sensitive to chlortetracycline. Previous studies have also shown that chlortetracycline exhibits a high degree of selectivity in its histamine-releasing action (Pearce *et al.*, 1983).

In sharp contrast to the rat, hamster peritoneal cells were essentially unresponsive to dextran and ATP. These agonists are thought to act through specific receptors on the cell membrane (Cockcroft & Gomperts, 1980; Moodley *et al.*, 1982) and the latter may be absent or uncoupled in the hamster cell. The present data extend previous studies showing that both substances exhibit considerable specificity in their action (Ennis & Pearce, 1980; Ennis, 1982; Pearce, 1982b).

In our hands, neither acetylcholine (alone or in the presence of the acetylcholinesterase inhibitor eserine) nor carbamylcholine produced any release of histamine from rat or hamster peritoneal mast cells. The effect of cholinergic agonists on mast cells is the subject of considerable controversy and some workers have reported a dramatic secretion of histamine (Fantozzi *et al.*, 1978; Schmutzler *et al.*, 1979) whereas others have failed to observe any response (Kazimierczak *et al.*, 1980; Foreman, 1981). Our results are in accord with the latter observations.

In summary, we have shown that there are considerable functional differences between peritoneal mast cells from the hamster and rat. These studies complement the recent investigations of ourselves and others using enzymically dispersed tissue mast cells (for reviews, see Pearce, 1982b; 1983). However, the latter studies require the use of extensive controls to exclude any possible modification of cellular function by the proteases used in the isolation procedure. These reservations do not apply in the present work as the cells are obtained in identical fashion by simple lavage. Hamster peritoneal cells should then provide an important additional tool for the investigation of mediator release.

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