

Human basophil/mast cell releasability.

XI. Heterogeneity of the effects of contrast media on mediator release

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Background: The activation of basophils and mast cells plays a role in the pathogenesis of anaphylactoid reactions occurring during the administration of iodinated radiocontrast media. **Methods:** We compared the effects of three contrast media (CM), Hexabrix (sodium and meglumine salts of ioxaglic acid), Telebrix (sodium and meglumine salts of ioxitalamic acid), and Optiray (ioversol) on the release of preformed (histamine and tryptase) and de novo synthesized (prostaglandin D₂ and leukotriene C₄) mediators from human basophils and mast cells isolated from lung, skin, and heart tissue. The commercial preparations were evaluated in parallel with the pure substances. Mannitol was used as a positive control inducing histamine release (HR) by hyperosmolar stimulation.

Results: Hexabrix (0.1 to 0.3 mol/L), Telebrix (0.1 to 0.5 mol/L), Optiray (0.2 to 0.5 mol/L), and the corresponding pure substances concentration-dependently induced HR from basophils. A positive correlation was found between CM osmolality and HR from basophils. Mast cells isolated from different anatomic sites responded differently to the three CM. Hexabrix and Optiray induced histamine and tryptase release from human lung mast cells, but not from human skin mast cells. No correlation was found between the osmolality of CM and HR from human lung mast cells. There was a significant correlation between the percent of histamine and tryptase release induced by CM from human lung mast cells. Hexabrix, Telebrix, and Optiray also induced histamine and tryptase release from human heart mast cells. None of the CM induced the de novo synthesis of leukotriene C₄ or prostaglandin D₂ from basophils or mast cells. The kinetics of HR caused by CM differed according to the drug used and the cell (basophils or human lung mast cells) examined. CM-induced HR from basophils and human lung mast cells was temperature-dependent, partially influenced by extracellular Ca²⁺ concentrations, and not modified by preincubation of basophils with IL-2 or IL-3.

Conclusions: These results provide evidence of the heterogeneity of the effects of CM on mediator release from human basophils and mast cells from different anatomic sites. They also suggest that hyperosmolality may be an important factor in the activation of basophils by CM, but less relevant for mast cells. CM induce only the release of preformed mediators. The measurement of plasma tryptase might be clinically useful for monitoring adverse reactions caused by CM. (*J ALLERGY CLIN IMMUNOL* 1996;97:838-50.)

Key words: Basophils, contrast media, histamine, leukotriene C₄, mast cells, prostaglandin D₂, tryptase

Iodinated radiocontrast media are invaluable tools that are widely used in several diagnostic procedures.¹ Most of the contrast media (CM)

currently used are ionic agents, derivatives of triiodinated benzoic acid, administered at an osmolality three to five times that of plasma.² Hyperosmolality is known to be responsible for some of the adverse effects caused by CM administration.¹⁻⁴ During the last decade new CM, characterized by a lower osmolality, have been developed.^{1,2} These novel preparations have a lower incidence of reactions compared with older hyperosmolar agents.^{1,3,5}

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Abbreviations used

Anti-IgE:	Goat IgG anti-human Fce
CM:	Contrast media
CMF-HBSS:	Ca ²⁺ - Mg ²⁺ -free Hank's balanced salt solution
EDTA:	Ethylenediaminetetraacetic acid
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
HHMCs:	Human heart mast cells
HLMCs:	Human lung mast cells
HR:	Histamine release
HSMCs:	Human skin mast cells
LTC ₄ :	Leukotriene C ₄
P buffer:	PIPES buffer, containing 25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl, pH 7.4
P ₂ CG:	P buffer containing 2.0 mmol/L CaCl ₂ and 1 gm/L D-glucose, pH 7.4
PGD ₂ :	Prostaglandin D ₂
PGMD:	P buffer containing 1 gm/L gelatin, 1 mmol/L MgCl, 20 mg/L DNase, pH 7.4
PIPES:	Piperazine-N, N'-bis (2-ethanesulfonic acid)

The overall incidence of adverse reactions to CM varies from 4.6% to 8.5%,² with fatalities occurring in 0.002% to 0.009% of all procedures.² Anaphylactoid reactions, ranging from urticaria, angioedema, pruritus, and dyspnea to hypotension or shock⁶ represent 20% to 30% of all reactions to CM.²

The pathogenesis of anaphylactoid reactions caused by CM is complex and remains unclear. Results from *in vivo*⁷⁻⁹ and *in vitro*¹⁰⁻¹² studies indicate that histamine release (HR) from basophils and tissue mast cells plays a major role in these reactions.

Previous studies have focused on the role of histamine in CM-induced anaphylactoid reactions, whereas a lack of knowledge exists about the effects of CM on other basophil/mast cell-derived mediators, such as tryptase, a marker of mast cell activation *in vivo*¹³ and eicosanoids (leukotriene C₄ [LTC₄] and prostaglandin D₂ [PGD₂]), which exert profound hemodynamic and bronchoconstrictor effects in human beings.^{14, 15} Furthermore, there is compelling evidence that human mast cells from different anatomic sites and basophils vary markedly in their functional responses to immunologic, nonimmunologic, and osmotic stimuli.¹⁶⁻¹⁹ Therefore this study was undertaken to systemati-

cally compare the capacity of a high osmolality CM (Telebrix: 1500 to 1600 mOsm/kg) with two CM with lower osmolality (Hexabrix: 580 mOsm/kg and Optiray: 702 mOsm/kg) to induce the release of preformed (histamine and tryptase) and *de novo* synthesized mediators (LTC₄ and PGD₂) from human basophils and mast cells isolated from lung, skin, and heart tissues. To evaluate the possible role of other components of the commercial preparations, they were evaluated in parallel with the corresponding pure substances: sodium and meglumine ioxithalamate (Telebrix), sodium and meglumine ioxaglate (Hexabrix), and ioversol (Optiray). Mannitol was used as a positive control that induced HR by hyperosmolar stimulation.¹⁹

METHODS

Reagents

The following were purchased: ethylenediaminetetraacetic acid (EDTA) disodium salt (J.T. Baker Chemical B.V., Deventer, The Netherlands); deoxyribonuclease and the Ca²⁺ ionophore A23187 (Calbiochem, La Jolla, Calif.); D-glucose, 70% HClO₄, CaCl₂, MgCl₂, KCl, NaCl, NaOH (Farmitalia Carlo Erba S.p.A., Milan, Italy); fetal calf serum, Eagle's minimal essential medium, gentamicin, and RPMI-1640 with 20 mmol/L (HEPES) (Flow Laboratories, Irvine, Scotland); Ca²⁺, Mg²⁺-free Hanks' balanced salt solution (CMF-HBSS), (Whittaker Bioproducts, Walkersville, Md.); tritiated PGD₂ (210 Ci/mmol) and tritiated-LTC₄ (39.3 Ci/mmol) (New England Nuclear Products, Boston, Mass.); dextran T70, Percoll (Pharmacia LKB Biotechnology AB, Uppsala, Sweden); piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES), hyaluronidase type III, collagenase type II, L-glutamine, bovine serum albumin, and synthetic PGD₂ and LTC₄ (Sigma Chemical Co., St. Louis, Mo.); recombinant human IL-2 (rhIL-2) (Boehringer Mannheim, Mannheim, Germany); gelatin (Serva Feinbiochemica, Heidelberg, Germany); Nytex cloth (Tetko, Elmsford, N.Y.). The following were kindly donated: ioxaglic acid, ioxitalamic acid, ioversol, N-methyl-D-glucamine, Hexabrix 320, Telebrix 38, Optiray 320 (Byk Gulden Italia S.p.A., Cormanio, Italy); goat IgG anti-human Fce (anti-IgE) (Drs. Teruko and Kimishige Ishizaka, La Jolla Institute for Allergy and Immunology, La Jolla, Calif.), anti-PGD₂ and anti-LTC₄ (Dr. Lawrence M. Lichtenstein, Johns Hopkins Asthma and Allergy Center, Baltimore, Md.), recombinant human IL-3 (rhIL-3) (Dr. Steven Gillis, Immunex, Seattle, Wash.). The tryptase radioimmunoassay kits (Pharmacia Tryptase RIACT 50, Kabi Pharmacia Diagnostics, Uppsala, Sweden) were kindly donated by Kabi Pharmacia Diagnostics, Milan, Italy.

Buffers

P buffer was made up of 25 mmol/L PIPES, 110 mmol/L NaCl, 5 mmol/L KCl. PGMD contains 1 gm/L

TABLE I. Osmolality of different molar concentrations of CM, mannitol, and buffers used in this study*

Mol/L	mOsm/kg	Mol/L	mOsm/kg
Ioxaglate		Hexabrix	
0.1	373	0.1	397
0.2	446	0.2	479
0.3	525	0.3	615
Ioxithalamate		Telebrix	
0.1	392	0.1	479
0.2	501	0.2	636
0.3	613	0.3	880
0.5	875	0.5	1100
Ioversol		Optiray	
0.2	393	0.2	376
0.3	441	0.3	462
0.5	540	0.5	629
0.8	740		
Mannitol			
0.1	377	P₂CG	295
0.3	541	P buffer	281
0.5	722		
0.8	1049		

*All substances were resuspended in P₂CG.

gelatin, 1 mmol/L MgCl₂, and 20 mg/L deoxyribonuclease in addition to P buffer; P₂CG contains 2.0 mmol/L CaCl₂ and 0.1% D-glucose in addition to P buffer.¹⁶ For all buffers, pH was titrated to 7.4 with NaOH.

Preparation of reagents

Ioxaglic acid and ioxitalamic acid in powder form were dissolved in P buffer together with NaOH (1 N) and N-methyl-D-glucamine to obtain their sodium and meglumine salts according to the instructions provided by CM manufacturers. The names of these salts, ioxaglate and ioxithalamate, will be used hereafter. The nonionic agent, ioversol, and mannitol in powder form were dissolved in P buffer. The pH of the stock solutions, prepared immediately before each experiment, was adjusted to 7.4. Each stock preparation was diluted into final molar concentrations in the appropriate buffer. The same concentrations were obtained for Hexabrix, Telebrix, and Optiray with the same buffer used for the pure substances. The osmolalities of each solution, obtained in duplicate by freezing point depression on a Vogel osmometer, were determined not only by the molar concentration of the CM used but also by the contribution of the buffer used. Table I shows the osmolality of molar concentrations measured for CM, mannitol, and buffers used in this study.

HR from human basophils

Informed consent was obtained from all subjects, and approximately 50 ml of blood was drawn into a final concentration of 0.008 mol/L EDTA and 1.1% dextran

70. The history of subjects' previous exposure to CM was unavailable. Approximately 50 ml of blood was drawn into a final concentration of 0.008 mol/L EDTA and 1.1% dextran 70. The erythrocytes were allowed to settle for 90 minutes at 22° C, and leukocyte-rich plasma was removed. The cells were separated by centrifugation at 180 g for 8 minutes, washed twice in P buffer, and counted in a Spiers-Levy chamber after staining with Alcian blue.²⁰ An aliquot of cells allowing $\sim 3 \times 10^4$ basophils per tube was resuspended in P₂CG; 20 μ l of the cell suspension was placed in Falcon 12 $\times$ 75 mm polyethylene tubes (Becton-Dickinson Labware, Lincoln Park, N.J.) and warmed to 37° C. Stimuli were added as 80 μ l aliquots, and incubation was continued at 37° C for 60 minutes. At the end of the incubation, the volume of the reaction mixtures was brought to 1 ml with cold P buffer. After centrifugation, the cell-free supernatant was assayed for histamine with an automated fluorometric technique.²¹ The net percentage release was calculated by subtracting the histamine released spontaneously by the unstimulated samples from the total histamine released by cells lysed with 2% HClO₄.¹⁶ Spontaneous release of histamine in P₂CG was $1.5\% \pm 0.4\%$ of the available basophil histamine content. The difference between replicate histamine measurements was less than 10%.

Isolation of human lung mast cells

Human lung tissue was obtained from patients undergoing thoracotomy and lung resection, mostly for treatment of lung cancer. The history of patients' previous exposure to CM was unavailable. 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.¹⁶ Yields with this technique ranged between 3×10^6 and 10×10^6 mast cells per gram of tissue, and purities ranged between 1% and 8%. For short-term (24-hour) cultures of human lung mast cells (HLMCs), the cells were resuspended in RPMI-1640 containing 20 mmol/L HEPES, 2 mmol/L L-glutamine, 1% gentamicin, and 10% fetal calf serum.¹⁶ In some experiments, HLMCs were partially purified by a multidensity Percoll gradient.¹⁷

Isolation of human skin mast cells

Skin was obtained from patients undergoing either mastectomy for treatment of breast cancer or elective cosmetic surgery procedures. The history of subjects' previous exposure to CM was unavailable. The specimen was immediately placed in minimal essential medium at 4° C and used within 1 hour. The skin was separated from the subcutaneous fat by blunt dissection. The remaining tissue was chopped into 1 to 2 mm fragments with scissors. The fragments were washed twice in CMF-HBSS at 22° C and incubated for 3 hours at 37° C under constant stirring in a solution of CMF-HBSS containing 2500 U collagenase per gram of wet weight of

tissue, 5 mg of hyaluronidase per gram of wet weight of tissue, and 1000 U/ml DNase.¹⁷ The isolated cells were separated from the partially digested tissue fragments by filtration through Nytex cloth and stored. The remaining tissue was digested a second time with a fresh batch of the enzymes for an additional 2 hours at 37° C. The cells were separated from the residual tissue fragments by filtration through Nytex cloth, washed twice, combined with the cells from the first digestion, and washed again in CMF-HBSS. Yields with this technique ranged between 1×10^6 and 9×10^6 mast cells per gram of tissue, and purities were between 1% and 5%. For short-term (24 hour) cultures of human skin mast cells (HSMCs), the cells were resuspended in RPMI-1640 containing 20 mmol/L HEPES, 2 mmol/L L-glutamine, 1% gentamicin, and 10% fetal calf serum.¹⁷ In some experiments, dispersed HSMCs were partially purified by a multidensity Percoll gradient, as previously described.¹⁷ Mast cells and basophils were stained with Alcian blue²⁰ and counted in a Spiers-Levy counter chamber.

Isolation of human heart mast cells

Heart tissue (100 to 600 gm) was obtained from patients undergoing heart transplantation, mostly for treatment of cardiomyopathy. The history of subjects' previous exposure to CM was unavailable. Fat tissue, large vessels, and pericardium were removed, and heart mast cells were isolated with a technique recently described.²² The tissue was finely minced with scissors into 2 to 5 mm fragments, suspended in P buffer (10 ml/gm of wet tissue), and washed three times (150 g, 4° C, 8 minutes, then twice at 150 g, 22° C, 8 minutes). After each centrifugation the heart fragments were filtered through Nytex cloth (150 μ m pore size). Fragments were weighed and incubated (15 minutes, 37° C) under constant stirring in P buffer containing 250 U collagenase per gram of wet tissue. At the end of the first incubation, the cell suspension was filtered through a Nytex cloth (150 μ m pore size). The residual tissue was weighed, and three additional cycles of enzymatic digestion were performed with a new preparation of collagenase each time. After the last enzymatic digestion, the cell suspension was centrifuged (150 g, 22° C, 8 minutes) and filtered twice through a Nytex cloth (150 μ m pore size, then 60 μ m pore size) to remove large particles and large cells (mostly myocytes, length >100 μ m). Cells were finally washed twice in PGMD (150 g, 22° C, 8 minutes). At the end of this procedure, Alcian blue-positive cells (mast cells) represented less than 0.1% of total cells. Cell pellets were resuspended in 250 ml of P buffer containing 2% bovine serum albumin and centrifuged (25 g, 22° C, 2 minutes) to remove sedimented myocytes. Supernatants containing endothelial cells, fibroblasts, and mast cells were collected and centrifuged (150 g, 22° C, 8 minutes). At this stage of purification, Alcian blue-positive cells represented less than 1% of total cells. Partial purification of human heart mast cells (HHMCs) was obtained with a multidensity Percoll

gradient.¹⁶ Purities ranged from 0.1% to 18%, with an average of $12\% \pm 6\%$.

Mediator release from HLMCs, HSMCs, and HHMCs

Aliquots of 20 μ l of the cell suspension containing $\sim 3 \times 10^4$ mast cells per tube were placed in Falcon 12 $\times$ 75 mm polyethylene tubes and warmed to 37° C. Eighty microliters of each releasing stimulus was added, and incubation was continued for 60 minutes. At the end of the incubation period, the sample volume was brought to 1 ml with cold P buffer. The remainder of the procedure and the calculation of HR were identical to the methods for basophils. In some experiments 200 μ l fractions were taken from supernatants for the analysis of tryptase, PGD₂, and LTC₄ concentrations. Spontaneous release of histamine in P₂CG was $11.4\% \pm 0.8\%$ of cellular content in HLMC preparations, $9.5\% \pm 1.0\%$ in HSMC preparations, and $13.6\% \pm 4.2\%$ in HHMC preparations.

Cell viability

Cell suspensions were tested for viability with the trypan blue exclusion method²⁸ after incubation (60 minutes, 37° C) with CM. Cell viability in the presence of these compounds was 95% or greater.

Histamine assay

Histamine levels in supernatants from basophils and lung, skin, or heart mast cell preparations were measured with the automated fluorometric method of Siraganian.²⁰ To avoid any interference with the fluorometric assay of histamine produced by CM,²³ all the experiments were performed in a volume of 100 μ l, which was brought, at the end of the incubation period, to a final volume of 1 ml with cold P buffer. After this 10-fold dilution, none of the CM interfered with the fluorometric assay of histamine. The addition of various concentrations of CM to known amounts of histamine (10 to 100 ng/ml) did not interfere with the extraction procedure.

Radioimmunoassay of PGD₂, LTC₄, and tryptase

PGD₂ and LTC₄ were evaluated by radioimmunoassay as described previously.²⁴ The rabbit anti-LTC₄ and anti-PGD₂ antisera have been characterized, and their cross-reactivity for heterologous ligands has been described.²⁵ Tryptase was analyzed by a solid-phase radioimmunoassay (Pharmacia Tryptase RIACT 50); duplicate samples or tryptase standards were added to plastic tubes coated with mouse monoclonal anti-tryptase antibodies. Then iodine 125-labeled anti-tryptase was added to the tubes,²⁶ which were incubated for 16 to 18 hours at 22° C. After washing (3 times in 2 ml of saline solution, 22° C), bound radioactivity was counted and produced a linear concentration-response curve within a range of 2 to 50 μ g/L. The net tryptase release was calculated by subtracting the spontaneous tryptase re-

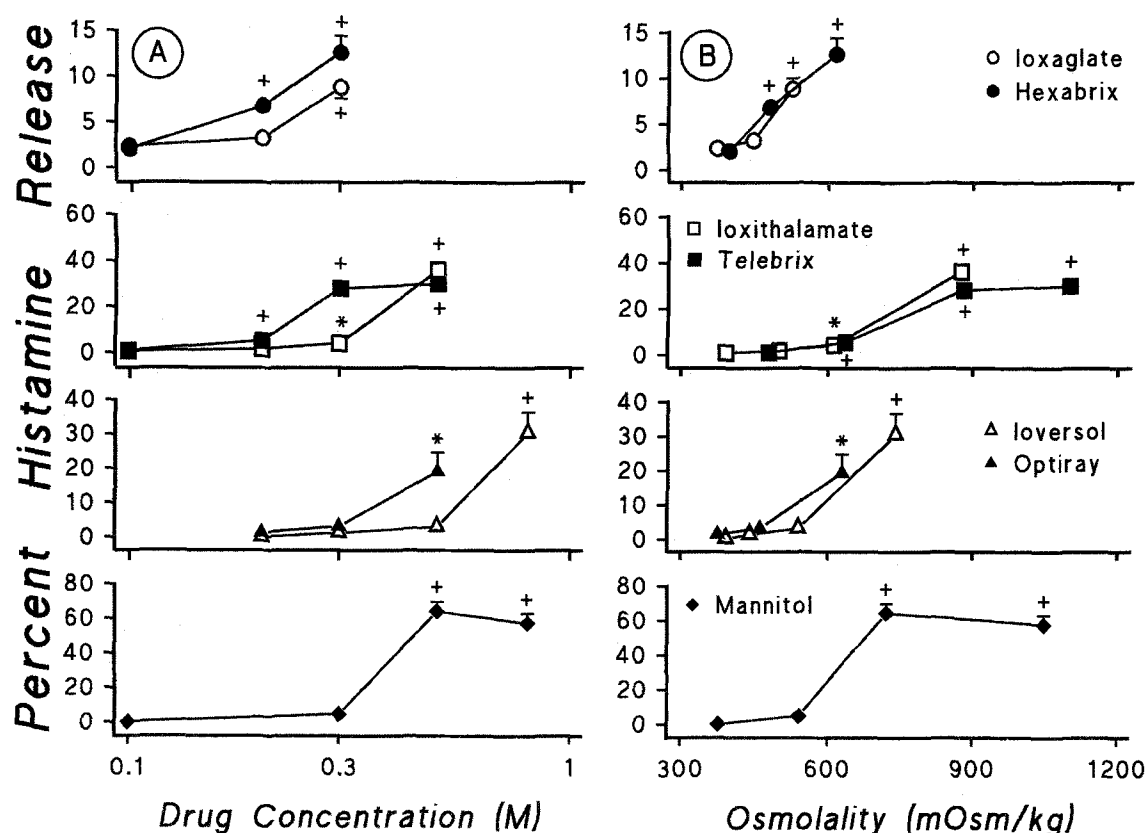


FIG. 1. A, Effect of increasing concentrations of ioxaglate (○), ioxithalamate (□), ioversol (△), their commercial preparations (Hexabrix (●), Telebrix (■), and Optiray (▲), respectively), and mannitol (◆) on HR from basophils. Results are expressed as means $\pm$ SEM obtained in 10 experiments. Error bars are not shown when graphically too small. $+p < 0.005$ compared with spontaneous release; $*p < 0.05$ compared with spontaneous release. B, Percent HR obtained from basophils in the experiments shown in A is plotted against the osmolality values of the molar concentrations used (see also Table I).

lease of the unstimulated cells from the values obtained for the CM-challenged samples.

Statistical analysis

The results are expressed as means $\pm$ SEM. Statistical significance was analyzed by two-way nonparametric analysis of variance (Friedman chi square test); the level of statistical significance was $p < 0.05$. Differences between matched pairs were analyzed by the Wilcoxon signed-rank test. Correlations were calculated by Spearman's nonparametric test.²⁷

RESULTS

Effects of ioxaglate, ioxithalamate, ioversol, and their commercial preparations on HR from human basophils

Fig. 1, A shows that ioxaglate (0.1 to 0.3 mol/L), ioxithalamate (0.1 to 0.5 mol/L), and ioversol (0.2 to 0.8 mol/L) concentration-dependently induced HR from basophils obtained from 10 normal do-

nors; maximum percent HR was $8.8\% \pm 1.2\%$, $36.4\% \pm 4.0\%$, and $30.4\% \pm 6.2\%$, respectively. The same concentrations of the commercial preparations induced similar, but not identical, dose-response curves, and the maximum percents of HR obtained with Hexabrix ($12.6\% \pm 1.8\%$) and Telebrix ($29.9\% \pm 3.2\%$) were similar to those obtained with the corresponding pure substances. The percent HR observed at the highest concentration attainable (0.5 mol/L) for Optiray ($19.2\% \pm 5.5\%$) differed from that induced by ioversol at the same concentration. Mannitol (0.1 to 0.8 mol/L) induced HR up to a maximum of $57.4\% \pm 5.7\%$. In these experiments, anti-IgE (0.3 $\mu\text{g/ml}$) and the Ca^{2+} ionophore A23187 (0.3 $\mu\text{g/ml}$) caused $51.4\% \pm 9.4\%$ and $30.2\% \pm 6.6\%$ of HR, respectively.

Hyperosmolality of CM can be responsible for their histamine-releasing activity.^{10, 11, 29} In Fig. 1,

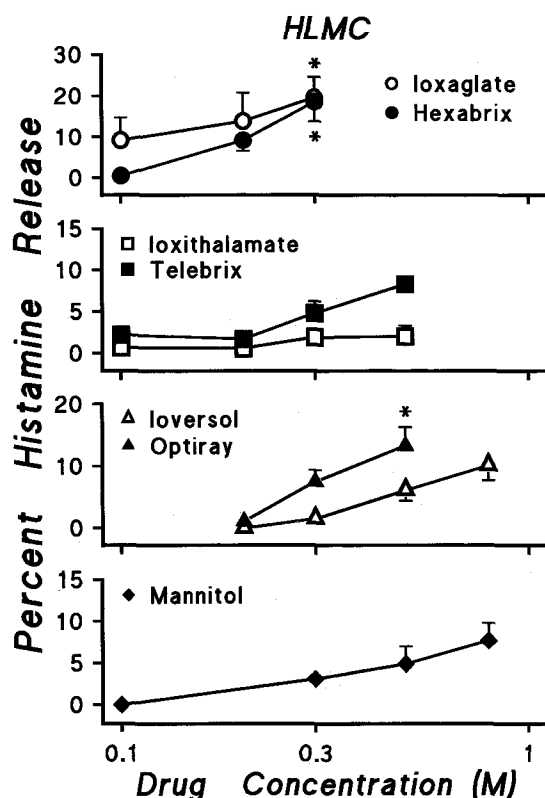


FIG. 2. Effect of increasing concentrations of ioxaglate (○), ioxithalamate (□), ioversol (△), their commercial preparations (Hexabrix [●], Telebrix [■], and Optiray [▲], respectively), and mannitol (◆) on HR from HLMCs. Results are expressed as means $\pm$ SEM obtained in 10 experiments. Error bars are not shown when graphically too small. * $p < 0.05$ compared with spontaneous release.

B the percent HR induced by CM and mannitol are plotted against the osmolality values of the molar concentrations used (Table I). Increasing the osmolality of the four agents tested caused an increase in the percent HR from basophils. However, a different percentage of histamine was released by the CM in the same osmolality range. For instance, the highest concentration of ioxaglate and Hexabrix induced significant HR at 525 and 615 mOsm/kg, respectively; whereas mannitol, ioxithalamate, and Telebrix at 541, 501, and 479 mOsm/kg, respectively, caused less than 5% HR. In addition, at osmolality values of 722 mOsm/kg or greater, the percent HR induced by ioxithalamate, Telebrix, and ioversol were markedly lower than that induced by mannitol.

Effects of CM on histamine and tryptase release from HLMCs, HSMCs, and HHMCs

In 10 experiments ioxaglate (0.1 to 0.3 mol/L) and ioversol (0.2 to 0.8 mol/L) induced concentra-

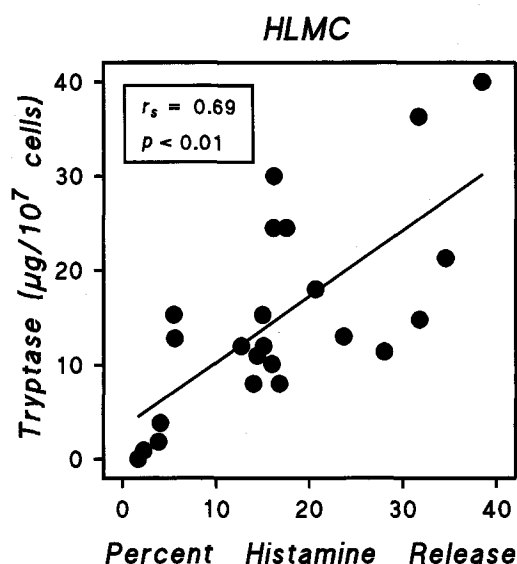


FIG. 3. Correlation between percent HR and tryptase secretion from HLMCs induced by the three CM used. Each point corresponds to the mean of duplicate samples.

tion-dependent HR from HLMCs up to a maximum of $19.6\% \pm 5.0\%$ and $10.1\% \pm 2.4\%$, respectively (Fig. 2). In contrast, the highest concentration of ioxithalamate used (0.5 mol/L) induced the release of less than 5% of the histamine content. Similar concentration-dependent curves were obtained with the three commercial preparations (Fig. 2). As previously reported,^{19,30} mannitol (0.1 to 0.8 mol/L) caused little HR from HLMCs ($7.7\% \pm 2.1\%$ at 0.8 mol/L). In these experiments anti-IgE (1 μ g/ml) and the Ca^{2+} ionophore A23187 (1 μ g/ml) caused $24.4\% \pm 7.2\%$ and $35.4\% \pm 4.7\%$ HR, respectively. Similar results were obtained in three experiments performed with enriched HLMC preparations (30%, 35%, and 58% of the total cell count) (data not shown). Ioxaglate, Hexabrix, and Optiray caused significant HR from HLMCs at osmolality values at which mannitol induced less than 5% HR (data not shown).

Tryptase is a neutral protease present in the granules of human mast cells.¹³ Ioxaglate and Hexabrix (0.2 and 0.3 mol/L), ioversol (0.5 and 0.8 mol/L), and Optiray (0.3 and 0.5 mol/L) induced, in addition to HR, concentration-dependent tryptase release from HLMCs (Table II). As expected, anti-IgE and the Ca^{2+} ionophore A23187 also induced the release of tryptase, in addition to histamine (Table II). There was a positive correlation between percent HR and tryptase release induced by CM ($r_s = 0.69$; $p < 0.01$) from HLMCs (Fig. 3).

TABLE II. Effects of CM, anti-IgE, and Ca^{2+} ionophore A23187 on HR and tryptase release from HLMCs

	No. of experiments	HR (%)	Tryptase release ($\mu\text{g}/10^7$ cells)
Ioxaglate (mol/L)			
0.2	2	17.8 ± 14	8.3 ± 6.5
0.3	3	18.2 ± 8.5	15.0 ± 3.2
Hexabrix (mol/L)			
0.2	2	15.0 ± 1	9.1 ± 1.1
0.3	4	21.8 ± 7.3	16.1 ± 8.1
Ioversol (mol/L)			
0.5	2	8.4 ± 6.7	6.0 ± 6.0
0.8	4	13.8 ± 3.2	18.8 ± 3.9
Optiray (mol/L)			
0.3	2	9.2 ± 6.9	12.7 ± 11.8
0.5	4	21.4 ± 5.2	25.4 ± 6.1
Anti-IgE ($\mu\text{g}/\text{ml}$)			
1	4	24.7 ± 6.7	14.9 ± 3.4
Ca^{2+} ionophore A23187 ($\mu\text{g}/\text{ml}$)			
1	2	39.2 ± 0.6	16.9 ± 2.6

Given the functional heterogeneity of human mast cells,¹⁶⁻¹⁹ we evaluated the effects of increasing concentrations of CM on HSMCs. In contrast to HLMCs and basophils in 10 experiments, none of the CM or mannitol induced the release of more than 5% of histamine content from mast cells isolated from skin tissues (data not shown). In these experiments HSMCs released $20.3\% \pm 9.1\%$ and $24.7\% \pm 2.6\%$ histamine when challenged with anti-IgE (3 $\mu\text{g}/\text{ml}$) and Ca^{2+} ionophore A23187 (1 $\mu\text{g}/\text{ml}$), respectively. Similar results were obtained in three experiments with enriched preparations (30%, 37%, and 70%) of HSMCs (data not shown). Similarly, tryptase was undetectable in the supernatants of HSMCs challenged with CM (data not shown).

We compared the effects of Hexabrix (0.2 and 0.3 mol/L), Telebrix (0.3 and 0.5 mol/L), Optiray (0.3 and 0.5 mol/L), and mannitol (0.1 to 0.8 mol/L) on the release of preformed mediators (histamine and tryptase) from HLMCs ($n = 3$). The three CM caused histamine and tryptase release, whereas hyperosmolar concentrations of mannitol did not induce the release of these mediators from HLMCs (Table III).

Correlation between osmolality and HR from basophils and HLMCs

Hyperosmolality of CM has been thought to be responsible for their basophil/mast cell-activating properties.^{10, 11, 29, 30} Fig. 4 shows that there was a positive correlation between the osmolality of CM used and the percent HR from basophils for

commercial preparations ($r_s = 0.76$, $p < 0.01$). Similar results were obtained for pure substances ($r_s = 0.64$, $p < 0.01$) (data not shown). In contrast, no significant correlation was found between osmolality and CM-induced HR from HLMCs ($r_s = 0.20$ and $r_s = 0.28$ for pure substances and commercial preparations, respectively).

Effect of CM on the de novo synthesis of eicosanoids from basophils and HLMCs

Cross-linking of IgE receptors on human basophils and lung mast cells, but not skin mast cells, leads to the de novo synthesis of LTC_4 ,^{24, 31} in addition to the secretion of preformed histamine. Both skin and lung mast cells, but not basophils, also synthesize PGD_2 .³¹ We investigated the effects of ioxaglate or Hexabrix (0.2 and 0.3 mol/L), ioxithalamate or Telebrix (0.3 and 0.5 mol/L), ioversol (0.5 and 0.8 mol/L), and Optiray (0.3 and 0.5 mol/L) on the de novo synthesis of eicosanoids from basophils and mast cells. Interference with the detection of eicosanoids by CM was tested. Only Hexabrix and ioxaglate interfered with the radioimmunoassay for LTC_4 and PGD_2 . Therefore the release of eicosanoids by these CM was not detectable. In three experiments none of the other CM used induced detectable amounts of LTC_4 from basophils, whereas they caused the secretion of histamine (data not shown). Similarly, the CM did not induce the synthesis of detectable amounts of LTC_4 or PGD_2 from HLMCs (data not shown). As reported by Findlay et al.,²⁹ mannitol did not induce the synthesis of eicosanoids from either

TABLE III. Histamine release and tryptase secretion induced by CM, mannitol, and Ca^{2+} ionophore A23187 from HLMCs

	Experiment 1		Experiment 2		Experiment 3	
	HR (%)	Tryptase ($\mu\text{g}/10^7$ cells)	HR (%)	Tryptase ($\mu\text{g}/10^7$ cells)	HR (%)	Tryptase ($\mu\text{g}/10^7$ cells)
Hexabrix (mol/L)						
0.2	2.3	0.9	ND	ND	4.8	10.2
0.3	3.0	2.0	23.9	22.5	27.4	21.9
Telebrix (mol/L)						
0.3	8.3	16.2	ND	ND	ND	
0.5	9.5	57.5	16.2	12.2	ND	
Optiray (mol/L)						
0.3	1.9	0.6	ND	ND	15.0	12.9
0.5	6.0	20.2	9.1	0	22.1	18.6
Mannitol (mol/L)						
0.3	0	0	0	0	ND	ND
0.5	0	0	0	0	0	0
0.8	0	0	0	0	0	0
A23187 ($\mu\text{g}/\text{ml}$)						
3	25.0	19.2	28.2	20.7	53.0	39.0

ND, Not done.

basophils or HLMCs (data not shown), whereas anti-IgE stimulated the de novo synthesis of LTC_4 from both cell types ($42.9 \pm 9.0 \text{ ng}/10^6$ cells from basophils and $158.8 \pm 15.6 \text{ ng}/10^6$ cells from HLMCs) or PGD_2 from HLMCs ($96.6 \pm 19.5 \text{ ng}/10^6$ cells, $n = 3$).

Characteristics of basophil and mast cell activation by CM

We investigated the kinetics and the temperature and Ca^{2+} dependence of CM-induced HR from basophils and HLMCs.

Optimal concentrations of ioxaglate, ioxithalamate, and ioversol induced HR from basophils with different time courses ($n = 4$). Ioxithalamate- and ioxaglate-induced HR was rapid, with a half-life of 2.3 ± 0.3 minutes and 5.1 ± 4.0 minutes, respectively, whereas histamine secretion induced by ioversol was slow (half-life = 22.1 ± 2.7 minutes). Similar results were obtained with the corresponding commercial preparations of CM (data not shown). Interestingly, the kinetics of ioxaglate-induced (0.3 mol/L) and ioversol-induced (0.8 mol/L) HR from HLMCs ($n = 4$) were strikingly different from those observed with basophils. In fact, the release induced by ioxaglate from HLMCs was significantly slower (half-life = 33.0 ± 4.6 minutes) than that in basophils ($p < 0.01$), whereas ioversol-induced release from HLMCs was faster (half-life = 2.3 ± 1.3 minutes) than that in basophils ($p < 0.01$) (Fig. 5).

We then investigated the temperature dependence of HR induced by CM, mannitol, and anti-IgE from basophils and HLMCs. The releasing activity of CM was optimal at 37°C , diminished at 22°C , and was abolished at 4°C ($n = 3$). For example, ioversol-induced (0.8 mol/L) and Optiray-induced (0.5 mol/L) HR was lowered from $41.7\% \pm 14.7\%$ and $35.7\% \pm 12.2\%$, respectively, to $0.5\% \pm 0.3\%$ and 0 in experiments with basophils and from $10.1\% \pm 2.8\%$ and $13.6\% \pm 5.5\%$ to $3.5\% \pm 1.2\%$ and $3.3\% \pm 0.7\%$, respectively, in experiments with HLMCs. Similar results were obtained with the other CM and with mannitol (data not shown). In these experiments the response of basophils and HLMCs to anti-IgE was abolished at 22°C (data not shown).

We next examined the effect of increasing concentrations of extracellular Ca^{2+} on HR from basophils and HLMCs caused by optimal concentrations of ioxaglate and Hexabrix, ioxithalamate and Telebrix, ioversol and Optiray, and mannitol. Fig. 6 shows that these agents caused HR from basophils incubated in buffer without the addition of extracellular Ca^{2+} . Increasing the extracellular Ca^{2+} concentrations from 0.3 to 5 mmol/L significantly increased the percent HR caused by ioxaglate, ioxithalamate, ioversol, and mannitol. Similar results were obtained with Hexabrix, Telebrix, and Optiray ($n = 5$). In a series of experiments performed with HLMCs, ioxaglate and Hexabrix ($n = 8$), ioversol and Optiray ($n = 10$), and

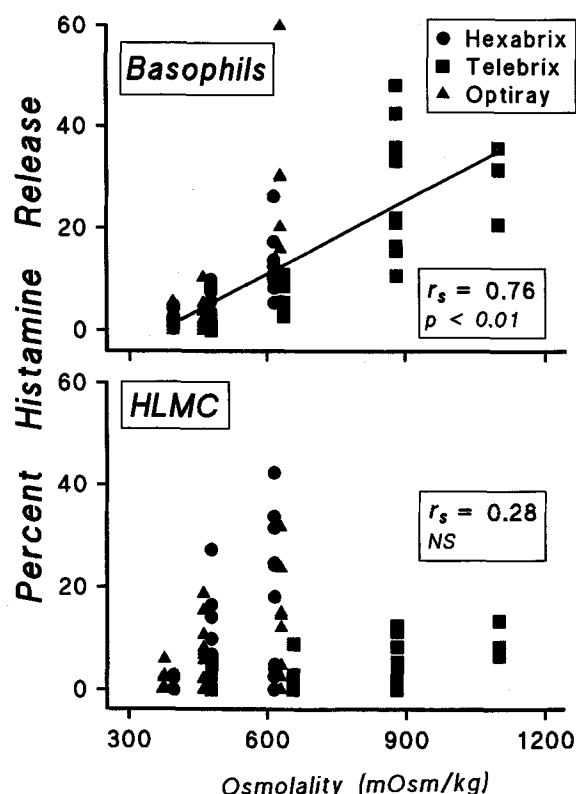


FIG. 4. Correlation between osmolality of different preparations of Hexabrix (●), Telebrix (■), and Optiray (▲) and percent HR from basophils (upper panel) and HLMCs (lower panel). Each point corresponds to the mean of duplicate samples from a separate experiment.

mannitol ($n = 9$) caused HR in buffer without the addition of extracellular Ca^{2+} ($12.6\% \pm 5.0\%$, $8.5\% \pm 2.6\%$, $12.3\% \pm 3.9\%$, $12.7\% \pm 3.3\%$, and $8.6\% \pm 3.7\%$, respectively). The addition of increasing extracellular Ca^{2+} concentrations (0.3 to 5 mmol/L) caused a variable degree of enhancement of HR, which was significant only when Optiray and mannitol were used as activating stimuli (data not shown).

Effect of IL-2 and IL-3 on basophil HR induced by CM

In vitro incubation of basophils with IL-3 enhances the release of histamine induced by all immunologic and nonimmunologic stimuli tested thus far.^{32,33} In vivo administration of IL-2 potentiates the release of histamine caused by CM.³⁴ We therefore evaluated the effects of preincubation of basophils with an optimal concentration of rhIL-2 (20 ng/ml) or rhIL-3 (10 ng/ml) on CM-, mannitol-, and anti-IgE-induced HR. Cells were preincubated (15 minutes, 37°C) with rhIL-2 or rhIL-3 and then challenged with Hexabrix (0.1 to 0.3 mol/L),

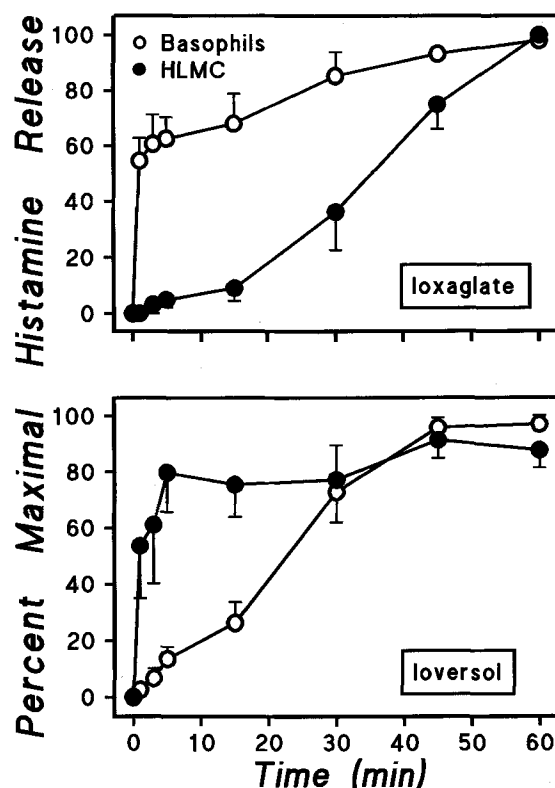


FIG. 5. Kinetics of HR induced by ioxaglate (0.3 mol/L) (upper panel) or ioversol (0.8 mol/L) (lower panel) from basophils (○) or HLMCs (●). Results are expressed as means $\pm$ SEM of the percent maximal HR (ioxaglate: $11.8\% \pm 2.4\%$ from basophils, $40.7\% \pm 3.4\%$ from HLMCs; ioversol: $44.8\% \pm 7.3\%$ from basophils, $15.7\% \pm 4.4\%$ from HLMCs, $n = 4$). Error bars are not shown when graphically too small.

Telebrix (0.2 to 0.5 mol/L), Optiray (0.2 to 0.5 mol/L), mannitol (0.3 to 0.8 mol/L), or anti-IgE (0.3 $\mu\text{g/ml}$). Human recombinant IL-2 and IL-3 did not increase basophil HR in response to any CM tested or to mannitol, whereas rhIL-3 produced a marked enhancement of anti-IgE-induced HR; rhIL-2 had no effect on IgE-mediated HR from basophils (data not shown).

DISCUSSION

Our results indicate that CM can induce the selective release of preformed chemical mediators from human basophils and mast cells isolated from different anatomic sites. There appear to be three levels of heterogeneity with regard to: (1) the releasing activity of different CM, (2) the capacity of basophils and mast cells isolated from different anatomic sites to respond to each CM, and (3) the different contributions of osmolality in CM-induced HR from basophils and mast cells.

During intravascular administration of CM for

radiographic procedures, peripheral blood basophils are rapidly exposed to high concentrations of CM. All of the CM used in this study, at concentrations including those administered intravascularly for diagnostic procedures, induced a concentration-dependent, noncytotoxic release of histamine from basophils obtained from normal donors.

Hyperosmolality is considered an important factor in the occurrence of adverse reactions to CM.^{1-4, 10, 11} The role of hyperosmolality in producing basophil and mast cell HR is well established.^{19, 29, 30} However, several reports indicate that anaphylactoid reactions still occur when new low-osmolality CM are used.^{1, 2, 35} We found a positive correlation between increasing osmolality and HR from basophils. However, at comparable osmolalities each CM induced a different amount of HR. Furthermore, the degrees of basophil activation caused by CM and mannitol were different. These findings suggest that hyperosmolality, although very important, is probably not the only mechanism by which CM activate basophils.

Ennis et al.⁹ and Amon et al.¹² demonstrated that rodent and canine mast cells isolated from different anatomic sites responded differently to various CM. We have extended these findings, showing that mast cells isolated from different human tissues are heterogeneous with respect to the releasing activity of different CM and that their sensitivity to these compounds differs from that of basophils. In particular, we found no correlation between the osmolality and the percent HR caused by CM from HLMCs. Hexabrix and Optiray, which have a lower osmolality than Telebrix, induced concentration-dependent release of preformed mediators (histamine and tryptase) from HLMCs. In contrast, Telebrix, which has the highest osmolality of the three CM tested, produced the highest percent HR from basophils but did not activate lung mast cells. These findings suggest that the relevance of hyperosmolality in CM-induced HR differs in basophils and mast cells and further emphasize the functional heterogeneity of these cells.^{16, 17}

An interesting finding of this study is the lack of activation of HSMCs by the CM used. Skin is often involved in the clinical manifestations (flushing, pruritus, and urticaria) of anaphylactoid reactions to CM.^{3, 4, 6} It is possible that the mechanism of cutaneous reactions to CM involves, in addition to direct activation of basophils, complement activation and anaphylatoxin formation,^{1, 2, 7} which may trigger activation of skin mast cells in vivo.

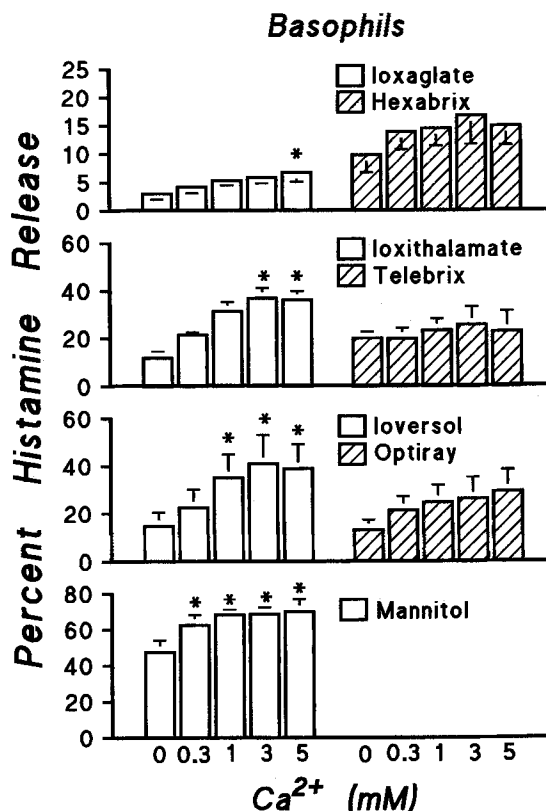


FIG. 6. HR from basophils incubated for 60 minutes at 37° C with ioxaglate (0.3 mol/L), Hexabrix (0.3 mol/L), ioversol (0.8 mol/L), Optiray (0.5 mol/L), or mannitol (0.8 mol/L) in the absence or presence of the indicated concentrations of extracellular Ca²⁺. Mean $\pm$ SEM of five experiments. **p* < 0.05 compared with HR in the absence of added extracellular Ca²⁺.

Another original finding is the activating property of CM on heart mast cells. Results with rodent mast cells indicate that heart mast cells can be activated by CM.⁹ Coronary artery spasm, hypotension, and shock can occur during intravascular administration of CM.^{3, 4, 6, 36} The density of mast cells around coronary vessels is increased in patients with cardiovascular disease,³⁷ and these subjects are at increased risk for CM-induced adverse reactions.^{4, 38} During coronary angiography, heart mast cells are exposed to high concentrations of CM, and our in vitro results indicate that heart mast cells exposed to these agents can release histamine and tryptase. Therefore a direct effect of vasoactive mediators released from heart mast cells and basophils during anaphylactoid reactions might contribute to the clinical manifestations of CM-related reactions.³⁹⁻⁴¹

We have attempted to shed some light on the characteristics of basophil/mast cell degranulation induced by CM. The kinetics of HR caused

by CM are heterogeneous with regard to the different CM and with regard to the cells (basophils or lung mast cells) challenged with each CM. For instance, ioxithalamate and ioxaglate caused the rapid release of histamine from basophils, whereas histamine secretion induced by ioversol was slow. In contrast, ioversol caused rapid secretion from HLMCs, and ioxaglate induced slow release from these cells. These striking differences point to different mechanisms of activation by CM in basophils and mast cells.

HR caused by CM from basophils and mast cells is only partially Ca^{2+} -dependent in that it occurs in the absence of added extracellular Ca^{2+} but can be enhanced to variable degrees in the presence of optimal Ca^{2+} concentrations. HLMCs appeared to be less sensitive than basophils to changes of extracellular Ca^{2+} concentrations.

The observation that in some cases HR follows slow kinetics and is not dependent on extracellular Ca^{2+} concentrations does not necessarily imply that this process is due to a toxic effect exerted by CM. Findlay et al.²⁹ demonstrated that hyperosmolar-induced HR from basophils is Ca^{2+} -independent and occurs slowly, but electron microscopic examination of basophils activated by hyperosmolar stimulation showed no sign of cell damage. Although in our study we have no direct evidence that CM did not selectively affect basophil and mast cell viability, several observations suggest that the HR we observed is not cytotoxic. First, the release of histamine was temperature-dependent; second, even after prolonged incubation, it never reached 100% of histamine content; and third, the viability of enriched preparations of HLMCs was comparable to that of unpurified HLMCs.

Our results provide the first evidence that CM can induce the release of tryptase from human mast cells *in vitro*. Once secreted, histamine is rapidly catabolized in plasma,⁴¹ whereas the increase in plasma tryptase concentrations induced by *in vivo* activation of mast cells has a half-life of ~2 hours.⁴² Therefore it might be clinically useful to measure plasma concentrations of tryptase to identify anaphylactoid reactions caused by CM administration.^{13, 43}

It has been suggested that lipid mediators synthesized *de novo* by basophils and mast cells (LTC_4 and PGD_2) play a role in the adverse reactions occurring during CM administration.⁴⁴ However, none of the CM tested in this study induced the *de novo* synthesis of eicosanoids from basophils and mast cells. Therefore these compounds appear to

be incomplete secretagogues, as previously reported for morphine¹⁸ and general anesthetic drugs.⁴⁵

Atopic individuals are more susceptible to anaphylactoid reactions to CM.^{1, 3, 4} Basophils obtained from atopic patients are more responsive to IL-3 than basophils from normal donors.⁴⁶ This cytokine also enhances the release of proinflammatory mediators from basophils induced by all immunologic and nonimmunologic stimuli tested thus far.^{32, 33} Recently, it has been reported that IL-2 administration *in vivo* potentiates the release of histamine caused by CM.³⁴ However, preincubation of basophils with rhIL-3 or rhIL-2 did not enhance the release of histamine induced by CM *in vitro*. Thus it appears that the metabolic pathways influenced by the activation of IL-3 or IL-2 receptors on basophils do not play a role in the release process caused by CM.

Finally, although our experimental procedures included extensive washing of lung, skin, and heart tissues and of the isolated cells, drugs used in premedication and for induction of anesthesia may be partly responsible for the differences found in the responses of mast cells and basophils to CM.

Although not directly applicable to the clinical arena, where manifestations of basophil/mast cell activation by CM are more episodic than we observed *in vitro*, our results have a number of implications. First, hyperosmolality is probably not the only biochemical factor responsible for basophil activation caused by CM and appears to be even less relevant for CM-induced mast cell activation. This implies that new CM characterized by lower osmolality should be tested *in vitro* for their histamine-releasing activity, not only on basophils but also on human mast cells isolated from different anatomic sites. Second, the CM used in this study are incomplete secretagogues, because they do not induce the *de novo* synthesis of eicosanoids. This might explain why anti- H_1 and anti- H_2 agents can attenuate anaphylactoid reactions caused by CM.^{2, 3, 5, 6, 8} Finally, the detection of plasma levels of tryptase may become a useful tool with which to identify mast cell activation *in vivo*^{13, 43} in adverse reactions occurring during CM administration.

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