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Heterogeneity of Human Mast Cells and Basophils in Response to Muscle Relaxants

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The authors studied the effects of increasing concentrations ($10^{-5} - 10^{-3}$ M) of four muscle relaxants (succinylcholine, *d*-tubocurarine, vecuronium, and atracurium) on histamine release from peripheral blood basophils and mast cells isolated from human lung parenchyma, skin tissues, and heart fragments. Basophil granulocytes released less than 5% of their histamine content when incubated with any one of the muscle relaxants tested. In contrast, mast cells showed a significant heterogeneity in response to different muscle relaxants. Succinylcholine did not induce histamine release from any type of mast cell, and only high concentrations of *d*-tubocurarine (10^{-3} M) caused histamine release from skin and lung mast cells. Vecuronium concentration-dependently induced histamine release from skin and lung—but not from heart mast cells—to a maximum of 7.2 ± 2.1% and 4.9 ± 1.4%, respectively. Atracurium concentration-dependently caused significant histamine release from skin and lung mast cells to a maximum of 46.2 ± 15.1% and 30.6 ± 6.0%, respectively. Atracurium ($5 \times 10^{-5} - 2 \times 10^{-4}$ M) also induced histamine release from heart mast cells. The histamine release process from both lung and skin mast cells caused by atracurium and vecuronium was extremely rapid ($t_{1/2}$ = < 1 min). The releasing activity of atracurium and vecuronium on lung and skin mast cells was not reduced, and not abolished, by lowering the temperature of the incubation buffer to 22° C and 4° C. Extracellular calcium did not affect the capacity of atracurium and vecuronium to induce histamine release from lung and skin mast cells. The releasing activity of atracurium on lung and skin mast cells was not reduced by metabolic impairment caused by preincubation with 2-deoxy-D-glucose plus antimycin A. Furthermore, this release process was not reduced by preincubation of lung and skin mast cells at 47° C for 20 min. These results confirm that there are functional differences between human basophils and mast cells and among mast cells isolated from different anatomic sites in response to the four muscle relaxants tested. (Key words: Histamine. Histamine-containing cells: basophils; mast cells. Muscle relaxants: atracurium; succinylcholine; *d*-tubocurarine; vecuronium.)

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MUSCLE RELAXANTS are the drugs most frequently implicated in anaphylactoid reactions occurring during general anesthesia.[1] Such reactions have been attributed to histamine release from human mast cells or basophils.[2,3] This is supported by findings that muscle relaxants might cause histamine release *in vitro*[4] and that histamine administration induces most of the metabolic and hemodynamic alterations observed during allergic reactions.[5–7]

The mechanism of basophil and mast cell activation by muscle relaxants is complex. In some cases, the drugs induce specific IgE synthesis and the release of mediators is the consequence of the interaction of drugs with specific IgE bound on basophils and mast cells.[2,8,9] Alternatively, muscle relaxants might induce histamine release by acting directly on human basophils and mast cells.[3,4,10,11]

Preliminary observations indicate that human basophils and mast cells isolated from different anatomic sites vary in their histamine-releasing capacity when challenged with several drugs. For example, *d*-tubocurarine induces histamine release from skin mast cells,[4] but not from basophils.[10] In addition, morphine causes histamine release from skin mast cells but not from lung and heart tissues.[12] The functional heterogeneity of human basophils and mast cells is of great practical importance, and, recently, techniques have become available to isolate mast cells from human lung parenchyma,[13] skin tissue,[14] and human heart fragments.[15] Increasing evidence suggests that mast cells from different tissues, or even from different anatomic sites within the same tissue, may vary significantly as to their morphologic, biochemical, and functional responses.[13] Therefore, it seems of interest to evaluate the effect of four muscle relaxants commonly used in clinical practice (succinylcholine, *d*-tubocurarine, vecuronium, and atracurium) with regard to their property to induce histamine release from human basophils and mast cells isolated from human skin tissue, lung parenchyma, and heart fragments.

Materials and Methods

REAGENTS

The following were purchased: piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), elastase type I, hyaluronidase, chymopapain, collagenase, 2-deoxy-D-glucose, and

antimycin A (Sigma Chemical Company, St. Louis, MO); Hanks' balanced salt solution (HBSS) and fetal calf serum (FCS) (Gibco, Grand Island, NY); DNase, pronase, and compound A23187 (Calbiochem, La Jolla, CA); and RPMI 1640 with 25 mM Hepes buffer and Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland). The following were donated: atracurium besylate, *d*-tubocurarine chloride, and succinylcholine chloride (Burroughs Wellcome, Pomezia, Italy); and vecuronium bromide (Organon Teknika, Rome, Italy). Rabbit IgG anti-human IgE is a generous gift of Drs. T. and K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA).

Histamine Release from Human Basophils

After informed consent was obtained from all subjects, approximately 50 ml blood was drawn into a final concentration of 0.008 M EDTA and 1.1% dextran 70. The erythrocytes were allowed to settle for 90 min at 22° C, and leukocyte-rich plasma was removed. The cells were separated by centrifugation at 180 × *g* for 8 min; washed twice in 23 mM PIPES (*p*H 7.35), 110 mM sodium chloride (NaCl), and 5 mM potassium chloride (KCl) (P); and resuspended in P plus 2.0 mM calcium chloride ($CaCl_2$) (PC); 0.4-ml aliquots of the cell suspension were placed in Falcon 12 × 75-mm polyethylene tubes and warmed to 37° C. All drugs were in powder form and were diluted immediately before each experiment. Each was diluted to final concentrations in the appropriate buffer and added to tubes in 0.2-ml aliquots; incubation was continued at 37° C for 45 min. After centrifugation, the cell-free supernatant was assayed for histamine with an automated fluorometric technique.[16] The net percentage of release was calculated by subtracting the histamine released spontaneously by the unstimulated aliquots from the total histamine release by cell aliquots lysed with 2% perchloric acid.[17] Mean spontaneous release of histamine in PC was 3.8% ± 0.8% (mean ± standard error of the mean [SEM]) of the available histamine content. The difference between replicate histamine measurements was less than 10%.

Isolation of Human Skin Mast Cells

Skin was obtained from patients undergoing either mastectomy for breast cancer or elective cosmetic surgery. Anesthesia in these patients was provided with the following drugs: thiopental, pancuronium, atropine, and fentanyl (premedication); and enflurane or isoflurane (anesthesia). Tissue was placed immediately in Eagle's MEM at 4° C and used within 1 h. The skin was separated from the subcutaneous fat by blunt dissection. The fragments were washed twice in calcium- and magnesium-free HBSS (CMF-HBSS) at 22° C and incubated in a solution of CMF-HBSS containing 20 mg collagenase/gram wet weight of tissue, 5 mg hyaluronidase/gram wet weight of tissue, and 1,000 U/ml DNase for 3 h at 37° C under constant stirring. The isolated cells were separated from the partially digested tissue fragments by filtration through Nytex® cloth (150-μm pore size) and reserved. The remaining tissue was digested a second time with a fresh batch of enzymes for an additional 2 h at 37° C. The cells were separated from the tissue fragments by filtration through Nytex® cloth, washed twice, combined with the cells from the first digestion, and washed again in CMF-HBSS. Yields obtained with this technique ranged between 1×10^6 and 5×10^6 mast cells, and purities ranged between 1% and 4%.

Isolation of Human Lung Mast Cells

Human lung tissue was obtained from patients undergoing thoracotomy and lung resection—in most cases for lung cancer. In these patients, anesthesia was provided with the following drugs: droperidol plus fentanyl and atropine (premedication); and droperidol plus fentanyl, thiopental, succinylcholine, and pancuronium (anesthesia). Macroscopically normal lung tissue was dissected free from pleura, bronchi, and blood vessels; minced into 5- to 10-mm fragments; and dispersed into single-cell suspension as previously described.[13] Yields obtained with this technique ranged between 3×10^6 and 8×10^6 mast cells, and purities ranged between 1% and 8%. Mast cells and basophils were stained with alcian blue and counted in a Spiers-Levy eosinophil counter.

Histamine Release from Human Skin and Lung Mast Cells

Next, 0.4-ml aliquots of the cell suspension, containing approximately 3×10^4 mast cells per tube, were placed in Falcon 12 × 75-mm polyethylene tubes and warmed to 30° C (skin mast cells) or 37° C (lung mast cells); 0.2 ml of each releasing stimulus was added, and incubation was continued at 30° C (skin mast cells) or 37° C (lung mast cells) for 30 min. Experiments with mast cells obtained from the skin of adult donors were performed at 30° C because adult skin mast cells release better at this temperature than at 37° C.[18] The rest of the procedure and the calculation of the percentage of histamine release were identical to the methods described above for human basophils. Mean spontaneous release was 9.2% ± 1.5% of the available histamine in human lung mast cell preparations and 6.3% ± 1.0% in human skin mast cell preparations.

Histamine Release from Human Heart Fragments

Human cardiac mast cell studies were performed on specimens of human right atrial appendages obtained from patients undergoing cardiac surgery for valvular correction or substitution, and aortocoronary vein graft.[15] Anesthesia in these patients was provided with the following drugs: thiopental or diazepam, morphine or fentanyl, pancuronium (premedication), and nitrous oxide (anesthesia). None of the patients had taken dipyridamole, aspirin, or any antiinflammatory medication for at least 7 days before the operation.

At the time of surgery, a fragment of atrial tissue removed from the atriotomy site immediately was placed in a polyethylene tube containing 10 ml calcium- and magnesium-free Tyrode's buffer (NaCl 8.0 g/l, KCl 0.2 g/l, $NaH_2PO_4H_2O$ 0.05 g/l, dextrose 1.0 g/l) at 4° C. The sample immediately was taken to the laboratory and processed within 90 min after removal. Only macroscopically normal areas of heart tissue were studied, and these areas meticulously were dissected free of adipose tissue. Cardiac tissue was minced into approximately 1-mm pieces with a tissue chopper equipped with a razor blade. The plate was rotated 90 degrees, and the chopping was repeated. The chopped tissue was washed with 5 ml ice-cold calcium- and magnesium-free Tyrode's buffer (three times) to remove contaminating peripheral blood cells.

Microscopic examination of thin sections of heart fragments demonstrated the absence of peripheral blood cells. Fragments of cardiac tissues randomly were placed in tubes containing either 1 or 2 ml Tyrode's buffer and incubated at 37° C in the presence of drugs or buffer. One sample was removed from each tube at the end of incubation. Each sample was centrifuged (1,000 × *g*, 3 min, 22° C) and frozen (−20° C) for the subsequent determination of histamine content. At the end of the experiments, the wet weight of the heart fragments in each tube was determined after the tissue was dried partially by blotting on filter paper. Residual tissue histamine content was determined after tissue was boiled in 2% perchloric acid for 10 min. The cytoplasmic enzyme, lactate dehydrogenase (LDH), was determined as previously described[19] and used as an indicator of cell viability. The drugs used did not induce release of LDH from cardiac tissue.

Histamine Assay

Histamine concentrations in supernatants from lung, skin, and cardiac mast cell preparations and from human basophils were measured with the automated fluorometric method of Siraganian.[16] The presence of histamine in the samples was confirmed by degradation with porcine histaminase, as described previously.[12] The percentage of histamine release from unstimulated cells (spontaneous release) was subtracted from the percentage of histamine release from stimulated cells to obtain the net stimulated histamine release.[17] Cell aliquots lysed with 2% perchloric acid were measured for total histamine content. At concentrations of 10^{-4} M and greater, atracurium fluoresced; therefore, for each atracurium concentration used, the peak height for each atracurium concentration at 0 ng/ml histamine was used as the background level and subtracted from the subsequent experimental data. Similar results were obtained when the percentage of histamine release was calculated by evaluating the residual histamine content of cell pellets exposed to various concentrations of atracurium.

Statistical Analysis

The results were expressed as the mean ± SEM. A Kruskal-Wallis test was used for comparisons between the groups. This test reduces to the Mann-Whitney (Wilcoxon) test when there are only two groups. $P < 0.05$ was considered statistically significant.

Results

In a first series of experiments, we evaluated the effects of increasing concentrations (10^{-5} to 10^{-3} M) of succinylcholine, *d*-tubocurarine, vecuronium, and atracurium on histamine release from peripheral blood basophils. Succinylcholine and *d*-tubocurarine did not induce histamine release from basophils obtained from 8 and 11 donors, respectively. The highest concentration (10^{-3} M) of vecuronium and atracurium induced more than 5% of histamine release from basophils in 1 of 9 and 5 of 12 donors, respectively (data not shown).

We next determined the effects of the same concentrations of the drugs on mast cells isolated from different anatomic sites. In eight experiments performed with skin mast cells, succinylcholine did not induce histamine release, whereas in seven experiments *d*-tubocurarine induced a maximum of 3.1% ± 1.3% of histamine release at the highest concentration used (10^{-3} M). Vecuronium concentration-dependently induced histamine release from 10 skin mast cell preparations up to a maximum of 7.2% ± 2.1% at 10^{-3} M ($P < 0.01$ when compared with spontaneous release). In nine experiments, atracurium caused histamine release that reached 46.2% ± 15.2% at the highest concentration used (10^{-3} M) ($P < 0.01$ when compared with spontaneous release) (fig. 1), which was significantly different from that obtained with the same concentration of vecuronium ($P < 0.05$), *d*-tubocurarine ($P < 0.005$), and succinylcholine ($P < 0.001$). It is important to realize that the maximum percentage of histamine release caused by 10^{-3} M atracurium in these experiments ranged between 2.7% and 100%. To assess the

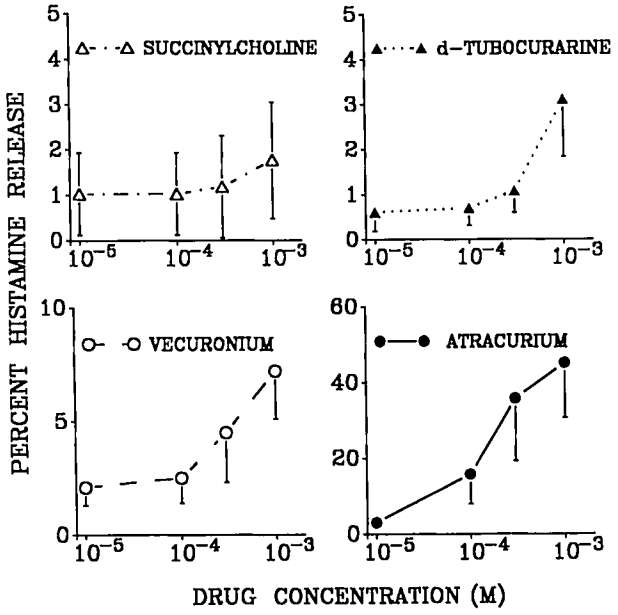

FIG. 1. Effect of increasing concentrations of succinylcholine, *d*-tubocurarine, vecuronium, and atracurium on histamine release from mast cells isolated from skin tissues incubated 45 min at 30° C. The results are the mean ± SEM. The bars corresponding to SEM are not shown when they are graphically too small.

ability of the skin mast cells to respond to IgE- and non-IgE-mediated stimuli, in each experiment cells also were challenged with an optimal concentration of anti-IgE (3 μg/ml) or Ca^{2+} ionophore A23187 (0.3 μg/ml). Anti-IgE and A23187 caused 18.1% ± 3.6% and 35.2% ± 6.0% of histamine release, respectively.

The effects of various concentrations of muscle relaxants on the release of histamine from mast cells isolated from lung parenchyma are shown in figure 2. In 8 experiments succinylcholine did not induce histamine release, whereas in 11 experiments the highest concentration of *d*-tubocurarine induced 5.9% ± 1.5% of histamine release ($P < 0.005$ when compared with spontaneous release). The highest concentration of vecuronium used (10^{-3} M) released 4.9% ± 1.4% of the histamine content of 9 lung mast cell preparations ($P < 0.05$ when compared with spontaneous release). Finally, atracurium concentrations greater than 10^{-5} M concentration-dependently induced histamine release from mast cells isolated from lung parenchyma. In 16 experiments, the maximum percentage of histamine release caused by 10^{-3} M atracurium ranged from 4 to 71%. The release of histamine caused by atracurium at this concentration (30.6% ± 6.1%, $P < 0.0005$ when compared with spontaneous release) was significantly different from that obtained with the same concentration of vecuronium ($P < 0.05$), *d*-tubocurarine ($P < 0.005$), and succinylcholine ($P < 0.005$). In contrast, the histamine release caused by vecuronium (10^{-3} M) was not statistically different from that caused by the same concentration of *d*-tubocurarine and succinylcholine.

As mentioned above, the histamine-releasing activity of atracurium in human skin and lung mast cells varies greatly from person to person. Figure 3 shows the individual dose-response curves obtained with various concentrations of atracurium (10^{-3} – 10^{-5} M) in mast cells isolated from skin tissue (n = 9) and lung parenchyma (n = 14).

We also measured the effect of these drugs on the release of histamine from mast cells from human heart, using a recently developed system.[15] We found that succinylcholine, *d*-tubocurarine, and vecuronium did not induce histamine release from human heart fragments *in vitro* (data not shown). In contrast, in 15 experiments, increasing concentrations of atracurium (10^{-5} to 2×10^{-4} M) concentration-dependently induced the release of histamine from human heart mast cells ($P < 0.001$ for histamine release obtained at 2×10^{-4} M when compared with spontaneous release) (fig. 4).

We next evaluated the mechanism of histamine release from skin and lung mast cells that was caused by atracurium and vecuronium. In a first group of three experiments, we evaluated the kinetics of histamine release from both lung and skin mast cells caused by optimal concentrations of atracurium or vecuronium. Figure 5*A* shows the results of three experiments in which we evaluated the effect of atracurium on lung mast cells. The release

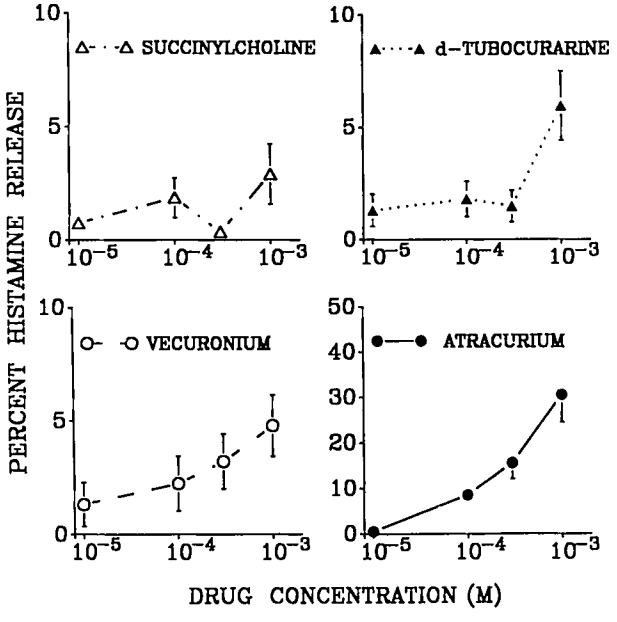

FIG. 2. Effect of increasing concentrations of succinylcholine, *d*-tubocurarine, vecuronium, and atracurium on histamine release from mast cells isolated from lung parenchyma incubated 45 min at 37° C. The results are the mean ± SEM. The bars corresponding to SEM are not shown when they are graphically too small.

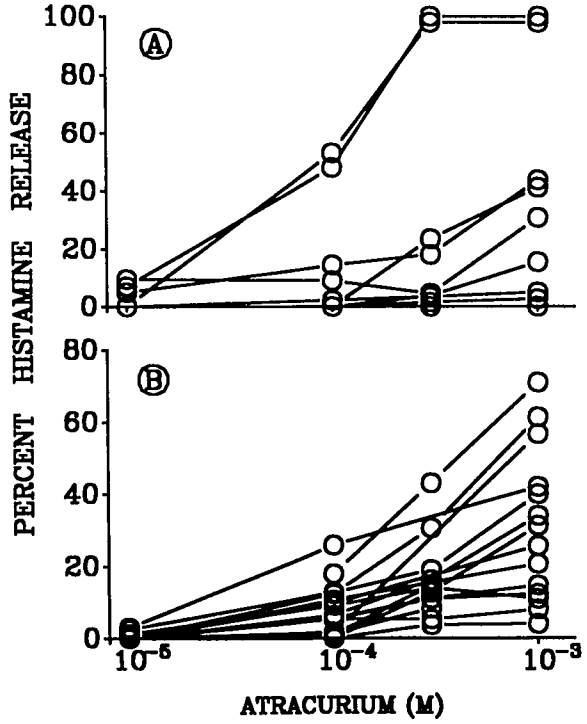

FIG. 3. Effect of increasing concentrations of atracurium on histamine release from mast cells isolated from skin tissue (*A*) and lung parenchyma (*B*) incubated 45 min at 30° C (skin mast cells) or 37° C (lung mast cells). Each line corresponds to the results obtained in an individual experiment.

process caused by atracurium (3×10^{-4} M) was fast, the $t_{1/2}$ being less than 1 min. Similar kinetics were obtained with vecuronium (10^{-3} M) (fig. 5*B*). Comparable time courses of histamine release from skin mast cells were obtained with atracurium or vecuronium (data not shown).

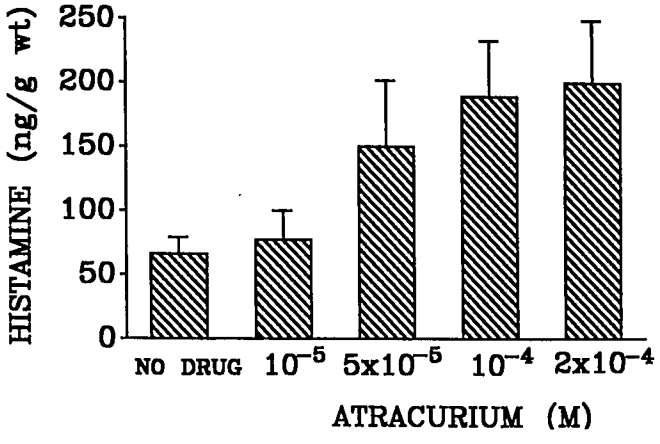

FIG. 4. Effect of increasing concentrations of atracurium on histamine release from human heart fragments incubated 45 min at 37° C. The results are the mean ± SEM obtained in 15 experiments.

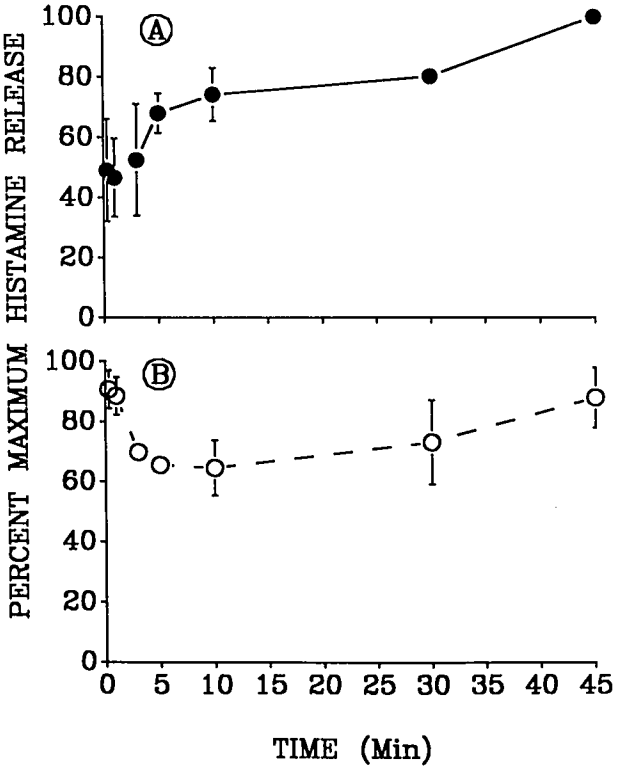

FIG. 5. Kinetics of histamine release induced by atracurium (3×10^{-4} M) (*A*), and vecuronium (10^{-3} M) (*B*) from human lung mast cells. Values are the means ± SEM of the percent maximum histamine release obtained in three experiments. The results are the mean ± SEM. The bars corresponding to SEM are not shown when they are graphically too small.

In six experiments, we evaluated the releasing activity of increasing concentrations of atracurium on mast cells isolated from lung parenchyma at 37° C, 22° C, and 4° C. As shown in table 1, the releasing activity of atracurium was decreased, but not abolished, by lowering the temperature of the incubation buffer from 37° C to 22° C or 4° C. Similar results were obtained in three experiments with mast cells isolated from human skin tissue (table 1). Also, similar results were obtained in a series of four experiments to evaluate the effect of the temperature of the incubation buffer on vecuronium's histamine-releasing activity in lung and skin mast cells (data not shown).

The effect of the presence of extracellular Ca^{2+} (1 mM) on the histamine-releasing activity of atracurium in mast cells isolated from lung parenchyma and skin tissues is shown in figure 6. In both systems, the releasing activity of atracurium was unaffected by the presence or absence of extracellular Ca^{2+}. The release from lung and skin mast cells that was caused by atracurium and vecuronium was not influenced by a wide range of extracellular Ca^{2+} concentrations (0.1–3 mM) (data not shown).

TABLE 1. Percent Histamine Release: Temperature Dependence of Effect of Atracurium

Temperature (°C)	Atracurium (M)				
	3×10^{-6}	10^{-5}	10^{-4}	3×10^{-4}	10^{-3}
Human lung mast cells*					
37	0.3 ± 0.2	0.7 ± 0.4	5.5 ± 2.0	11.1 ± 2.5	21.8 ± 8.4
22	0.0 ± 0.0	1.0 ± 0.6	2.6 ± 1.0	5.1 ± 1.7	7.8 ± 2.4
4	0.1 ± 0.9	0.9 ± 0.2	2.6 ± 0.7	4.8 ± 1.7	3.7 ± 2.2
Human skin mast cells†					
30		0.0 ± 0.0	2.2 ± 1.2	2.0 ± 0.7	7.8 ± 4.1
4		0.0 ± 0.0	2.3 ± 2.3	1.7 ± 1.2	4.5 ± 2.8

* Mean ± SEM obtained from six experiments.

† Mean ± SEM obtained from three experiments.

The biochemical events underlying the histamine release from human mast cells caused by anesthetics are unknown. We evaluated the effect of metabolic impairment on atracurium-induced histamine release from human lung and skin mast cells. Table 2 shows that a 20-min preincubation of human lung mast cells with 10 mM 2-deoxy-D-glucose and 1 μM antimycin A did not affect the histamine release caused by two concentrations of atracurium. In contrast, in the same experiments, preincubation with 2-deoxy-D-glucose plus antimycin A inhibited the release of histamine induced by the Ca^{2+} ionophore A23187 by more than 80%.[20] Similarly, in four experiments, preincubation of skin mast cells with 2-deoxy-D-glucose plus antimycin A significantly inhibited the releasing activity of the same concentrations of compound A23187 but did not affect the releasing activity caused by the same concentrations of atracurium (data not shown).

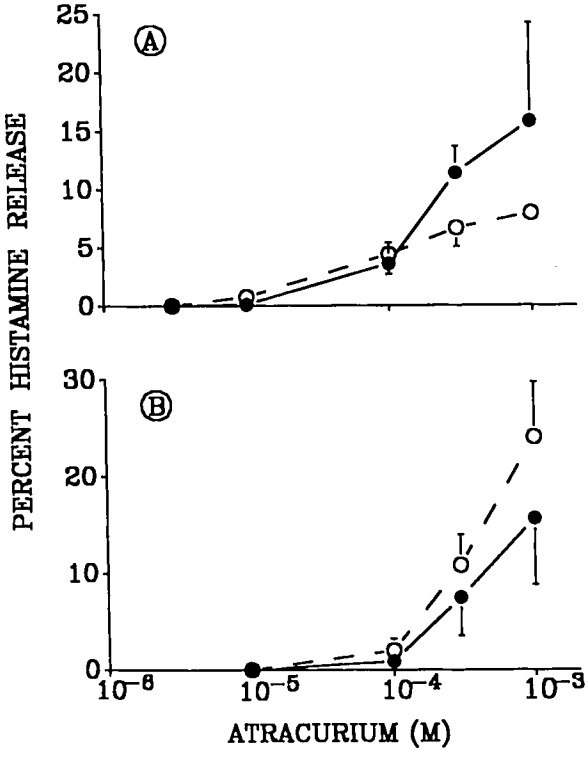

FIG. 6. Effects of various concentrations of atracurium on histamine release from human lung (*A*) or skin (*B*) mast cells incubated in the absence (open circles) or in the presence (filled circles) of extracellular Ca^{2+} (1 mM). Values are the mean ± SEM obtained in five experiments. The results are the mean ± SEM. The bars corresponding to SEM are not shown when they are graphically too small.

Heating cells to 47° C for 20 min denatures proteins and inactivates the cellular metabolic processes.[21] Table 3 shows that this treatment significantly suppressed the releasing activity of compound A23187 in lung mast cells, whereas it did not affect the releasing activity of two concentrations of atracurium. Similar results were obtained when skin mast cells were subjected to the same protocol.

Discussion

The pioneering studies by Hirshman *et al.* and Tharpe *et al.* clearly demonstrated that mast cells present in human skin tissue are exquisitely sensitive to such diverse drugs as atracurium, *d*-tubocurarine, and morphine.[4,12,22] Our results extend their observations by demonstrating the existence of two levels of heterogeneity: 1) with respect to the histamine-releasing activity of different muscle relaxants, and 2) with respect to the capacity of primary effector cells of allergic reactions in humans (*e.g.*, basophil granulocytes and mast cells isolated from different anatomic sites) to respond to each muscle relaxant.

Peripheral blood basophils are essentially unresponsive to a wide spectrum of concentrations of the muscle relaxants used in this study. Therefore, the *in vitro* model of basophil histamine release appears to be useful in detecting patients who have specific IgE against a common epitope present in several muscle relaxants.[9] Mast cells obtained from different human tissues showed a pattern of sensitivity to various muscle relaxants clearly different than that of basophils. Furthermore, mast cells isolated from different sites showed a remarkable degree of heterogeneity in response to a single muscle relaxant. In particular, succinylcholine did not activate mast cells isolated from

TABLE 2. Effect of Metabolic Impairment on Atracurium- and A23187-induced Histamine Release from Human Lung Mast Cells

	Number of Experiments	Net Histamine Release (%)		Inhibition of Histamine Release (%)
		Control	+2dDG/Antimycin A	
Atracurium (M)				
10^{-3}	6	21.6 ± 4.6	23.3 ± 3.9	0
3×10^{-4}	6	12.8 ± 4.2	13.2 ± 4.0	0
A23187 (μg/ml)				
1	6	55.0 ± 4.9	5.1 ± 1.6*	90.7
0.3	6	22.3 ± 5.4	2.9 ± 1.2†	86.9

Human lung mast cells were incubated 20 min at 37° C in the presence of buffer (control) or 2-deoxy-D-glucose (2dDG) 10 mM + antimycin A 1 μM. Then the cells were challenged with the indicated concentrations of atracurium or compound A23187, and release was allowed to proceed for 30 min at 37° C. Results are presented as the mean ± SEM obtained from six experiments.

* $P < 0.05$ when compared to control.

† $P < 0.05$ when compared to control.

lung parenchyma or skin tissue or present in cardiac fragments, whereas vecuronium and *d*-tubocurarine induced the release of a small percentage of histamine from skin mast cells.

These results appear to contrast with the findings reported by North *et al.*[4] However, the system used by North *et al.* to challenge skin preparations with muscle relaxants differed from our system. In fact, they used fragments of foreskin suspended in buffer, whereas we used enzymatically dispersed mast cells. As previously demonstrated,[18,23] enzymatic dispersion does not affect the ability of cells to respond to IgE- and non–IgE-mediated stimuli. In our experiments, anti-IgE and Ca^{2+} ionophore A23187 always were included as a positive control. Furthermore, they used infant foreskins, whereas we used mast cells from skin tissue obtained from adults. There is compelling evidence that the age of cell donors significantly influences the releasability of human basophils[17] and mast cells,[18,24] and *in vivo* studies have demonstrated that histamine release after administration of atracurium or vecuronium in children differs from that in adults.[25] Taken together, these observations emphasize that studies aimed at evaluating the *in vivo* or *in vitro* releasing capacity of muscle relaxants and general anesthetics in general should consider donor age.

The histamine-releasing capacity of atracurium has not been evaluated systematically in previous *in vitro* studies with human basophils and mast cells. However, several clinical observations[25,26] and an *in vivo* study in rabbits[27] suggest that histamine release occurs after atracurium administration. Our results demonstrate that atracurium concentration-dependently induced histamine release from the three types of mast cells used in this study. In particular, atracurium was a potent stimulus for histamine release from mast cells isolated from lung parenchyma and skin tissues. It is interesting that atracurium's histamine-releasing activity in human skin and lung mast cells varied remarkably between different preparations (see fig. 3). This *in vitro* observation might be related to the extreme variability in histamine release observed after the administration of atracurium *in vivo*.[25,26]

The biochemical pathways underlying the nonimmunologic histamine release from human mast cells induced by general anesthetics are essentially unknown. In this study we have attempted to shed some light on the biochemical characteristics of atracurium- and vecuronium-induced histamine release from human mast cells. Our results indicate that histamine release from skin and lung mast cells that was evoked by both muscle relaxants has a time course that is extremely rapid and certainly more

TABLE 3. Percent Histamine Release: Effect of Pretreatment at 47° C

	HLMC				HSMC			
	37° C		47° C		37° C		47° C	
	a	b	a	b	a	b	a	b
Atracurium (M)								
10^{-3}	16.6	16.5	33.6	16.0	6.8	24.9	7.1	18.0
3×10^{-4}	4.6		9.4		10.9		10.7	
A23187 (μg/ml)								
1	54.0	49.2	0	21.5	30.8	20.3	0	17.5
0.3	49.0	19.4	0	4.5	15.4	9.2	0	7.0

Results are presented as the mean of duplicate samples. a and b refers to separate experiments.

HLMC = human lung mast cells; HSMC = human skin mast cells.

rapid than that of the release process initiated by IgE cross-linking.[20,23]

Also, the role of extracellular calcium in histamine release induced by atracurium and vecuronium differs from its involvement in the actions of compound A23187 or anti-IgE.[14,21] In fact, the releasing activity of atracurium and vecuronium in lung and skin mast cells is Ca^{2+} independent. It has been demonstrated previously that the absence of extracellular calcium has little effect on the histamine release from human skin mast cells that is caused by morphine.[28] It remains to be evaluated whether this is a common biochemical characteristic of the histamine release caused by various drugs used during general anesthesia.

Another original finding of this study is the peculiar effect of the temperature of the incubation buffer on the releasing activity of atracurium and vecuronium. In fact, the releasing activity of atracurium and vecuronium in lung and skin mast cells is reduced but not blocked by decreasing the temperature of the incubation buffer to 22° C or 4° C. These observations raise the possibility that these drugs induce the release of histamine from mast cells through a biochemical mechanism that is, at least in part, temperature independent.

Two series of experiments also were conducted to investigate whether the histamine-releasing capacity of atracurium in human lung and skin mast cells is energy dependent. Inhibition of glycolytic and oxidative metabolism within mast cells with the combination of 2-deoxy-D-glucose plus antimycin A abolished the response of these cells to compound A23187. In contrast, the activity of atracurium was unaffected by metabolic impairment caused by this procedure. Additional evidence that atracurium induces histamine release independently from intact biochemical processes of the cells was provided by the experiments demonstrating that heating mast cells to 47° C for 20 min prevented the response to the Ca^{2+} ionophore while not inhibiting the histamine release induced by atracurium. These observations could suggest that atracurium, and probably other muscle relaxants, induces a leakage of histamine from certain types of mast cells but not from human basophils. Alternatively, atracurium and, presumably, vecuronium induce histamine release from human mast cells through the activation of biochemical pathways that are influenced, albeit slightly, by temperature, extracellular calcium, and glycolytic and oxidative metabolism. Additional studies are necessary to clarify the biochemical effects of atracurium and vecuronium on human mast cells.

The *in vitro* concentrations of drugs that we have used are higher than those usually found in the peripheral blood of patients undergoing general anesthesia performed with the four drugs tested.[29] However, it is possible that the local concentrations of these drugs during or immediately after intravenous infusion might be relevant, considering the variability in the releasing activity of cells from different donors and variations of pharmacokinetics in different clinical conditions.[29] In addition, succinylcholine,[9] *d*-tubocurarine,[3] vecuronium,[30–33] and atracurium[25,26] can produce allergic reactions and hemodynamic effects caused by release of histamine and other mediators from basophils and mast cells. Lastly, although our experimental procedures included extensive washing of lung parenchyma, heart fragments, skin tissues, and the isolated mast cells, we cannot exclude that drugs used in premedication and for anesthesia could be responsible—at least in part—for the differences found in the response to muscle relaxants between mast cells and basophils.

The observation that the histamine-releasing activity of various muscle relaxants varies with respect to the drug and the type of cell examined may have practical implications. We suggest that future studies of the histamine-releasing capacity of new drugs of this class of compounds or other general anesthetics should include the systematic evaluation of the capacity to induce the release of histamine from peripheral blood basophils and tissue mast cells isolated from lung parenchyma, skin, and cardiac tissues. Furthermore, we suggest that the evaluation of the mediator-releasing activity of general anesthetics should be evaluated on other mediators, such as peptide leukotrienes, synthesized by human basophils and mast cells[14,34] that exert profound hemodynamic effects in humans.[35] The latter observation is particularly important because previous work has demonstrated that prior administration of H_1 and H_2 histamine antagonists can significantly attenuate the effects of histamine release associated with morphine administration during cardiac surgery.[36] However, a recent study has demonstrated that the hemodynamic changes associated with *d*-tubocurarine administration are antagonized only partially by histamine antagonists.[37] This raises the possibility that muscle relaxants have direct cardiovascular effects independent of their ability to release histamine or other mediators.

Additional *in vitro* studies using the experimental models described here might be useful to extend current knowledge of the capacity of muscle relaxants and general anesthetics to release histamine and other chemical mediators from human basophils and mast cells.

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