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Research Article

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The cells of all donors released histamine *in vitro* in response to hyperosmolar (0.2-0.7 M) solutions of a number of solutes including mannitol. That this was not a toxic process was supported by a number of criteria, including inhibition of release by excess stimulus at 37°C and a lack of release at 4°C. Furthermore, electron microscopic studies revealed that hyperosmolar stimulation did not disrupt the cell membrane or lead to any signs of cytotoxicity. In contrast to antigen-stimulated release, where granules fuse only with the cell membrane, granules in mannitol-stimulated cells, in addition to fusing with the cell membrane, may also be extruded into a common intracellular sac before exteriorization.

Characteristics similar to antigen-induced histamine release included the time-course for release, inhibition by drugs that modify phospholipid metabolism, *p*-bromophenacyl bromide, and *eicosa*-5,8,11,14-tetraenoic acid, and augmentation of release by deuterium oxide (D₂O). The release process differed from antigen-induced release by a number of criteria, including independence from immunoglobulin (Ig)E-related mechanisms, insensitivity to [...]

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ABSTRACT Idiopathic reactions occurring during the infusion of hyperosmolar solutions, such as radiocontrast dyes, cause a significant number of deaths each year. These reactions are similar to those which follow mediator release during allergen-induced anaphylaxis. In attempting to explain these nonimmunologic reactions, we examined the direct effect of hyperosmolarity on normal human basophils with emphasis on release induced by mannitol.

The cells of all donors released histamine *in vitro* in response to hyperosmolar (0.2–0.7 M) solutions of a number of solutes including mannitol. That this was not a toxic process was supported by a number of criteria, including inhibition of release by excess stimulus at 37°C and a lack of release at 4°C. Furthermore, electron microscopic studies revealed that hyperosmolar stimulation did not disrupt the cell membrane or lead to any signs of cytotoxicity. In contrast to antigen-stimulated release, where granules fuse only with the cell membrane, granules in mannitol-stimulated cells, in addition to fusing with the cell membrane, may also be extruded into a common intracellular sac before exteriorization.

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vate intracellular cyclic AMP, minimal dependence on extracellular calcium, lack of inhibition by 2-deoxyglucose and theophylline, and a temperature optimum of 32°C.

We conclude that this noncytotoxic hyperosmolar release process is different from IgE-mediated secretory events and may well play a role in the idiopathic reactions which occur secondary to the infusion of hyperosmolar solutions in man.

INTRODUCTION

The etiologic role of basophil and mast cell mediators such as histamine in immunoglobulin (Ig)E-mediated anaphylactic reactions is now widely recognized (1). A similar type of "anaphylactoid" or idiopathic reaction is associated with the infusion of hyperosmolar solutions pre-operatively or during radiographic procedures. Patients often receive 30–50 cm³ of contrast media or mannitol at concentrations >1 M. Although the idiopathic reactions described in the literature do not appear to be due to IgE-related mechanisms, the signs and symptoms of these syndromes mimic precisely the reactions which are secondary to the antigenic release of mediators (2, 3). In attempting to explain these nonimmunologic reactions, both direct and indirect mechanisms for the release of basophil and/or mast cell mediators have been invoked (4, 5).

We have examined the direct effects of hyperosmolarity on normal human basophils. It was found that mannitol and similar solutes release histamine *in vitro* from the cells of all donors at concentrations >0.1 M. This release mechanism is not cytotoxic, but appears to be different from any described heretofore.

METHODS

Subjects. Blood donors were all healthy volunteers between the ages of 21 and 50 yr. None of the donors had displayed idiopathic or allergic reactions to drugs.

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Reagents. The goat anti-human IgE Fc (anti-IgE) was a gift from Dr. Kimishige Ishizaka, Baltimore, Md., and *eicosa-5,8,11,14-tetraynoic acid* (ETYA)¹ was the gift of Dr. W. E. Scott, Hoffman-LaRoche, Inc., Nutley, N. J. The following were purchased: piperazine-*N,N'*bis(2-ethane-sulfonic acid) (Pipes free acid), deuterium oxide (D₂O), theophylline, 2-deoxy-D-glucose, D-mannitol, *p*-bromophenacyl bromide (BPB), lactate dehydrogenase (LDH) assay kits (Sigma Chemical Co., St. Louis, Mo.); EDTA (Fisher Scientific Co., Pittsburgh, Pa.); perchloric acid 60% (J. T. Baker Chemical Co., Phillipsburg, N. J.); dextran 70 (Cutter Laboratories, Berkeley, Calif.); Hypaque 50 (Winthrop Laboratories, New York). Pipes buffer used in these experiments consisted of NaCl 110 mM, KCl 5 mM, Pipes 25 mM (pH 7.4, adjusted using sodium hydroxide). PCM consisted of Pipes buffer plus 0.6 mM CaCl₂ and 1 mM MgCl₂. D₂O-containing buffer stock solutions were composed of 88% D₂O and 12% H₂O, vol/vol.

Histamine release and assay. Blood was drawn from normal donors and mixed to a final concentration of 0.01 M EDTA and 20% vol/vol dextran 70. Erythrocytes were allowed to sediment for 90 min at room temperature, and the leukocyte-rich plasma was removed. The cells were separated from the plasma by centrifugation at 180 *g* at 4°C for 10 min and were washed twice in Pipes buffer. The cells were then resuspended in PCM and brought to a 37°C reaction temperature unless otherwise specified. 0.5 ml of the leukocyte suspension was added to 75 × 10-mm Falcon tubes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif.) which contained prewarmed test solutions. After an incubation of 45 min at 37°C (unless otherwise specified), the cells were separated from the reaction mixture by centrifugation. The supernates were assayed for histamine using the automated fluorometric technique of Siraganian (6). The total quantity of intracellular histamine was determined from cell aliquots lysed with 2% perchloric acid. The amount of histamine released was normalized by calculation of net percent histamine released. Unstimulated cells released no more than 2% histamine. The leukocytes in 1 ml of reaction volume equaled the number of cells obtained from 0.5 ml of whole blood. Purified basophils were prepared as previously described (7). BPB and ETYA were solubilized in acetone and dimethyl sulfoxide, respectively, and controls for solvent effects were included in all experiments. None of the reagents used, including mannitol, fluoresced, nor did they interfere with the fluorometric assay for histamine. Hemolysis was not observed at any concentration of mannitol (or other solutes) tested.

Electron microscopy. For morphologic studies a 10-fold excess of dilute Karnovsky's fixative (8) was added directly to cell suspensions, thus halting the degranulation process instantaneously. Cells were fixed at room temperature for 1 h and then washed in 0.1 M sodium cacodylate buffer, 4°C, pH 7.4. The cells were then suspended in 1 cm³ of Hanks' balanced salt solution containing 50 μl of cationized ferritin (Miles Laboratories, Inc., Ames Div., Elkhart, Ind.) (9) and placed on a rotary shaker set at low speed for 30 min at room temperature. They were then washed again in 0.1 M sodium cacodylate buffer, transferred to microtubes, and centrifuged at 1,500 *g* for 1 min. Cell pellets were suspended in warm 2% agar and recentrifuged. The agar cell pellet was then post-fixed for 2 h at 4°C in 2% aqueous osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M sodium phos-

phate buffer, pH 6.0 (OPF method) (10). Thereafter, cell pellets were dehydrated in a graded series of alcohols and embedded in a propylene oxide-Epon sequence. Sections were cut with diamond knives, placed on copper grids, and stained with lead citrate. A single time-interval (60 min) following exposure to 0.75 M mannitol (without D₂O) at 37°C and appropriate buffer-treated controls were studied by electron microscopy.

The question of cytotoxicity induced by mannitol was studied primarily by electron micrography. LDH studies were done on two occasions, first with basophils purified (58%) from a patient with chronic myelogenous leukemia; the basophils had normal amounts of histamine and responded well to anti-IgE. The second experiment was with normal basophils purified by the technique of MacGlashan and Lichtenstein (7) to the point of 50% purity. In each case, the cells were exposed to 0.75 M mannitol for 45 min. In the first instance, there was 16 ± 1.6% histamine release by mannitol. The net release of LDH was 2.7 ± 2.5%. On the second occasion, there was 46.3 ± 3% histamine release. Net LDH release of the mannitol-challenged cells was 6.9 ± 1.2%, with a control value of 7.4 ± 0.9%.

RESULTS

Basophil histamine release in the presence and absence of D₂O. The dose-response relationship between the molar concentration of mannitol and the percent histamine release in the presence and absence of 44% D₂O are represented in Fig. 1. The maximal histamine release occurred at 0.75 M mannitol (50 ± 5%, mean ± SE) in the presence of D₂O; at higher concentra-

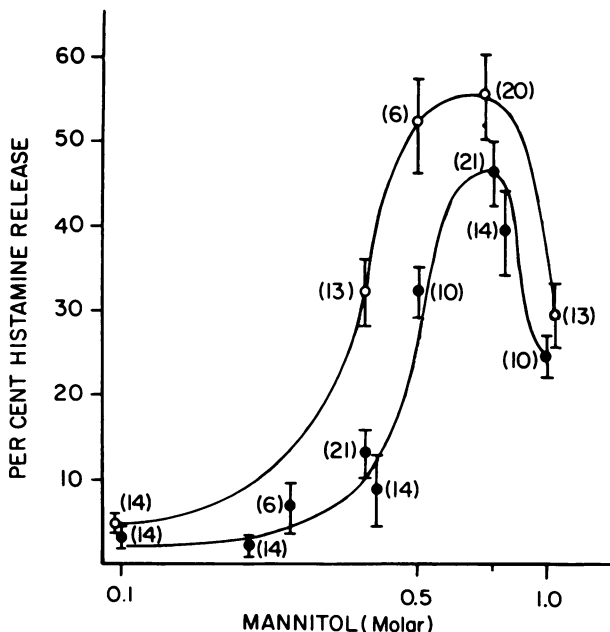


FIGURE 1 The percent histamine release from human basophils in vitro at various concentrations of mannitol. The results were obtained using both PCM (closed circles) or 44% D₂O PCM (open circles). Numbers accompanying each point represent the number of separate experiments used in the calculation of the mean and standard error values.

¹ Abbreviations used in this paper: BPB, *p*-bromophenacyl bromide; ETYA, *eicosa-5,8,11,14-tetraynoic acid*; ID₅₀, inhibitory dose, 50%; LDH, lactate dehydrogenase; PCM, Pipes buffer plus 0.6 mM CaCl₂ and 1 mM MgCl₂; Pipes free acid, piperazine-*N,N'* bis (2-ethane-sulfonic acid); SRS, slow-reacting substance.

tions there was a progressive decrease in histamine release. A concentration of 0.375 M mannitol induced little release ($13 \pm 6\%$), but the response was augmented considerably ($32 \pm 6\%$) if 44% D_2O was present. It has been shown previously that concentrations of antigen that barely reach a threshold for histamine release are greatly potentiated if D_2O is substituted for H_2O in the buffer solutions (11). Mannitol- and antigen-induced release are therefore analogous in that both processes are augmented by D_2O , although mannitol release appears to be increased somewhat less than antigen release.

It should be noted that in the routine preparations, basophils represent $<1\%$ of the leukocyte population. In an experiment using partially purified basophils (37% basophils, 63% mononuclear cells) histamine release after 45 min reached 36% using 0.375 M mannitol. Thus, following a 100-fold purification of basophils, removal of eosinophils and polymorphonuclear leukocytes, and with a ratio of mononuclear cells of basophils

of 2:1 rather than the usual 100:1, the response induced by mannitol was not affected (data not shown).

To see the effects of normal human serum, dose-response curves using 0.1, 0.375, 0.75, and 1 M mannitol were carried out without serum and in the presence of 5, 15, and 25% serum. The dose-response curves were essentially identical, with slightly more release being noted in the presence of serum in one experiment and slightly less release observed in the other (data not shown).

Electron microscopy of basophils exposed to hyperosmolarity. Normal human leukocytes were exposed to 0.75 M mannitol or buffer at $37^\circ C$ for 60 min in the absence of D_2O , and the basophils were studied ultrastructurally. Net histamine release was 53%. The majority of the basophils present in each preparation were partially granulated (type II cells [12]). These cells were characterized by a mixture of cytoplasmic granules filled with particulate material and membrane-bound vacuoles which were empty or contained a

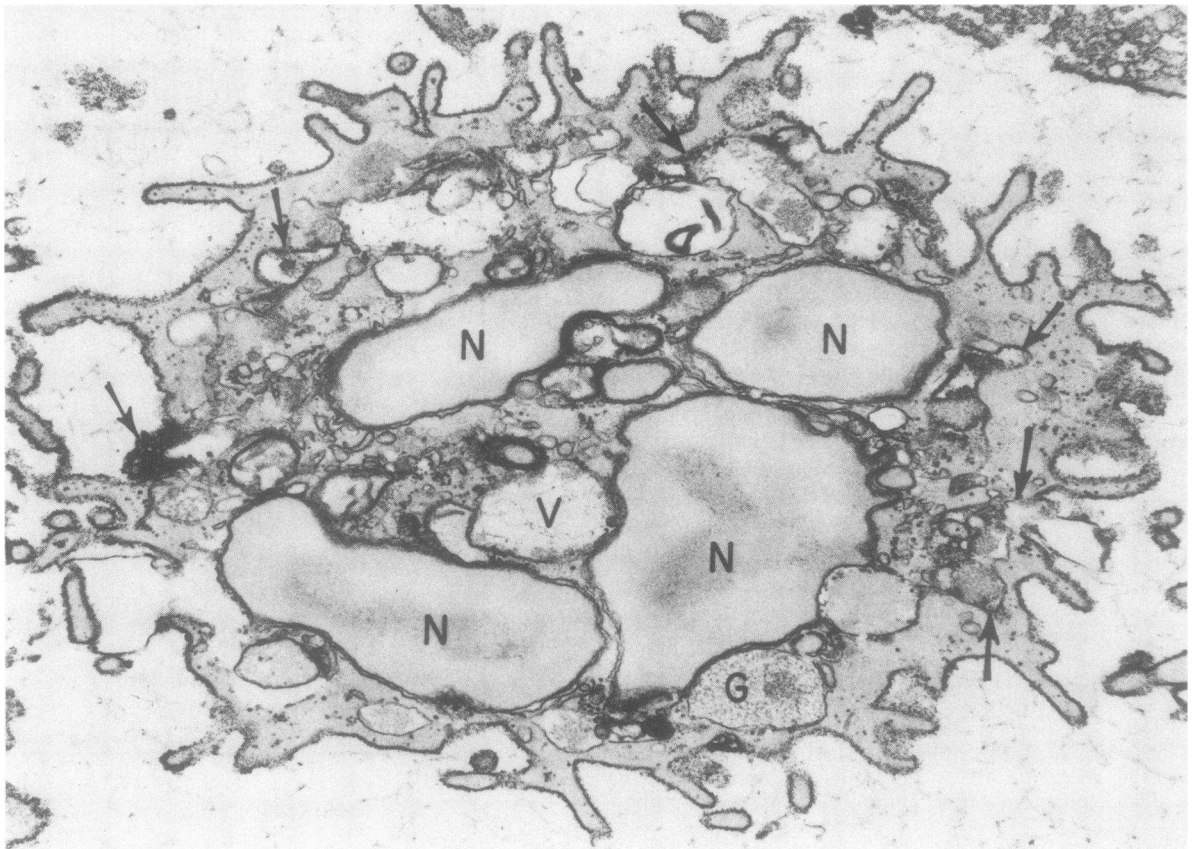


FIGURE 2 After a 60-min exposure to 0.75 M mannitol (53% histamine release), this electron micrograph of a basophil (type II, see text) shows individual extrusion of membrane-free granules into cationized, ferritin-filled spaces, which are in continuity with the cell surface (arrows). Some particle-filled cytoplasmic granules (G) and empty granule-vacuoles (V) remain. N, nucleus. $\times 22,000$.

variable amount of granule material (Fig. 2). Mannitol caused no cytotoxic ultrastructural changes in basophils (Fig. 2), i.e., the cell membrane was intact, and the cytoplasm did not contain the extracellular marker ferritin. Cells exposed to mannitol showed exocytosis of individual granules (Figs. 2, 3A, and 4A). Extruded granules retained their particulate nature, were covered with the cationized ferritin tracer, and remained adherent to the surface of the cell (Figs. 3A and 4A). Many cytoplasmic granule-vacuoles also displayed ferritin-labeled membranes, some of which were being extruded (Fig. 3B). In some cases basophils had extruded their membrane-free granules into a centrally located, separate, membrane-bound sac (Fig. 4B). These large cytoplasmic spaces were not yet in continuity with the exterior, since these structures were not labeled with ferritin. The subplasma membrane cytoplasm of most basophils was generally devoid of granules (Fig. 4C) (except those in the active extrusion process) and was filled with a fine filamentous meshwork.

Kinetics of histamine release induced by hyperosmolar mannitol. Antigen-induced histamine release is complete (depending on the degree of antigenic stimulation) in 30–45 min (13). The time-course for mannitol-induced histamine release also varied inversely with the stimulus concentration and ranged between 45 and 60 min. Fig. 5 demonstrates the time-dependent release of histamine at two different concentrations of mannitol with and without D₂O. D₂O accel-

erated the release process, especially at early times, when 0.75 M mannitol was used. Histamine release at the lower concentration (0.375 M) was low and occurred very slowly in buffer alone. Both the rate and magnitude of release were markedly augmented in the presence of D₂O. All subsequent reactions were performed using the 45-min incubation period.

Temperature dependence of histamine release by hyperosmolar mannitol. As previously reported (13), the optimum temperature for histamine release induced by anti-IgE or antigen was 37°C (Fig. 6A). In contrast, the optimum temperature for mannitol-induced histamine release at several concentrations was 32°C (Fig. 6 B–D). At 4°C, release did not occur with any concentration of mannitol tested.

Desensitization with anti-IgE and its effect on the hyperosmolar release of histamine by mannitol. In three separate experiments, one of which is shown in Fig. 7, normal leukocytes were nonspecifically desensitized with a high concentration of anti-IgE in the absence of calcium. This procedure is nonspecific because it deactivates all IgE-dependent release mechanisms (14). In each experiment, rechallenge of the leukocytes revealed that anti-IgE no longer caused histamine release (Fig. 7, upper left, insert). When these desensitized cells were challenged with hyperosmolar concentrations of mannitol, however, the cells were quite responsive. In fact, the desensitized cells were consistently slightly more reactive than non-desensitized cells (arrows, Fig. 7). This was true whether

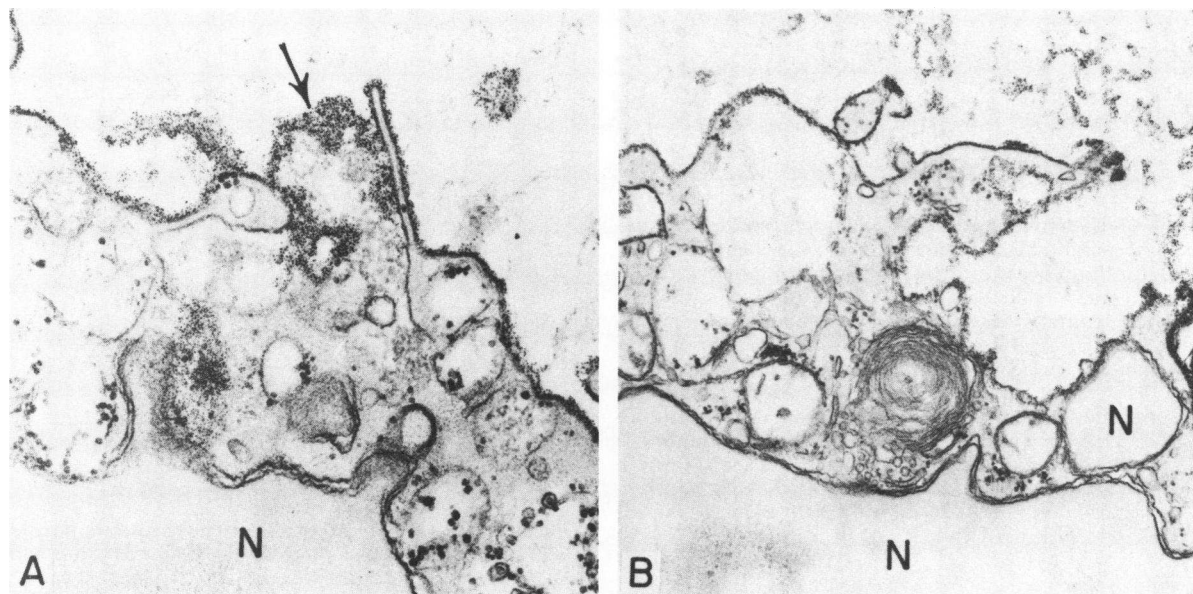


FIGURE 3 Electron microphotographs of two basophils prepared as in Fig. 2. In Fig. 3A, cationized ferritin covers the extruded membrane-free granule on the cell surface (arrow), as well as the surface of the basophil. In Fig. 3B, large empty granule-vacuole spaces are lined by the tracer and are being extruded. N, nucleus. A, $\times 42,000$; B, $\times 26,000$.

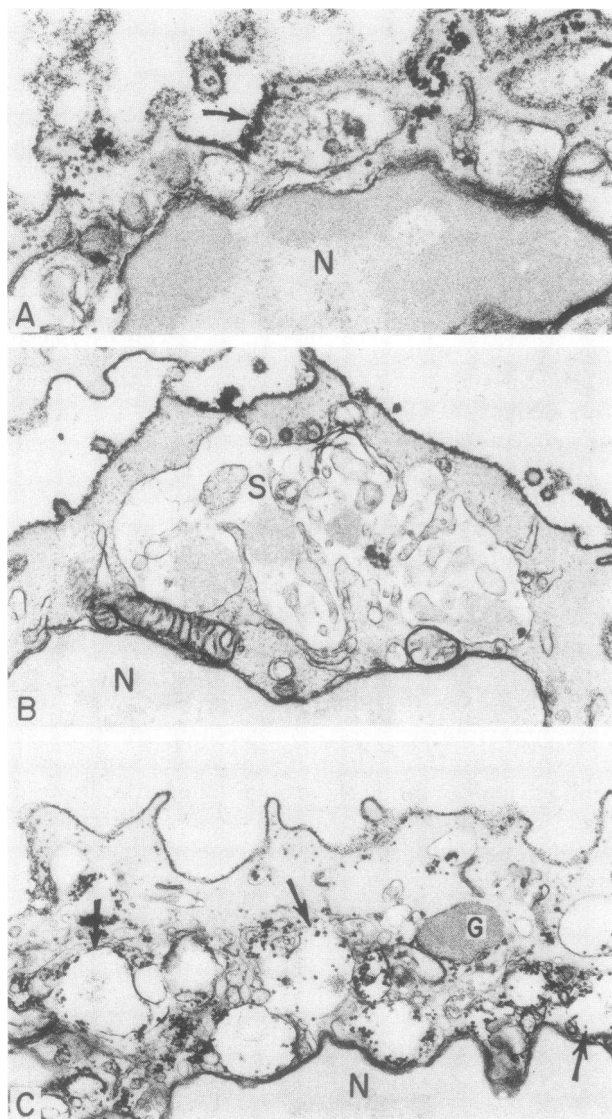


FIGURE 4 Electron micrographs of basophils after exposure to 0.75 M mannitol for 60 min. In Fig. 4A, a membrane-free granule in the process of extrusion is in continuity with the cell surface and is labeled with cationic ferritin (arrow). In Fig. 4B, a centrally located, membrane-bound cytoplasmic sac(s) contains membrane-free granules. Cationized ferritin labels the cell surface, but has not entered this space, indicating that it is not yet open to the exterior of the cell. In 4C, the subplasma membrane cytoplasmic area is devoid of granules. Deeper within the cell, an unaltered granule (G) and several partially filled granule-vacuoles are seen (arrows). No ferritin is present in these. They are therefore not yet open to the exterior. N, nucleus. A, $\times 42,000$; B, $\times 21,000$; C, $\times 22,000$.

the cells were challenged with mannitol in the presence or absence of D_2O .

Modulation of hyperosmolar, mannitol-induced histamine release. In contrasting the hyperosmolar

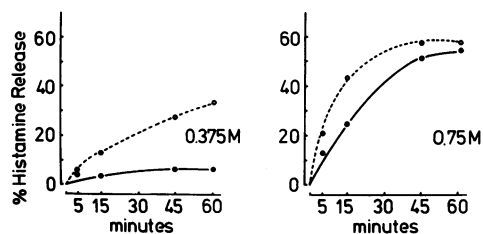


FIGURE 5 The time-dependent release of histamine from normal leukocytes at two concentrations of mannitol in a single experiment, using PCM (closed circles) or 44% D_2O PCM (open circles). Similar results were obtained in three separate experiments using leukocytes from different donors.

dependent release process with anti-IgE-induced release, we characterized each process using (a) the divalent cation chelator EDTA; (b) heat pretreatment of cells; (c) the metabolic inhibitor 2-deoxyglucose; and (d) the cyclic AMP-active drug theophylline (Table I). As shown, anti-IgE-induced release is almost completely inhibited under each condition. Unlike the anti-IgE-dependent process, either pretreatment of cells with heat (30 min at $47^\circ C$) or the addition of EDTA (1 mM) only partially reduced the histamine release response to 1.0 and 0.75 M mannitol. At the lowest

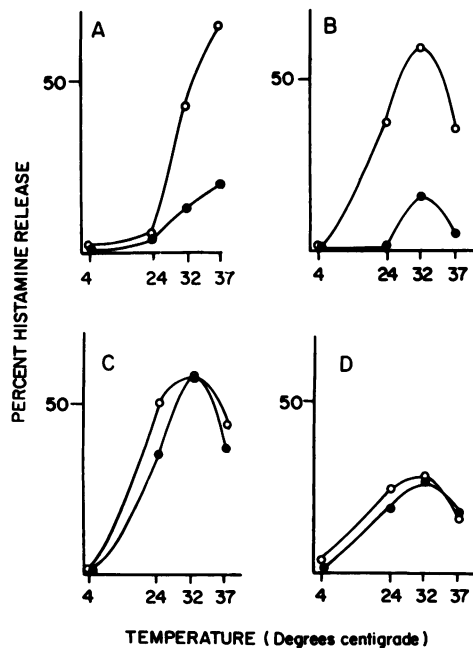


FIGURE 6 The temperature dependence of histamine release elicited by anti-IgE or hyperosmolar mannitol using normal leukocytes. Similar results were obtained in three separate experiments, one of which is depicted. Panel A depicts the percent release obtained using 0.1 $\mu g/ml$ anti-IgE. Panels B, C, and D depict percent release obtained using 0.375, 0.75, and 1 M mannitol, respectively, in the absence (closed circles) and presence (open circles) of D_2O .

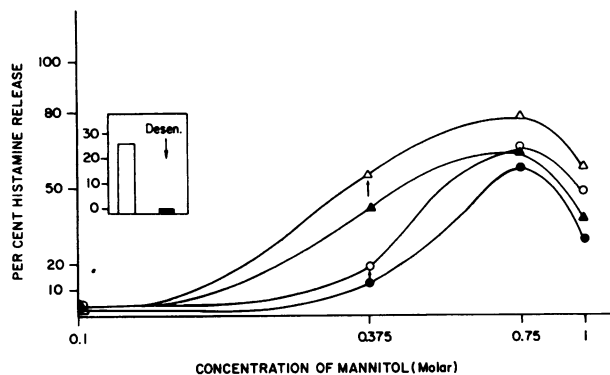


FIGURE 7 The percent histamine release in response to mannitol in normal non-desensitized and anti-IgE-desensitized leukocytes is shown. The insert shows that normal, non-desensitized cells, released 26% histamine (open bar). After desensitization, the leukocytes did not release histamine (closed bar). Desensitizing conditions were anti-IgE 1 $\mu\text{g/ml}$, EDTA 1 mM, at 37°C for 30 min in Pipes buffer. Non-desensitized cells were incubated similarly but without anti-IgE. Cells were washed twice in Pipes buffer prior to challenge with 0.1 $\mu\text{g/ml}$ anti-IgE or mannitol. When suspended in PCM (circles) or in 44% D₂O PCM (triangles), desensitized cells (open symbols) released more histamine (arrows) than non-desensitized cells (closed symbols).

concentration of mannitol tested (0.375 M), treatment with EDTA and heat markedly reduced histamine release; theophylline had no effect, and 2-deoxyglucose enhanced release. Thus, 2-deoxyglucose (10^{-3} 1 mM) and theophylline (1 mM) had no inhibitory effect on mannitol-induced histamine release at any concentration tested. Also, in three successive experiments, changing the external calcium concentration (0–1 mM) did not influence mannitol-induced release (data not shown). The data contained in Table I suggest that more than one release mechanism is triggered by hyperosmolarity. For example, with high concentrations of mannitol (1.0 M), release is statistically unal-

tered by the presence of EDTA, heat, or 2-deoxyglucose. On the other hand, inhibition by EDTA and heat exceeds 50% at the low concentration (0.375 M). This suggestion is strengthened by the observation that, at high concentrations of mannitol, 2-deoxyglucose had little effect, whereas at the lower concentration, there was a consistent augmentation, seen in three of three experiments.

The effects of inhibitors of phospholipid metabolism on hyperosmolar histamine release. Although the inhibitors and conditions cited above had little or no effect on the hyperosmolar release reaction, the effects of drugs which purportedly act on phospholipid metabolism were similar to those observed on IgE-mediated release. ETYA, which blocks both the cyclooxygenase and the lipoxygenase pathways of arachidonic acid metabolism (15, 16), inhibits anti-IgE-induced histamine release with an inhibitory dose, 50% (ID₅₀), of 40 μM (Fig. 8a). A similar effect of ETYA is seen on hyperosmolar release with an ID₅₀ at a threefold higher concentration (120 μM). BPB, a phospholipase A₂ inhibitor (17), blocks release by both types of stimuli at concentrations in the range of 0.4–0.8 M (Fig. 8b).

Histamine release as a function of osmolarity. To determine whether osmolarity alone could explain the histamine release obtained in vitro, we challenged leukocytes with several different hyperosmolar solutions. Fig. 9 presents the result of one of three experiments comparing histamine release in response to hyperosmolar solutions of sodium chloride, dextrose, Hypaque 50, and mannitol. Hyperosmolar sodium chloride was much less effective than the other compounds tested in releasing histamine. In similar experiments, mannitol was compared with its isomer, sorbitol, and to other polyhydric alcohols—erythritol (C₄), xylitol (C₅), and persitol (C₇). Both in the presence of D₂O and in PCM, all of these agents gave similar or identical release at equivalent molar concentrations (data not shown).

Release of mediators other than histamine. 13 ex-

TABLE I
Comparison of the Effect of EDTA, Heat, and Drugs on Histamine Release by Mannitol and Anti-IgE

Mannitol concentration in PCM	EDTA (1 mM)		Heat (47°C)		2-Deoxyglucose (1 mM)		Theophylline (1 mM)	
	-	+	-	+	-	+	-	+
M	% release							
1.0	22.7±3*	21.6±5*	20.5†	20.0†	24.6±1*	22.0±4*	24.6±1*	27.0±1*
0.75	52.6±8§	41.0±8§	51.5±11	19.0±7	39.7±3*	39.8±3*	39.7±3*	41.0±2*
0.375	19.6±9§	6.8±4§	18.0±13	9.0±2	9.7±5*	30.0±4*	9.7±5*	8.7±7*
Anti-IgE (0.1 $\mu\text{g/ml}$)	57.6±10§	1.8±1§	53.0±12	2.5±1	51.6±4*	7.6±4*	51.6±4*	9.3±6*

Values are the mean alone or mean±SE.

* n = 3.

† n = 2.

§ n = 5.

|| n = 4.

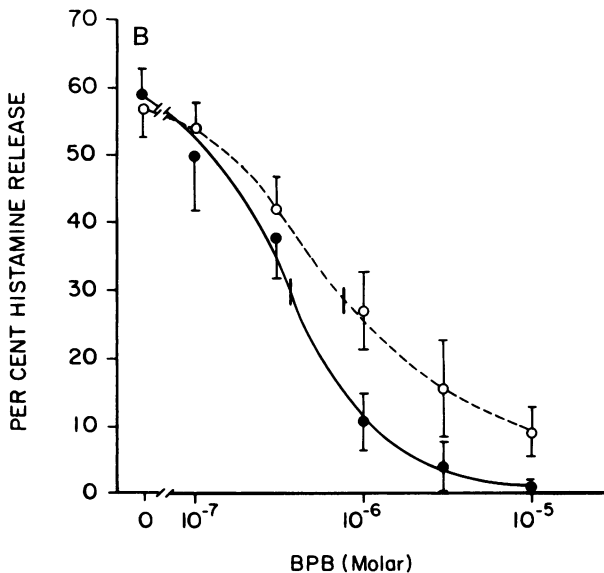
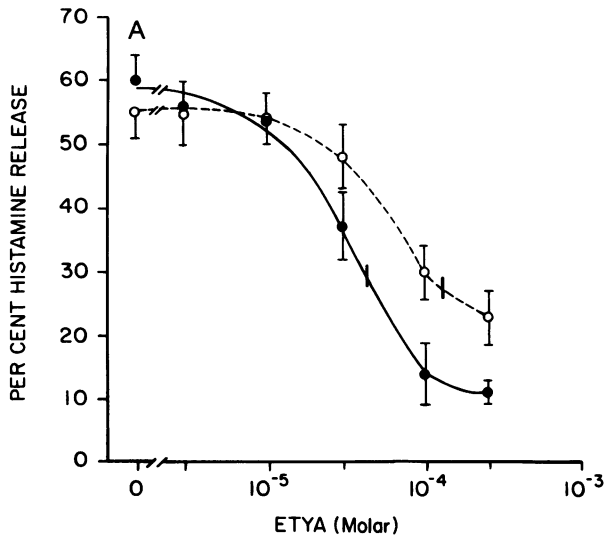


FIGURE 8 (A) The release of histamine in the absence or presence of ETYA (3×10^{-6} to 3×10^{-4} M). In four separate experiments, mannitol (0.75 M) or anti-IgE (0.1 $\mu\text{g}/\text{ml}$) was used to challenge leukocytes which had been pretreated with drug for 10 min. The ID_{50} was about 120 μM for mannitol (open circles) and 40 μM for anti-IgE (solid circles). With both stimuli incubations proceeded at 37°C for 45 min. Brackets represent the standard error of the mean values. The vertical bars on each log dose-response curve mark 50% inhibition of release. (B) The release of histamine in the presence of BPB (10^{-7} – 10^{-5} M). Concentrations of stimuli, incubation time, temperature, and symbols are the same as in Fig. 8A. The ID_{50} for mannitol was 0.78 μM , and for anti-IgE, 0.37 μM . Standard error brackets enclose mean values from four experiments.

periments were carried out to ascertain whether mannitol released slow reacting substance (SRS) (18). The leukocytes were used at concentrations ranging from

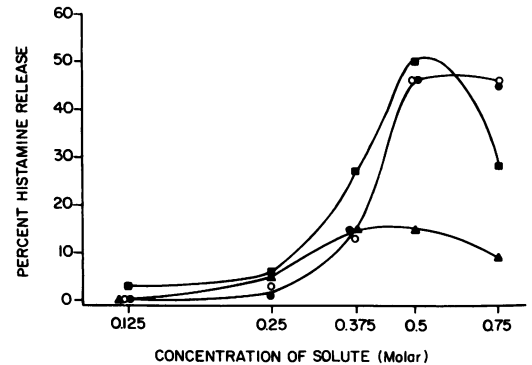


FIGURE 9 The net percent histamine release in response to hyperosmolar solutions. Sodium chloride (\blacktriangle), Hypaque 50 (\blacksquare), dextrose (\bullet), and mannitol (\circ) were incubated with leukocytes at 37°C for 45 min. Two other experiments gave similar patterns of release.

5×10^7 to $10^8/\text{ml}$, and the concentration of mannitol was 0.75 M. The histamine release averaged $44 \pm 30.7\%$, with a range from 9 to 95%. SRS was undetectable in any of these specimens; the limit of SRS detection was 70 U/ml. With similar amounts of histamine release induced by antigen or anti-IgE, there would have been detectable SRS (18). Three experiments were carried out to see whether another preformed mediator, *p*-toluene sulfonyl-L-arginine (TAME) esterase, was released (19, 20). In each case, the dose-response curves for histamine and esterase release were similar in shape, although, as previously reported, there was no quantitative relationship between the two (data not shown). Thus, like C5a, mannitol appears to release preformed, but not newly synthesized, basophil mediators.

DISCUSSION

Idiopathic reactions to a number of substances which are infused into humans at high molar concentrations, e.g., mannitol and radiocontrast media, appear to be independent of classic anaphylactic mechanisms. We have attempted to determine the characteristics of these reactions by studying the direct effects of hyperosmolar solutions of mannitol on human basophils. In every donor tested, histamine release was obtained at concentrations between 0.3 and 1 M mannitol. A number of polyhydric alcohols were found to substitute readily for mannitol. Hyperosmolar sodium chloride was, however, not as effective. The reasons for this are not clear, but may lie in the relative ease of transmembrane diffusion of these ions as compared with the other solutes. It is also possible that the high concentrations of these ions play an independent inhibitory role, thus preventing hyperosmolar triggering of release.

The release process in normal donors does not appear to be toxic or lytic and does not depend on basophil-bound IgE. These conclusions were based on the

following data: (a) the release process was inhibited by concentrations of mannitol >0.75 M; (b) release did not occur at 4°C and was inhibited at temperatures of $>32^{\circ}\text{C}$; (c) release was partially inhibited by heat pretreatment and EDTA; (d) 100% release was never observed ($n = 21$), nor was hemolysis noted; (e) inhibitors of phospholipid and arachidonate metabolism (BPB, ETYA) very effectively inhibited release by mannitol; (f) cells desensitized nonspecifically by anti-IgE released histamine in response to mannitol; (g) LDH release from human basophils was minimal following mannitol exposure; and (h) electron micrographic studies of basophils exposed to hyperosmolar mannitol (0.75 M) showed exclusion of generalized cytoplasmic staining with the tracer ferritin. The basophils in this study also showed none of the characteristics of damaged cells, such as membrane breakage, swollen organelles, lucent cytoplasm, or nuclear pyknosis. The most conclusive evidence that the cells in these studies were not damaged is, however, that they totally excluded the extracellular tracer ferritin.

It is of considerable interest that drugs which affect glucose metabolism (2-deoxyglucose) or intracellular cyclic AMP levels do not inhibit hyperosmolar release. This is in marked contrast to IgE-mediated release. On the other hand, ETYA and BPB, which impair phospholipid and arachidonate metabolism, inhibited hyperosmolar release at concentrations similar to those effective in IgE-mediated reactions. The only precedent for this pattern of inhibition can be found in recent experiments carried out in our laboratory with phorbol myristate acetate-induced release. This release is a slow, noncytotoxic process that is variably affected by cyclic AMP-active drugs and is only partially dependent upon calcium. Phorbol myristate acetate-induced release is, however, completely inhibited by ETYA and BPB. Possibly both phorbol myristate acetate and mannitol induce release by a unique mechanism which involves the activation of phospholipid metabolism.

The experiment with partially purified basophils (37%) suggests that cell-cell interaction plays a minor role in mannitol-induced release of basophil histamine. The basophils responded very well in the absence of polymorphonuclear leukocytes and eosinophils, and with greatly reduced numbers of mononuclear cells relative to basophils.

Previously, using similar techniques, we described the morphology of antigen-induced IgE-mediated degranulation of human basophils (12). In that study it was useful to classify basophils before antigenic stimulation, but after isolation procedures, as type I (fully granulated), type II (partially granulated), or type III (cytoplasm-containing granule-vacuoles that were completely devoid of granule particulate content). Following exposure to antigen, individual granules fused with

the cell membrane at multiple points, and the granule contents were extruded, often remaining attached to the cell membrane for a short time. This process paralleled the release of histamine, and resulted in viable cells which were devoid of all granules, except for a smaller, less dense granule type which was not extruded. When histamine release was completed and high ($>90\%$), one could no longer find any type II cells.

Both significant differences and similarities between the antigen-stimulated process described above and the mannitol-induced degranulation were noted. Using normal donor cells, we found nearly all basophils exposed to mannitol (0.75 M) to be type II, i.e., partially granulated. In other words, in nearly all of these cells individual granule extrusion was present, but the cells also retained numerous granules and granule-vacuoles. Cationized, ferritin-stained, empty vacuoles were numerous, indicating continuity with the cell surface. Extrusion of the membranes of previously emptied granules also was a prominent process. Granule extrusion was never seen in control cells.

Many of the remaining granules were centrally located. Often this process left the cytoplasm beneath the cell surface devoid of granules and filled with a more extensive filamentous array than was seen in control cells or in cells undergoing degranulation in response to antigen. A small number of basophils were present with centrally placed sacs filled with membrane-free granules. These sacs were not yet in continuity with the exterior, as the cationic ferritin tracer was not present in these sacs. These central granule-containing sacs induced by mannitol are similar to those found after the anaphylactic degranulation of guinea pig basophils, a process which is morphologically dissimilar to that of human basophils (21, 22). These morphological differences between IgE-mediated and mannitol-induced degranulation of human basophils, although subtle, clearly distinguish between these release mechanisms.

We chose to study the direct effect of mannitol and other hyperosmolar solutions on basophil histamine release in the absence of serum since we observed no differences in release with or without serum. Also, although involvement of the complement system has been implicated both *in vivo* and *in vitro* (23, 24) in animals (25) and in man (26), no definitive correlations have been established between the changes noted and clinical reactions. Too, controversy exists as to the pathway activated (23, 27) and whether the decrease in complement components is due to true activation or to nonsequential cleavage of components of this system by the contrast media or by proteases involved in the clotting cascade (28).

In summary, we have described a unique noncytotoxic mechanism for histamine release from basophils exposed to hyperosmolar solutions. When considering

the clinical implications of this work, it should be noted that the hyperosmolar-dependent histamine release occurred using cells of all donors. We would therefore expect all patients receiving a rapid hyperosmolar infusion to release histamine. In fact, this has recently been demonstrated in patients undergoing cardiac catheterization (27). Anaphylactoid reactions in response to hyperosmolar glucose infusions have also recently been reported (29). It is likely that the mast cell, another IgE-bearing, histamine-containing cell is responsible for such reactions in vivo. Rockoff and co-workers (30) suggested that contrast media released histamine from mast cells due to their hyperosmolarity and Mann (31) in the 1960's first suggested the involvement of histamine in anaphylactoid reactions to radio-contrast media (31). More recently, in vivo histamine release has been found in rabbits following infusion of hyperosmolar solutions (25). Additionally, our data, as well as those of Rockoff et al. (30), show that, of the solutes used, NaCl was the least effective in causing mediator release or changes in complement components. Whether a hyperosmolar-dependent release process is principally responsible for severe anaphylactic-like reactions has not yet been established. It has been noted, however, that there are marked differences in the "releasability" of histamine and other mediators in the cells of patients with asthma and urticaria (32). Therefore, there may exist individuals who are abnormally sensitive to hyperosmolar stimuli. We have observed one such patient whose bronchospastic reaction to mannitol infusion was associated with an increased (twofold) in vitro sensitivity of his basophils to mannitol. It was, in fact, this patient who initiated the studies described above (manuscript in preparation).

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