

Structural and secretory characteristics of bovine lung and skin mast cells: evidence for the existence of heterogeneity

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Summary

We have examined cells dispersed enzymatically from three different sites in the bovine lung (tracheal mucosa, bronchial mucosa and parenchyma) and the skin, in order to ascertain whether the bovine model could be used to study mast cell heterogeneity. Histochemically there were two sub-populations of mast cells present in both lung and skin (on the basis of toluidine blue staining and the sensitivity to formalin fixation), but their proportions were similar in all sites studied. Skin mast cells contained approximately twice the amount of histamine than their counterparts in the lung ($P < 0.05$). Functional heterogeneity was examined by *in vitro* release of histamine following secretagogue challenge. Calcium ionophore induced a substantial release of histamine; skin mast cells releasing significantly more histamine than any of the lung mast cells (at $10 \mu\text{M}$ ionophore, 37.1% and 20.7% net histamine release, respectively, $P < 0.05$), although the time-course of release from the two tissues was similar. The neuropeptides vasoactive intestinal peptide and somatostatin induced a modest but statistically significant release of histamine from both skin and lung mast cells, whilst substance P only induced histamine secretion from skin mast cells. A range of other potential immunological and non-immunological secretagogues was unsuccessful in eliciting histamine release from mast cells in any of the tissues. We conclude that there were no convincing histochemical differences between mast cells from the sites examined in the lung or skin. Additionally, there was no discernable functional heterogeneity between mast cells within the lung, but functional differences were evident between mast cells of the bovine lung and skin. However, in the absence of a suitable immunological stimulus the bovine model cannot be regarded as a good model of mast cell heterogeneity.

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Introduction

Heterogeneity of rat mast cells is well established, with the existence of two major sub-populations represented by the peritoneal (connective tissue type; CTMC) and intestinal mucosal mast cell (mucosal type; MMC). These sub-populations are distinguishable by histochemical and functional criteria [1].

In humans, sub-populations of mast cells have also been reported, but their existence is less well defined than in rodents [1–3]. Skin mast cells, for example, in addition

to releasing mediators in response to an immunological stimulus can also be stimulated by challenge with neuropeptides (substance P, vasoactive intestinal peptide and somatostatin), polybasic peptides (compound 48/80, polylysine), opiates and the anaphylatoxin C5a [3]. In comparison, human lung mast cells, although also responsive to immunological challenge, are refractory to stimulation with all of the non-immunological secretagogues [4]. Additionally, although human lung mast cells can be activated by anti-IgE, they require prior passive sensitization with IgE for optimal histamine release [5]. Mast cells from bronchoalveolar lavage (BAL), however, are more sensitive to immunological stimulation and do not require sensitization for maximal response. Whilst

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histamine release from lung parenchymal mast cells is relatively insensitive to sodium cromoglycate, that from BAL mast cells is inhibited by this chromone [5].

There is also heterogeneity with respect to the content of the two major mast cell proteases: tryptase and chymase. In human lung parenchyma the predominant mast cell contains only tryptase (MC_T) with few tryptase- and chymase-containing mast cells (MC_{TC}). In the skin the converse is true with mainly MC_{TC} and only few MC_T [2]. Several studies have examined the properties of mast cells from the human lung, but with the exception of BAL mast cells these have invariably been derived from the lung parenchyma [6–9].

Mast cells are implicated in the pathogenesis of asthma, particularly in the early response, a condition which is primarily a conducting airways phenomenon, consequently it is important to know whether mast cells resident in the conducting airways differ in any way to those in the parenchymal tissue in their responsiveness to secretagogues as well as their inhibition by drugs. Unfortunately, human airway tissue is difficult to acquire in sufficient quantities, and for this reason it was decided to use the bovine lung as a model for examining region-specific mast cell heterogeneity.

Mast cells have been identified in various tissue sites in bovine species, including the lungs and the skin [10,11], and have been demonstrated to contain histamine, dopamine and 5-hydroxytryptamine. Lung fragments from sensitized calves release all of these mediators and SRS-A upon challenge with antigen [12–15]. The capacity of these species to produce a reaginic homocytotropic immunoglobulin has also been clearly shown [16,17].

We have dispersed mast cells enzymatically from three different sites in the bovine lung: tracheal mucosa, bronchial mucosa and parenchyma. Bovine skin mast cells were also examined for comparison. Heterogeneity in these sites was assessed by examination of both histochemical and functional parameters.

Materials and Methods

Reagents

The following chemicals and enzymes were used, and unless otherwise stated were of analytical grade and obtained from Sigma, Poole, U.K. Collagenase (type I), hyaluronidase (type I-S), protease (type XIV), chymopapain, deoxyribonuclease-I. HEPES-balanced salt solution (HBSS) contained the following: ($g\ l^{-1}$) NaCl 8.0, KCl 2.0, NaH_2PO_4 0.06, $MgCl_2 \cdot 6H_2O$ 0.26, $CaCl_2$ 0.22, D-glucose 1.0, HEPES (BDH Chemicals, Poole, U.K.) 2.38, bovine serum albumin (BSA) (Cohn fraction V) 0.1, pH adjusted to 7.4. Eagle's minimum essential

medium (MEM: containing 25 mM HEPES buffer and L-glutamine; GIBCO, Irvine, U.K.). Fetal calf serum (heat-inactivated, GIBCO). Disodium EDTA (BDH).

Secretagogues

Non-immunological secretagogues included the following: formyl methionine leucyl phenylalanine (fMLP), platelet-activating factor (hexadecyl PAF, Novabiochem, Nottingham, U.K.), substance P, vasoactive intestinal peptide (VIP; porcine sequence), somatostatin, calcium ionophore, interleukin-1 (IL-1) alpha and beta. (Recombinant; Koch-Light, Haverhill, U.K.), dextran (GMW 72 200), compound 48/80, poly-L-lysine. (GMW about 55 000). All stock solutions were diluted to the required concentration in freshly made HBSS prior to challenge.

Mast cell dispersion

Bovine lungs and foreskin from freshly killed male animals were obtained from a local abattoir and tissue dissected within 2 hr of death. Bronchial and tracheal mucosa were stripped from the airways, and parenchymal lung tissue excised and placed directly in MEM. All tissue was chopped finely into $0.5\ mm^2$ -pieces and rinsed briefly in MEM. To disperse mast cells, fragments were incubated in a shaking water-bath for 40 min at $37^\circ C$ in MEM ($3\ ml/g^{-1}$ tissue) containing 2% FCS, DNase ($200\ mg\ l^{-1}$) and, in the case of bronchial and tracheal mucosae: collagenase ($450\ Uml^{-1}$) and hyaluronidase ($148\ Uml^{-1}$). Parenchymal fragments were incubated with protease ($10\ Uml^{-1}$) and chymopapain ($10\ Uml^{-1}$).

After incubation for 40 min, dispersed cells were harvested from undissociated tissue by sequential filtration through $800\text{-}\mu m$ and $60\text{-}\mu m$ nylon gauze filters. Undissociated tissue was incubated for a further 40 min with fresh enzyme solution after which dispersed cells were collected again. Mast cells from skin were dispersed in a similar fashion except that $10\ ml\ MEM\ g^{-1}$ tissue containing collagenase, hyaluronidase and protease were used, and two 1-hr incubations were performed. Following dispersion cells were washed three times in MEM (containing FCS and DNase). In each case cells from the second dispersion were pooled with those from the first. Mast cell counts were made by light microscopy following metachromatic staining with Kimura's stain [18]. Viability, ascertained by trypan blue exclusion, was $>95\%$ in all cases.

Tracheal mucosal mast cells were only used in secretagogue challenge experiments and for assessment of histamine content. No histochemical or morphological studies were performed using these cells.

Mast cell activation

Before secretagogue challenge, cells were washed once more in HBSS and then resuspended in this buffer. Aliquots (100 μ l) of the cell suspension containing at least 10^4 mast cells were dispensed into 3-ml polypropylene tubes then made up to 900 μ l with HBSS. After 5 min of pre-incubation at 37°C, 100 μ l of the secretagogue were added to each duplicate tube. To quantify spontaneous histamine release, 100 μ l of HBSS alone were added to a duplicate tube. The tubes were briefly shaken and further incubated for 20 min at 37°C. After this, the reaction was stopped by centrifugation (5 min at 500 *g* and 4°C) and the supernatant immediately removed from the cell pellet (except where the total histamine content of the pellet was required). TCA was added to the samples to a final concentration of 5% prior to histamine analysis. Histamine content was assayed by an automated spectrofluorometric assay [19]. Net histamine release was expressed as a percentage of total cell-associated histamine, following correction for spontaneous release assessed in quadruplicate samples in the absence of secretagogue.

In experiments to analyse the time course of histamine release, the above procedure was modified so that reactions were stopped at appropriate times by addition of 1 ml of ice cold Ca^{2+} -free HBSS which contained 2 mM EDTA, followed immediately by centrifugation. Supernatant and cell-associated histamine were measured as described above.

Histochemistry

Cytospin preparations (Shandon, London, U.K.) were made for histochemical studies on dispersed cells. Slides were air-dried, fixed for 5 min in either 10% neutral buffered formalin or Carnoy's, and stained with 0.5% toluidine blue (pH 0.5, 30 min). Mast cells were enumerated by counting a minimum of 1000 cells in random fields of view, and results were expressed as a percentage of the total number of nucleated cells.

Tissue blocks were also processed for histology. Small samples (1 mm²) of each tissue were fixed for 24 hr in buffered formalin or Carnoy's, dehydrated and embedded in paraffin wax, sections (5 μ m) were cut and then stained as described above with toluidine blue or haematoxylin and eosin.

Electron microscopy

For electron microscopy, tissue removed from animal within 5 min of death, was fixed in ice-cold Karnovsky's fixative [20], post-fixed in osmium tetroxide, block-stained with uranyl acetate, dehydrated and then embed-

Table 1. Influence of fixation on the toluidine blue staining of bovine mast cells

Fixative	Parenchyma (<i>n</i> = 6)	Bronchial mucosa (<i>n</i> = 5)	Skin (<i>n</i> = 8)
Carnoy	8.2 $\pm$ 1.0*	4.5 $\pm$ 1.3*	1.0 $\pm$ 0.7*
Formalin	3.0 $\pm$ 0.7	2.1 $\pm$ 0.8	0.4 $\pm$ 0.3

**P* < 0.05 compared with formalin-fixed cells.

Mast cell number as percentage of nucleated cells (mean $\pm$ s.e.m.).

ded in Spurr resin. Thick and thin sections were cut on a Reichert OM3 ultramicrotome, stained with lead citrate and examined in a Hitachi H7000 electron microscope. Cells dispersed as described above (except that only one 40-min (lung) or one 60-min digestion (skin) was used), were fixed in 2.5% glutaraldehyde and then processed as for tissue.

Statistical analysis

Histamine release values are the mean ($\pm$ s.e.m.) net histamine release expressed as a percentage of total histamine content. Differences between means were tested for significance with Student's *t*-test for paired or unpaired data as appropriate. Differences were considered significant at *P* < 0.05.

Results

When cytopins made from suspensions of cells from bronchial mucosa, parenchyma and skin were stained with toluidine blue after fixation in Carnoy's or formalin and mast cells counted, there were significantly fewer stained mast cells present on the slides fixed in formalin (Table 1). Thus, in these three sites a formalin-sensitive population of mast cells existed which predominated over the formalin-insensitive population. There was no significant difference between the ratios of formalin-sensitive to formalin-insensitive mast cells in each of the sites examined, which indicated that the proportions of the mast cell populations was similar within the lung and between the lung and skin. No obvious ultrastructural differences were found between mast cells from the different sites either in tissue or in dispersed tissue preparations (Fig. 1). In the bronchial mucosa, mast cells were predominantly found in the lamina propria (defined as the region between the basement membrane of the epithelium and the underlying bronchial smooth muscle), usually in intimate association with connective tissue fibrils

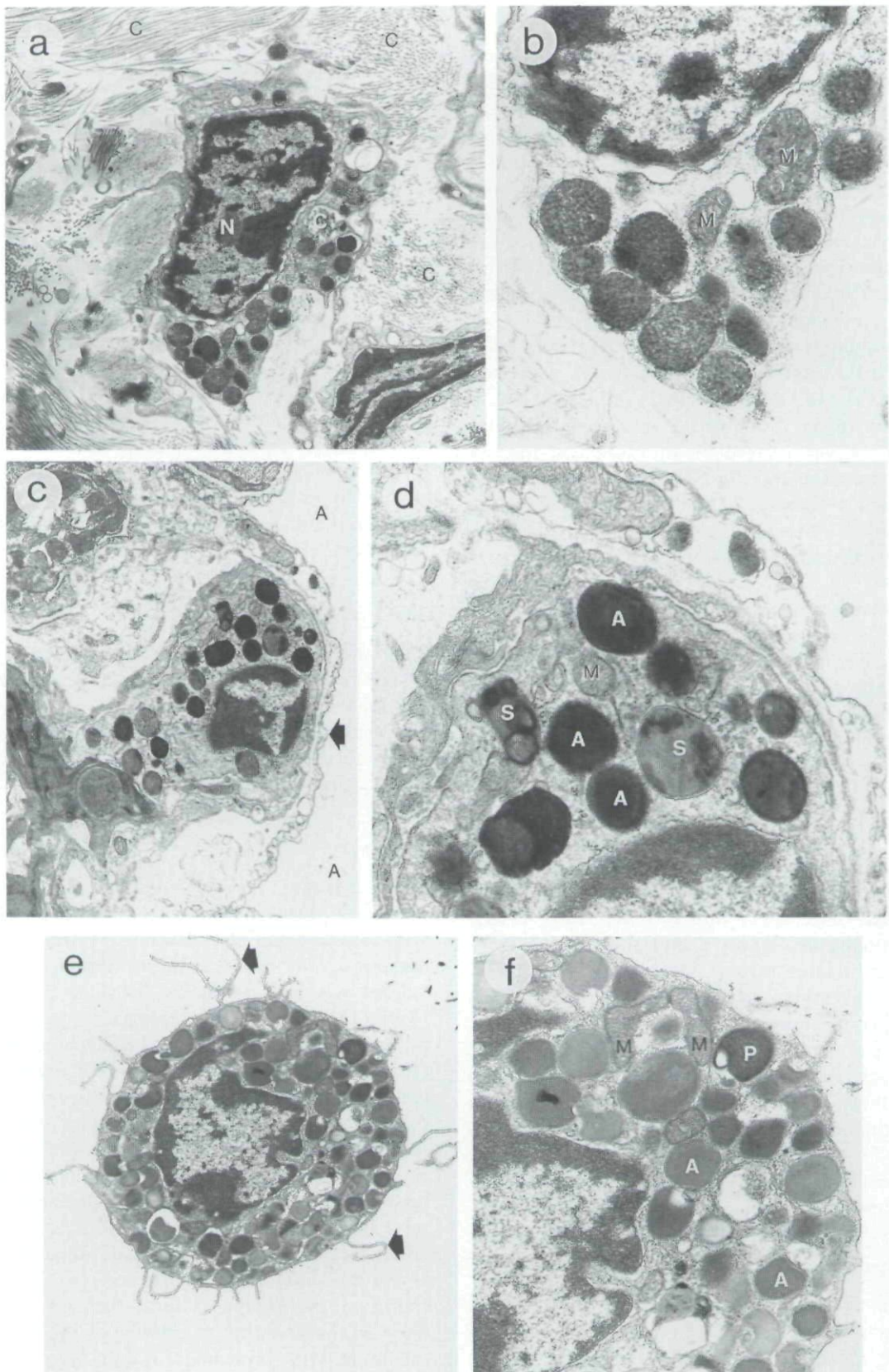


Table 2. Histamine content of mast cells dispersed from bovine lungs and skin (mean $\pm$ s.e.)

	Parenchyma (<i>n</i> = 26)	Tracheal mucosa (<i>n</i> = 6)	Bronchial mucosa (<i>n</i> = 39)	Skin (<i>n</i> = 27)
Histamine content pg/mast cell	3.9 $\pm$ 0.7	4.8 $\pm$ 0.5	4.2 $\pm$ 0.8	9.3 $\pm$ 1.0*

**P* < 0.05 compared with each lung site.

n, number of animals from which mast cells were obtained.

(Fig. 1a, b). They were often seen immediately beneath the reticulin fibres of the basement membrane of the epithelium, but only rarely in the epithelium itself. Mast cells were seen more frequently in the lung parenchymal tissue than in the bronchial mucosa, and were usually sited in the interalveolar septa adjacent to pulmonary capillaries (Fig. 1c & d). In foreskin, mast cells were invariably found in the dermis and, as in the bronchial lamina propria they were in close contact with connective tissue fibrils. In tissue sections mast cells were seen to be elongated and distorted and interspersed between connective tissue elements (Fig. 1a).

Following their dispersal, mast cells from all three sites assumed a spherical shape with numerous fine protruding pseudopodia (e.g. Fig. 1e). The nuclei of the mast cells were either spherical, indented or even bilobed, whilst the cytoplasm was replete with granules, which were often heterogeneous in character. Three granule types were seen in the bovine mast cells. The first had an amorphous content and varied in degree of electron density (examples of which can be seen in cells in Fig. 1c-f). the second was identifiable by the particulate appearance of its content (e.g. Fig. 1b, f). The third granule population was characterised by its inclusion of sub-structures (e.g. Fig. 1d). However, these did not resemble the scrolls or other configurations described in human mast cells [21].

The mean content of histamine per mast cell dispersed from the various sites in the lung and the skin was

Table 3. Effect of non-immunological secretagogues on histamine release from bovine dispersed lung and skin mast cells

	Range of net histamine release	
	Lung mast cells	Skin mast cells
A23187 Ionophore	14.9–34.8†	31.7–46.4*
Substance P	0.1–4.1 (NS)	5.5–17.7‡
Somatostatin	–0.3–12.9§	6.2–15.6‡
VIP	2.5–8.5§	3.0–11.5‡
Poly-L-lysine	1.2–10.9§	1.0–9.1‡
Compound 48/80	–1.5–3.1 (NS)	–0.5–0.1 (NS)
Dextran	–0.6–1.3 (NS)	ND
Interleukin-1	–0.7–0.4 (NS)	ND
Platelet-activating factor	–0.7–0.8 (NS)	ND
f-met-leu-phe	–0.5–0.8 (NS)	ND

* 25–40% net histamine release.

† 15–25% net histamine release.

‡ 5–15% net histamine release.

§ 0–5% net histamine release.

NS, no significant release (*P* > 0.05); ND, not determined.

Data for mean net histamine release are presented as scores (denoted by the above symbols) for ease of comparison with skin mast cells. The scores for lung mast cells are composite values derived from experiments with mast cells derived from tracheal and bronchial mucosae and parenchyma (there being no significant difference in the values obtained with cells from each site). Range of net histamine release from lung tissues and skin.

determined (Table 2). No significant difference was found in the histamine content of mast cells dispersed from the three different sites in the lung, but the histamine content of skin mast cells was more than twice that of lung mast cells (*P* < 0.05).

To optimize the mast cell yield, it was necessary to employ different procedures of enzymatic digestion for each of the tissues used. However, there was no correlation in our results between use of a particular enzyme and the sensitivity, or indeed lack of sensitivity of mast cells to a particular secretagogue.

Fig. 1. (a) Mast cell in the lamina propria of bronchial mucosa (magnification $\times$ 7500). Note abundant connective tissue (C) surrounding the cell. N, nucleolus; (b) detail from (a) (magnification $\times$ 22 000). This cell contains granules with a particulate content. Note mitochondria (M); (c) mast cell underlying alveolar wall in lung parenchyma (magnification $\times$ 7500). Note alveolar space (A) and attenuated cytoplasm of a type I pneumocyte which forms the lining of the alveolar sac (arrow); (d) detail of (c) (magnification $\times$ 22 000). Note the granules of varying electron density in the mast cell cytoplasm. Some of these have an amorphous content (A) and others contain sub-structures (S). M, mitochondrion; (e) mast cell dispersed from bovine foreskin (magnification $\times$ 7500). The cytoplasm is replete with granules of varying electron density. Numerous fine pseudopodia are present at the cell surface (arrows); (f) detail from (e) (magnification $\times$ 22 000). Note granules, some of which have an amorphous content (A) and others a particulate content (P). M, mitochondrion.

Histamine release by non-immunological mechanisms

Lung mast cells. Table 3 summarizes the results of experiments using non-immunological mast cell secretagogues. None of the following stimuli elicited significant net release of histamine from any of the lung mast cells: PAF (10^{-6} – 10^{-9} M), fMLP (10^{-4} – 10^{-9} M), dextran (1 – 10 mg ml $^{-1}$), compound 48/80 (1 – 100 mg ml $^{-1}$), IL-1 α and IL-1 β (50 U ml $^{-1}$) and fMLP (10^{-4} – 10^{-9} M). Of the non-immunological secretagogues, the neuropeptides soma-

tostatin and VIP, but not substance P, induced a small but significant net release of histamine from lung mast cells in all sites tested. For example, mast cells dispersed from tracheal mucosa gave a net release of $5.1 \pm 1.6\%$ ($P < 0.05$, $n = 4$) when challenged with VIP (30 μ M) and $3.5 \pm 1.7\%$ ($P < 0.05$, $n = 8$) with somatostatin (30 μ M). Poly-L-lysine (10 μ M) also elicited a net histamine release from tracheal mucosal mast cells ($6.4 \pm 2.0\%$; $P < 0.01$, $n = 4$). These results are representative of mast cells from other sites in the lung. The only secretagogue that elicited

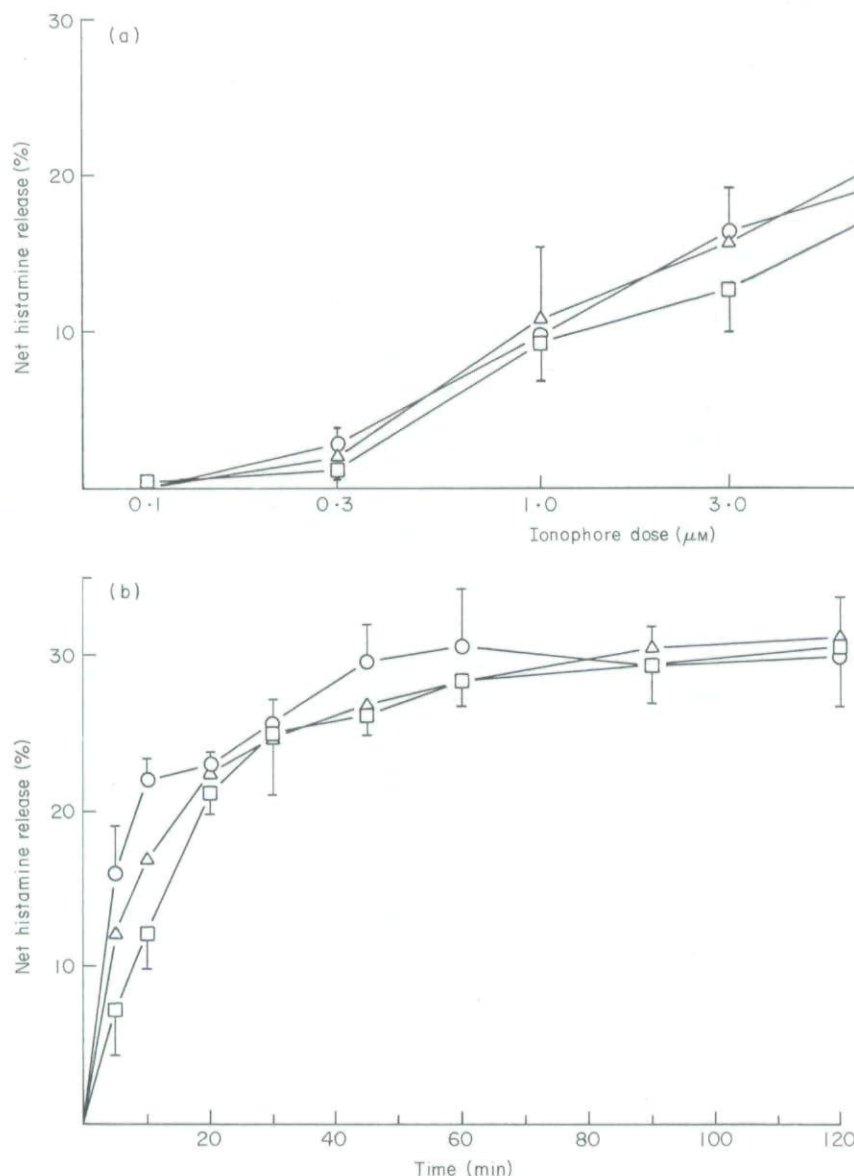


Fig. 2. (a) Effect of increasing concentrations of calcium ionophore A23187 on net histamine release from mast cells dispersed from the parenchyma (Δ), tracheal mucosa ($\square$) and bronchial mucosa ($\circ$). Each point represents the mean $\pm$ s.e.m. for cells from four to five different animals; (b) time course of net histamine release from bovine lung mast cells dispersed from the parenchyma (Δ), tracheal mucosa ($\square$) and bronchial mucosa ($\circ$) activated by a single concentration of A23187 (10 μ M). Each point represents the mean $\pm$ s.e.m. for cells from three different animals.

substantial release of histamine from bovine lung mast cells was calcium ionophore A23187 (Fig. 2a). In the presence of Ca^{2+} , A23187 ionophore induced a significant ($P < 0.01$) and concentration dependent ($0.3\text{--}30\text{ }\mu\text{M}$) release from cells isolated from all three sites in the lung. The maximum value for all three mast cell dispersates was 28–30% with no significant differences being observed in the dose response characteristics, which in general

plateaued at an ionophore concentration of $30\text{ }\mu\text{M}$. After addition of ionophore, histamine release was rapid in onset and continued for 20 min (Fig. 2b), thereafter, any further increase was due to a gradual rise in spontaneous release (data not shown). There were no significant differences in the time-course profile of histamine release between mast cells isolated from any of the three lung sites. In control studies the metabolic inhibitors 2-deoxy-

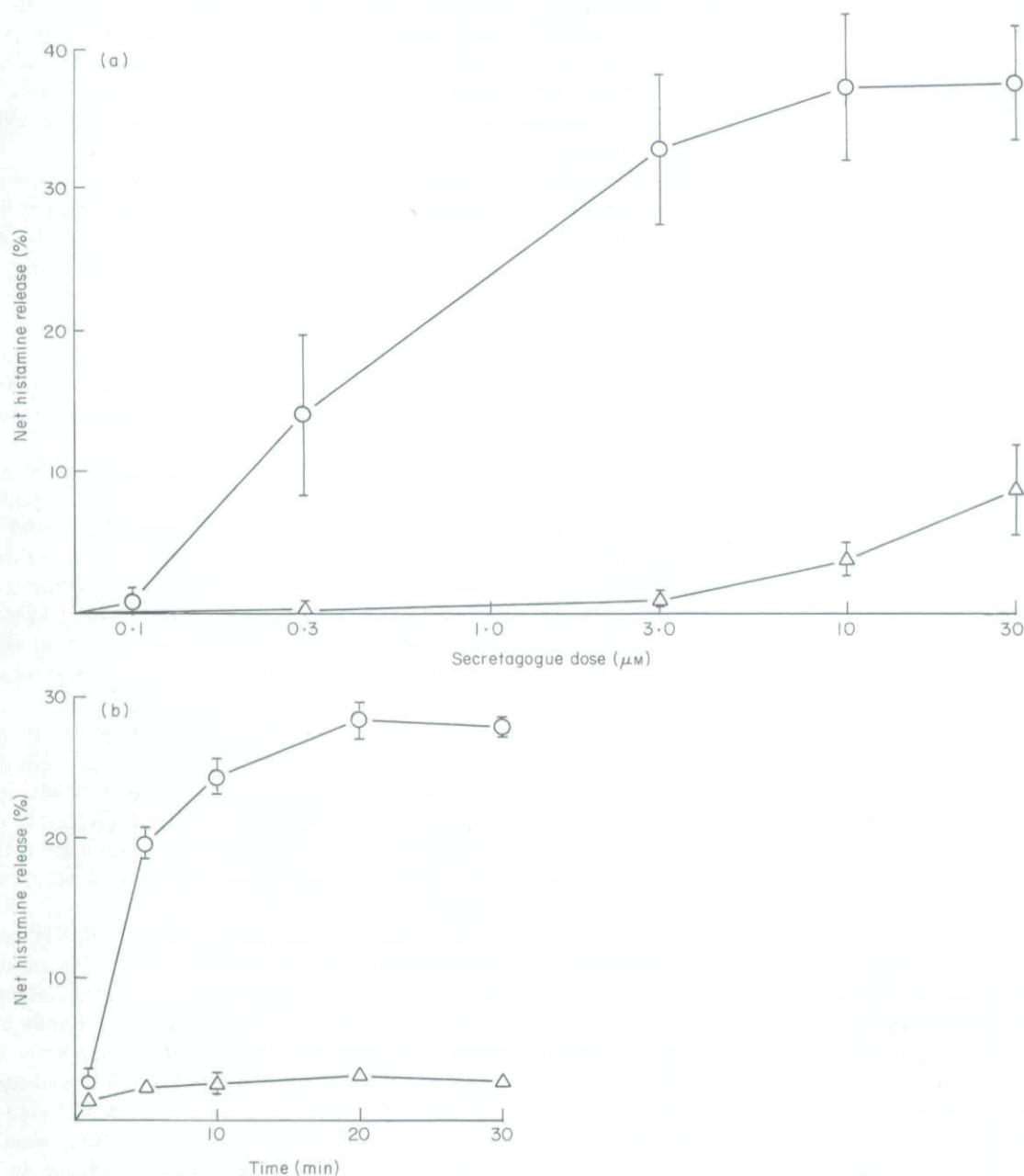


Fig. 3. (a) Response of bovine dispersed skin mast cells to increasing concentrations of calcium ionophore A23187 (○) and substance P (Δ). Each point represents the mean $\pm$ s.e.m. for cells from four to five different animals; (b) time course of histamine secretion from bovine dispersed skin mast cells activated with substance P (Δ) ($30\text{ }\mu\text{M}$) and calcium ionophore A23187 (○) ($10\text{ }\mu\text{M}$). Each point represents the mean $\pm$ s.e.m. for cells from four to five different animals.

D-glucose (10 mM) and antimycin A (1 μ M) inhibited ionophore induced histamine release (>95%) indicating it to be non-cytotoxic.

Skin mast cells. A smaller range of secretagogues was tested against dispersed skin mast cells (Table 3). While compound 48/80 did not elicit histamine release, 1 μ M and 10 μ M concentrations of polylysine caused a small but significant ($P < 0.05$) net release of histamine amounting to 2.7 ± 1.0 and $5.6 \pm 1.5\%$, respectively. All three neuropeptides tested, substance P, VIP and somatostatin, also induced histamine release (Table 3). The concentration dependency of histamine release to substance P is shown in Fig. 3a and the time course of substance P induced histamine release is also shown (Fig. 3b). Although significant release was seen as early as 5 sec after addition of substance P, maximal release was not attained until 20 min. Skin mast cells also responded to A23187 ionophore challenge (0.3–30 μ M) with significant histamine release (Fig. 3a, b). The histamine release of the skin mast cells was significantly greater ($P < 0.05$) than that of lung mast cells challenged with identical concentrations, while the time course (Fig. 3b) was similar to that observed for lung cells.

Discussion

The restricted availability of tissue makes it very difficult to examine for regio-specific heterogeneity of mast cells within the human lung. Use of bovine lung has enabled us to do this, and to comment on the suitability of the bovine system as a general model for mast cell heterogeneity.

Cellular heterogeneity can be examined in several ways. It has long been known that rodent mast cell populations can be discerned histochemically on the basis of their sensitivity to formalin. The mucosal mast cell (MMC) population is formalin sensitive, i.e. metachromatic staining is abolished after formalin fixation, whereas connective tissue mast cells (CTMC) are formalin resistant. This is a reflection of the glycosaminoglycan (GAG) content of the rat mast cell granules [1].

In the bovine skin, and in the two sites examined in the lung, there appeared to be two populations of mast cells discernable on the basis of their toluidine blue staining after formalin fixation. Differences in the ratio of formalin-sensitive to formalin-resistant mast cells were not statistically significant (data not shown) indicating that the proportions of the two mast cell populations was similar in each tissue examined. These results are in accordance with observations made in human lung and skin which also contain a population of formalin-sensitive mast cells [22,23]. Somewhat surprising was the high proportion of formalin-sensitive mast cells dispersed

from bovine skin; these predominated over the formalin-insensitive mast cells normally associated with connective tissue sites, for example, in human skin 90% of the mast cells are formalin insensitive [3,24].

Mast cells may also be heterogeneous in relation to their histamine content, for example, in rodents, the histamine content of MMC (about 1 pg cell⁻¹) is appreciably lower than that in CTMC (about 10 pg cell⁻¹) [25]. The situation regarding histamine content of human mast cells is more complex [7,8,25]. In dispersed preparations of bovine lung and skin mast cells, the latter was found to contain significantly more histamine, but within the different anatomical sites in the lung no differences were apparent.

Mast cell heterogeneity can also be discerned at a functional level. Attempts were made to elicit histamine release from dispersed bovine mast cells by use of a number of immunological stimuli including anti-IgE antisera, lectins, bacterial protein and a bovine lungworm extract, but these were unsuccessful. We were unable to determine the precise reason for the failure to observe histamine release; for this reason the results of the immunological challenge were regarded as inconclusive and so are not presented here.

Mast cells may be activated for secretion by a number of non-immunological stimuli, e.g. neuropeptides, poly-L-lysine, compound 48/80, dextran, IL-1, PAF [1,4,25–29]. Compound 48/80 stimulates release of histamine from human skin and rodent CTMC, but not from mast cells isolated from human lung or rodent MMC [4,26]. We found compound 48/80 to be inactive at concentrations up to 100 μ g/ml with both bovine lung and skin mast cells.

Poly-L-lysine, a highly basic peptide, is a potent secretagogue of rodent CTMC and mast cells dispersed from human skin, but not from rodent MMC or human lung mast cells [4,30–32]. Surprisingly, mast cells dispersed from bovine lung and skin responded with a weak but statistically significant release of histamine to this peptide.

Neuropeptides, including substance P, VIP and somatostatin have been shown to elicit good histamine release from mast cells of human skin and from rodent CTMC [1,4,29] but not from human lung mast cells or rodent MMC. In the present study we showed a weak secretory response of bovine lung mast cells to VIP and somatostatin, but not to substance P. Pearce *et al.* [33] also reported a similar weak response of human lung mast cells to somatostatin with 9% net histamine release. In addition to somatostatin and VIP, bovine skin mast cells also responded to challenge with substance P with a modest release of histamine. However, the magnitude of this response was considerably less than that of human skin

mast cells. Histamine release from human skin mast cells elicited by substance P reaches a maximum after only 20 sec, whereas with anti-IgE 5–7 min is required before maximal histamine release is attained [34]. However, in bovine skin mast cells maximal histamine release occurred 20 min after substance P challenge and they were approximately 10-fold less sensitive to the secretory effect of substance P than mast cells from human skin [34].

In bovine lung and skin two histochemically distinct subpopulations of mast cells have been identified. Formalin-sensitive mast cells predominated in all sites examined. Mast cells from the sites within the lung did not differ significantly in the relative number of formalin resistant and formalin sensitive mast cells, histamine content, granule ultrastructure or in their response to the A23187 calcium ionophore. When compared with mast cells of humans and other species, bovine mast cells appeared to be remarkably resistant to activation. Bovine skin mast cells contained more histamine and were more responsive to activation by A23187 and substance P than lung mast cells. Thus, while lung and skin mast cells in humans differ to a marked extent, bovine functional differences are less evident. Histochemical parameters have been shown to be of limited worth in discerning mast cell heterogeneity in humans, and this would also appear to be the case in this species. In the bovine model, as in other animals and humans, it is likely that phenotypic differences between mast cells in different tissues, and even at different sites within the same tissue are expressed at a number of levels. These include variation in granular constituents, cell surface receptors and in the ability to activate secretory pathways. This heterogeneity is likely to reflect the microenvironment in which the mast cell is situated. Our results show that the value of the bovine as a model system for the examination of mast cell heterogeneity is limited by the lack of a suitable immunological stimulus to induce histamine release from its mast cells.

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