A method has been developed for the enzymic dissociation of rat skin into its component cells. The resulting suspensions contained 3–5% mast cells. The latter were intact as judged by light microscopy and exhibited a low spontaneous release of histamine. Cells obtained from actively sensitized animals released histamine on challenge with specific antigen. The process was rapid, being essentially complete within 1 min, and was both calcium- and temperature-dependent. The cells also responded to antirat IgE and to calcium ionophores but showed a selective, time-dependent reactivity toward defined chemical histamine liberators. On the basis of these results the properties of the cutaneous mast cell are compared with those previously reported for mastocytes from other sources and discussed in terms of the general heterogeneity of this cell population.

The release of chemical mediators from cutaneous mast cells has been implicated in many diseases of the skin, including eczema, contact dermatitis, and several forms of urticaria [1]. However, little is known about the precise mechanisms of anaphylaxis in this tissue. Studies in vitro have been carried out on skin slices, either from the rat [2] or human [3], and the release process has been shown to be relatively rapid and inhibited by extremes of temperature. In addition, human cutaneous anaphylaxis is totally dependent on the presence of calcium ions [4,5] and may be inhibited by agents that raise intracellular levels of cyclic AMP [6]. However, such experiments present some problems of interpretation since it is not possible to control precisely the environment of the cells of interest. Factors such as diffusion, accessibility of reagents, nonspecific adsorption, and masking of receptor sites may all influence results. It was of interest, therefore, to develop a method for the preparation of single cell suspensions from rat skin and to determine some of the properties of the cells so obtained. Such a study might be hoped to provide a useful model of cutaneous allergy.

MATERIALS AND METHODS

Sprague-Dawley rats of either sex (150–250 g) were used throughout. The animals were killed by ether anesthesia followed by exsanguination. The abdominal skin was shaved and a section (usually ca. 4 × 3 cm, weighing ca. 1 g) was excised. The skin was dissection free of underlying fat, then washed in a modified Tyrode’s buffer having the composition (mM): NaCl 137, glucose 5.6, HEPES 10, KCl 2.7, Na-HPO₄ 0.4, CaCl₂ 1, MgCl₂ 1. The tissue was then chopped finely into fragments of ca. 1 mm², and incubated (4 h, 37°C) in a solution (ca. 25 ml per g of skin) of collagenase (Sigma Type I, 1 mg/ml) and hyaluronidase (Sigma Type I, 1 mg/ml) in Hanks’ balanced salt solution (Gibco Biocult) buffered with HEPES (10 mM, pH 7.2–7.4) and supplemented with fetal calf serum (Gibco Biocult, 20%). The incubation mixture was gently gassed throughout with a mixture of oxygen (95%) and carbon dioxide (5%). Following the 4-h period, the suspension was stirred with a magnetic follower (45 min, 37°C) and remaining tissue was then disrupted by expression through a syringe. The mixture was filtered through gauze and the cells recovered by centrifugation (5 min, 150 g, 4°C) and washed once in ice-cold Tyrode’s buffer. Finally, the cells were resuspended in the volume of buffer required for the experiment and passed through a poroous plastic filter (Vyon F, 0.75 mm, Porvair Ltd, Norfolk) to remove debris and clumps of cells.

The histamine-releasing properties of several agents were determined in this system as detailed previously [7]. Briefly, suspensions of cells (1 ml containing ca. 10⁶ mast cells) were allowed to equilibrate (5 min, 37°C unless otherwise indicated) in a metabolic shaker with gentle mechanical agitation and were then challenged by addition of the agent under study in a minimum volume. After incubation for the specified periods of time (10 min unless otherwise stated), release was terminated by the addition of ice-cold Tyrode’s solution (2 ml) and the cells were immediately separated from the supernatant by centrifugation (5 min, 150 g, 4°C). The cell pellets were resuspended in buffer (3 ml) and residual histamine was released by lysis with perchloric acid (to a final concentration of 0.4 M) followed by boiling (10 min). No additional histamine was released by sonication of the samples (5 min, 50 kHz) or by repeated (5 cycles) of freeze-thawing from liquid nitrogen. Histamine was measured spectrophotometrically, in both the cellular and supernatant fractions. Release is expressed as a percentage of the total histamine initially in the cells, corrected for the spontaneous release occurring in the absence of secretagogues. Values given as mean ± SEM for the number (n) of observations noted throughout this paper, each observation represents a separate experiment on a different preparation of mast cells. All statistical analyses are based on t-tests for related measures.

The specificity of the assay procedure was confirmed by determining the susceptibility of the apparent tissue histamine to degradation by diamine oxidase (histaminase) as described by Thrapp et al [5]. Samples were incubated with the enzyme (0.1 units/ml, HEPES-Tyrode’s buffer, pH 7.4) and residual histamine was determined as described. Parallel experiments with authentic histamine standards were also carried out. Further control experiments showed that none of the test reagents employed in the present study interfered with the assay under the conditions used.

In some experiments, rat peritoneal mast cells were obtained by direct lavage [7] and histamine release was determined as above. For comparative purposes, samples of these cells were treated with collagenase and hyaluronidase under conditions identical to those used in the isolation of the cutaneous cells. The subsequent effect of this procedure on their reactivity was then examined.

For experiments involving an anaphylactic stimulus, rats were sensitized to the nematode *Nippostrongylus brasiliensis* as detailed previously [8]. Mast cells from these animals were challenged with specific secretory allergen quantitated in terms of worm equivalents (WE) as previously described [8].

Slides for microscopic examination were prepared by the method of Mota and Vugman [9]. These slides were then scanned systematically and the mean (average of maximum and minimum) diameters of 400 mast cells (identified by their pronounced metachromatic staining) were measured using a graduated eye piece (15× GR, Wild Heerbrug).

The number of mast cells on a given slide was also determined as a percentage of the nucleated cells, and a count of the total cells in the...
initial suspension was made using an improved Neubauer hemocytometer. Comparison of the histamine content of the suspension with standards allowed determination of the histamine content per mast cell. Analysis of the histamine content of undissociated skin permitted calculation of the recovery of the amine through the dispersion process.

ATP, chlorotetracycline hydrochloride, diamine oxidase (histaminase), poly-L-lysine hydrobromide (M, 65,000), concanavalin A (all from Sigma), sheep antirat IgE serum (Miles Laboratories Ltd., Slough), ionophore A23187 (donated by the Lilly Research Centre, Windsleham, Surrey), ionomycin (donated by the Squibb Institute, Princeton, New Jersey), Br-X537A (donated by Roche Products Ltd., Welwyn Garden City, Herts), compound 48/80 (donated by the Wellcome Research Laboratories, Beckenham, Kent), peptide 401 (generously provided by Dr. A. J. Garman, University College London), dextran (M, 110,000, Fisons), phosphatidylserine (PS) (Lipid Products, Redhill, Surrey), and trypan blue dye (Gibco Biscuit) were obtained from the sources indicated.

RESULTS

Some properties of the cells obtained as described are given in Table I. The histamine content of the undispersed skin was 21.0 ± 2.8 ng/mg wet weight (n = 4). The identity of the apparent histamine present in the isolated cell preparations was confirmed by incubation with histaminase. Treatment with the enzyme resulted in an essentially complete degradation of both the cutaneous amine and authentic histamine standards (Table II). The free mast cells were intact as judged by light microscopy and the nucleated cells exhibited a viability of greater than 95% as judged by exclusion of trypan blue dye (0.1%). The size distribution of the mast cells is shown in Fig 1.

Cutaneous mast cells from rats actively sensitized to N. brasilienis released histamine in a dose-dependent fashion on challenge with specific antigen (Fig 2). The release was not potentiated by PS but the lipid (15 μg/ml) increased the antigen-induced secretion of histamine from rat peritoneal cells from 37.5 ± 3.2% to 67.3 ± 2.2% (n = 4). The anaphylactic release from the skin cells was rapid, with a half-life of approximately 20 s and being essentially complete within 1 min (Fig 3). Similar kinetics have been previously reported for peritoneal cells [8]. The process was temperature-dependent, being maximal at 37°C, reduced at 25°C, and abolished at 4°C or 45°C (Table III). In contrast, the spontaneous release increased over this temperature range (Table III, footnote). The release was also calcium-dependent (Fig 4). Secretion was optimal at a calcium concentration of 1 mM and was suppressed at higher and lower values. Interestingly, brief pretreatment (5 min) with the chelating agent EDTA (0.1 mM) increased the release in a simple calcium-free medium from 6.5 ± 1.7% to 14.9 ± 1.2% (n = 4, p < 0.05).

The cells also released histamine on challenge with antirat IgE and the lectin concanavalin A. The time-course of secretion appeared to be slower than with antigen and the release continued to increase over a 60-min period (Table IV). Similar results were observed with high concentrations (1 mM) of ATP and moderate, but more rapid, releases of histamine were produced by the polysaccharide dextran. The cutaneous cells were weakly responsive to low concentrations (<10 μg/ml) of the basic secretagogues compound 48/80, peptide 401, and polylysine, but higher concentrations induced a release which again increased progressively with time.

<table>
<thead>
<tr>
<th>Table I. Some properties of cell suspensions obtained from rat skin</th>
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<tr>
<td>Spontaneous histamine release (% total)</td>
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<tr>
<td>Mast cells (% total nucleated cells)</td>
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<tr>
<td>Mast cells recovered per g skin (10⁷)</td>
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<tr>
<td>Histamine content per mast cell (pg)</td>
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<td>Histamine recovery (% total)</td>
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Values are means ± SEM for the number of different experiments shown in parentheses.

<table>
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<th>Table II. Validation of the spectrophotometric assay procedure by enzymatic degradation of apparent skin histamine</th>
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<tr>
<td>Sample</td>
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<tr>
<td></td>
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<tr>
<td>Buffer control</td>
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<tr>
<td>Authentic histamine</td>
</tr>
<tr>
<td>Skin sample I</td>
</tr>
<tr>
<td>Skin sample II</td>
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Samples were incubated (60 min, 37°C, pH 2.4) with freshly prepared histaminase (0.1 units/ml), heat-inactivated (100°C, 10 min) histaminase, or buffer alone. Histamine was determined spectrophotometrically as described in the text. Values are means ± SEM for triplicate histamine determinations. The limit of detection in these experiments (5 ng/ml), in which relatively large amounts of histamine were employed to test fully the specificity of the procedure, does not reflect the maximum sensitivity of the method (see text). Skin samples were derived from boiled suspensions of isolated cutaneous cells.
Fig. 3. Kinetics of histamine release from rat skin mast cells challenged with specific allergen (20 WE/ml). Values are the means from 4 different experiments and vertical bars denote SEM. The spontaneous release did not increase significantly over the time period of the experiment and was 9.9 ± 1.9% (0 s), 10.8 ± 2.0% (600 s), and 10.3 ± 2.7% (1800 s).

Table III. Effect of temperature on allergen-induced histamine release from rat skin mast cells

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Histamine release (%)</th>
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<tr>
<td>4</td>
<td>1.3 ± 0.6*</td>
</tr>
<tr>
<td>25</td>
<td>10.3 ± 2.0</td>
</tr>
<tr>
<td>37</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>45</td>
<td>2.3 ± 0.8*</td>
</tr>
</tbody>
</table>

Values are means ± SEM for 4 different experiments. Secretion was assessed after a 10-min period of incubation following challenge of sensitized mast cells with specific allergen (20 WE/ml). All values are corrected for the appropriate spontaneous release (4°C, 4.4 ± 1.2%; 25°C, 7.1 ± 1.6%; 37°C, 7.5 ± 1.8%; and 45°C, 15.1 ± 4.3%).* Denotes values of the spontaneous and corrected, induced releases of histamine which are significantly different (p < 0.05) from those at 37°C.

The cutaneous cells were moderately responsive to the calcium ionophores A23187, ionomycin, and chlortetracycline, releasing a maximum of approximately 30% of the total histamine in each case (Table V). In contrast, large releases of histamine were evoked by Br-X573A. The kinetics of the ionophore-induced releases were less systematically investigated than those of the receptor-mediated ligands but the secretion produced by A23187 (1 μM: 10 min, 17.1 ± 2.6%; 30 min, 21.9 ± 4.5%; 60 min, 28.2 ± 6.1%; n = 3) and chlortetracycline (1000 μM: 10 min, 28.9 ± 4.9%; 30 min, 43.2 ± 1.9%; 60 min, 42.7 ± 4.9%; n = 4) again increased with time over a 60-min period.

In general, the cutaneous cells were markedly less responsive to all the histamine liberators tested than were peritoneal cells from the same animals. The latter released larger amounts of histamine and at lower concentrations of secretagogue. Moreover, treatment of the peritoneal cells with the proteases used in the tissue dispersion procedure did not significantly alter their reactivity (Table VI).

DISCUSSION

The present study provides a simple method for the preparation of mixed suspensions containing viable and functionally responsive mast cells from rat skin. The mast cells were obtained in acceptable yield, comprised 3–5% of the total nucleated population, and exhibited a low spontaneous release of histamine. The isolation procedure is unlikely to lead to any alteration in functional properties as extensive control experiments showed that identical enzyme treatments had a negligible effect on the responsiveness or histamine content of rat peritoneal mast cells from the same animals. These results are in accord with our previous findings and those of others [10,11].

The histamine content per mast cell (4–5 pg) was less than that found for rat peritoneal cells (ca. 20 pg) but comparable to that of other tissue mast cells [12–14]. The amount of histamine found in intact skin (ca. 20 ng/mg) was similar to that reported by others [2].
Previous finely observed the same phenomenon with rat medium is normally attributed to the mobilization of intracellular stores of the cation and we have argued that this mobilization may be facilitated by destabilization of the membrane following limited treatment with chelating agents [8]. Conversely, supramaximal concentrations of calcium may increase the rigidity of the membrane and prevent fluxes of the cation [8]. Such conditions thereby inhibit mediator release. However, the cutaneous cells (Fig 4) appeared to be somewhat less sensitive to this effect than were their peritoneal counterparts where calcium concentrations of 5 mM or greater produce a very marked inhibition of release [8]. These findings may indicate subtle differences in the membrane composition or cation-binding properties of the two cell types.

The rat skin cells were moderately reactive toward the polysaccharide dextran but responded to ATP and to the basic secretagogues compound 48/80, polylysine, and peptide 401 only at relatively high concentrations. Under these conditions, release may be due to cell damage. Purification of the mast cells and measurement of the loss of characteristic cytosolic markers would be required unambiguously to resolve this point. Notwithstanding the marked differences in the kinetics of the release process in the two cell types (see below), the cutaneous cells were in general much less reactive than peritoneal cells from the same animals. The latter released much larger amounts of histamine and at lower concentrations of secretagogue. These differences cannot be attributed to the tissue dispersion process since treatment of the peritoneal cells in the same way did not significantly alter their reactivity. The effects of the named secretagogues on the peritoneal cells are believed to be mediated through specific membrane receptors [18–20] and these binding sites may be absent or present in modified or uncoupled form in the cutaneous cells.

Antigen-induced release from the skin cells was rapid, being essentially complete within 1 min. However, the secretion induced by anti-IgE, concanavalin A, compound 48/80, peptide 401, polylysine, and ATP was much slower and, in most cases, was apparently still ongoing after a period of 60 min. This is in sharp contrast to the rat peritoneal cell in which secretion evoked by most ligands is usually complete within minutes or even seconds [21]. However, even in this cell type [21], as well as in the human basophil leucocyte [22] and now the rat cutaneous mast cell, the release produced by antigen is significantly more rapid than that evoked by other IgE-directed ligands. The reasons for this distinction are not known but it has been suggested that there may be subtle mechanistic differences in the biochemical events following the various stimuli [22].

The cutaneous cells were responsive to the action of the ionophores A23187, ionomycin, and chlortetracycline although the magnitude of release was again less than that from rat peritoneal cells. In general, tissue mast cells are hyporesponsive to ionophores [12–14,23,24]. These agents are thought to trigger secretion by directly transporting calcium ions across the cell membrane [25,26], suggesting that the tissue cells might have more efficient mechanisms for controlling their intracellular calcium concentrations than do their peritoneal counterparts. Alternatively, the differences may reflect variations in the membrane lipid composition, favoring dissolution of the ionophore in one cell type above the other. In contrast, the ionophore Br-X537A produced large releases of histamine from the skin cells. This agent may act by transporting monovalent ions, or even histamine itself [25]. Its activity in the present system, relative to the selective calcium ionophores, presumably reflects its nonspecific nature.

In summary, the present study provides a rapid method for the production of suspensions of single cells from rat skin. It also further emphasizes the heterogeneity that exists between mast cells from different sites [27,28]. This heterogeneity extends to the susceptibility of the release process to modulation by antiallergic drugs, which might account for the conflicting data on the efficacy in allergic skin diseases of compounds.
developed using the rat peritoneal mast cell model [1]. The availability of isolated cutaneous mast cells may then facilitate the further study of the role of this cell in skin allergy and provide a convenient test system for the screening of compounds directed against this condition.

REFERENCES