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MECHANISM OF ACTION OF DISODIUM CROMOGLYCATE—MAST CELL CALCIUM ION INFLUX AFTER A HISTAMINE-RELEASING STIMULUS

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Abstract—Rat peritoneal mast cells release histamine and accumulate ^{45}Ca in a dose-dependent manner when concentrations of compound 48/80 ranging from 0·1 to 1·0 μg/ml are incubated with suspensions of the cells for 5 min at 37°. Influx of ^{45}Ca stimulated by compound 48/80 can be inhibited to varying degrees by prior addition of disodium cromoglycate. Inhibition was dependent on the concentration of both disodium cromoglycate and compound 48/80. The electrokinetic properties of intact rat mast cells are described; disodium cromoglycate caused a plasma membrane alteration possibly related to Ca^{2+} influx. Cromoglycate increased mast cell electrophoretic mobility but decreased the electrophoretic mobility of rat erythrocytes. The net electrophoretic mobility was a function of terminal sialic acid residues, ionic strength, and pH. Binding of disodium cromoglycate to Ca^{2+} could not be demonstrated by a variety of sensitive physical techniques. The data support the theory that secretion of mast cell histamine is coupled to Ca^{2+} influx. It is suggested that disodium cromoglycate prevents mast cell histamine release by a plasma membrane alteration which prevents an increase in membrane permeability to Ca^{2+} stimulated by compound 48/80.

Disodium cromoglycate is effective in the treatment of certain types of asthma [1]. The drug appears to have a direct protective effect on mast cells [2]. Disodium cromoglycate *in vitro* prevents histamine release from mast cells caused by compound 48/80 [3], dextran [4], surface-active agents such as Tween 20 [2], and the addition of antigen to previously sensitized cells [5].

The mechanism of mast cell histamine release has been extensively studied, and it is known that calcium is required for release by compound 48/80 [6], or release induced by the antigen–antibody reaction [7]. It has been suggested that, after the attachment of the releasing stimulus to the mast cell membrane, there is a change in the calcium permeability of the cell membrane followed by an influx of calcium into the cell [8]. The importance of Ca^{2+} in the coupling of stimulus to secretion is well established in many systems [9]. Recently it has been demonstrated that injection of Ca^{2+} into mast cells causes histamine release in the absence of any other stimulus to secretion [10]. Calcium ionophores, A2318 and X537A, have also been used to demonstrate that increasing the level of intracellular Ca^{2+} is sufficient to cause histamine release [11].

The available evidence indicates that Ca^{2+} ions are absolutely required and alone are sufficient to cause histamine release from mast cells. The mechanism of disodium cromoglycate's protective effect on mast cells is unknown, but the drug does not interfere with the binding of antigen to previously sensitized cells [5]. The present studies were undertaken to determine if disodium cromoglycate might prevent histamine release from rat peritoneal mast cells by preventing calcium influx in response to a histamine-releasing stimulus. Compound 48/80 was used to stimulate histamine release because at low concentrations its effect is similar to the antigen antibody reaction [3]. Before the effect of cromoglycate on mast cell Ca^{2+} influx could be studied, it was necessary to demonstrate that compound 48/80 caused increases in mast cell Ca^{2+} levels which were related to histamine release. The electrokinetic properties of the mast cell surface were studied to determine if an alteration in the mast cell plasma membrane could be correlated with a change in ion permeability.

MATERIALS AND METHODS

For each experiment mast cells were obtained from the peritoneal cavities of several female Holtzman rats (300–400 g) by the procedure of Lagunoff [12]. After decapitation, 15 ml of phosphate-buffered salt solution (150 mM NaCl, 1 mM $CaCl_2$, 4 mM Na_2HPO_4, 2·5 mM KH_2PO_4; 5·6 mM dextrose, and 0·1% albumin, pH 6·8) was injected into the peritoneal cavity, and after 1–2 min of gentle massage, the cavity was opened and the fluid removed with a glass pipette. The mast cells were collected by centrifugation at 200 *g* for 5 min at 5°. To separate the mast cells from other peritoneal cells, the cell pellet from four rats was resuspended in 5 ml of the phosphate-buffered salt solution and layered over 5 ml of 35% albumin. After centrifugation at 200 *g* for 20 min at 5°, the top 7 ml of fluid and cells was carefully removed from the gradients. The bottom 3 ml of albumin solution, which contained the mast cell pellet, was diluted 1:1 with phosphate-buffered salt solution. The cell pellet was resuspended and the mast cells were collected by centrifugation at 200 *g* for 5 min at 5°. The cells were washed twice with the phosphate-buffered salt

solution and then resuspended in the buffer at approximately 5×10^5 cells/ml. This procedure routinely yielded mast cells of greater than 80 per cent purity. It has been established that sedimentation of mast cells through high concentrations of albumin only slightly affects histamine content and does not affect the ultrastructural appearance of the cells [12].

For studying Ca^{2+} uptake into purified mast cells, 2 ml of cells (containing approximately 10^6 cells) was incubated at 37° in phosphate-buffered salt solution, pH 6·8, for 5 min prior to the addition at time zero of 5 μl ^{45}Ca (1·25 μCi) obtained from New England Nuclear. At 1 min, small volume additions were made of various concentrations of disodium cromoglycate, the disodium salt of 1,3-bis-(2-carboxychromon-5-yloxy)-2-hydroxypropane, a gift of Fisons Pharmaceuticals. Immediately after the addition of disodium cromoglycate, compound 48/80 (approximately 10 μl of various concentrations) was added to the appropriate tubes. Compound 48/80, a gift of Burroughs Wellcome, is a condensation product of *N*-methylhomoanisylamine, formalin and HCl. After addition of 48/80, the incubation was continued for 5 min at 37°. To terminate incorporation, 4·0 ml of ice-cold phosphate-buffered salt solution was added to each tube and the cells were collected by centrifugation at 500 *g* for 3 min. The cells were rapidly washed twice with cold phosphate- buffered salt solution. This procedure was also used to study the effect of disodium cromoglycate on the ^{45}Ca uptake of embryonic 3T3 mouse fibroblasts maintained in monolayer culture and harvested with a rubber policeman at confluency. The cells were maintained in Dulbecco's medium plus 10% calf serum as previously described [13]. The final cell pellets were solubilized in 0·2 ml of 1·0 N NaOH and radioactivity was determined by liquid scintillation counting. Results are expressed as cpm/10^6 cells. Cell numbers were determined with a Coulter Counter Research Model B.

To measure mast cell histamine release during incubation, after the cells were removed by centrifugation, the undiluted supernatants were assayed spectrofluorometrically according to the procedure of Loeffler *et al.* [14]. Results were expressed as the per cent of total cell histamine obtained by boiling the mast cell suspensions for 5 min.

Measurements of electrophoretic mobility were performed as previously described [15]. Mobilities of the particles were calculated in $\mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$; each value was obtained by timing the movement of at least 100 mast cells. Determinations of mobility were made either in a solution of 0·0145 M NaCl, 4·5% sorbitol, 0·6 mM $NaHCO_3$, pH 7·2 (termed saline sorbitol) or in a solution of 0·145 M NaCl containing 0·6 mM $NaHCO_3$, pH 7·2 (termed saline). To study drug effects on electrophoretic mobility, disodium cromoglycate was either added to the cells and removed by washing prior to mobility determinations or the drug was added to the solutions used for electrophoresis. The ionic strengths of the saline and saline sorbitol were kept constant. To study the effect of varying the pH of the measuring solution on cell mobility, isotonic solutions of NaOH and HCl for adjusting the pH were prepared as described by Heard and Seaman [16]. In some cases the mast cells were treated with neuraminidase (*Clostridium perfringens*, EC 3.2.1.18), obtained from Worthington Biochemical Corp. To remove terminal cell surface sialic acid residues, neuraminidase treatment was for 30 min with 40 μg/ml of enzyme.

Two procedures were utilized in an attempt to demonstrate binding of Ca^{2+} by disodium cromoglycate. According to the method of gel filtration described by Colman [17], a known amount of drug, 1·2 mg, was dissolved in buffer, 0·1 M ammonium acetate containing a known concentration of calcium ion, 10^{-6} M to 10^{-2} M, with tracer ^{45}Ca added, approximately 50,000 cpm/ml. The drug solution was then applied to a column of Sephadex G-10 equilibrated and eluted with the ammonium acetate buffer containing the same concentration of free calcium ion which was used to dissolve the drug. The concentration of drug emerging from the column was monitored by its ultraviolet absorption at 239 nm. If binding occurs, the ultraviolet absorption peak is accompanied by a rise in the concentration of ^{45}Ca above the baseline level as determined by liquid scintillation counting.

Murexide (J. T. Baker Chemical Co.), a metallochromic indicator, and a dual wavelength, double beam Aminco Chance spectrophotometer were employed according to the method of Ohnishi *et al.* [18] to measure Ca^{2+} binding by disodium cromoglycate. The experimental conditions used were: 70 μM murexide, 40 mM HEPES (pH 7·0), 85 mM KCl with varying concentrations of cromoglycate (1–10 mM) present. The difference in optical density between 507 and 535 nm was measured after small increments of Ca^{2+} were added to the cuvette to a final Ca^{2+} concentration of 0·1 mM.

RESULTS

The incubation of rat peritoneal mast cells with small amounts of compound 48/80 for 5 min at 37° resulted in a dose-dependent increase in cellular ^{45}Ca (Table 1). The influx of ^{45}Ca after the addition of compound 48/80 could be detected after short incubation times, 1–3 min, but the results were most consistent after a time interval of 5 min. More variable results were obtained with the low concentration of compound 48/80 (0·1 μg/ml) and with concentrations above 1·0 μg/ml (data for concentrations higher than 1·0 μg/ml not reported). Since it was necessary to wash the cells in buffer minus ^{45}Ca, it is possible that calcium exchange occurred before radioactivity was determined. Although the probability of ^{45}Ca loss negates the possibility of quantifying Ca^{2+} influx stimulated by compound 48/80, accurate qualitative statements are justified by the consistency of duplicates within an experiment and the reproducibility of the amount of drug-stimulated ^{45}Ca influx between experiments. All data are means ± 1 S. D. of four experiments performed in duplicate.

Incubation of rat peritoneal mast cells with disodium cromoglycate, at the concentrations tested, decreased accumulation of ^{45}Ca as compared with untreated, control cells (Table 1). The observation that cromoglycate could prevent influx of ^{45}Ca into untreated cells was confirmed with 3T3 mouse fibroblasts. Using the same conditions of temperature, time, cell and drug concentrations that affected mast

Table 1. Rat mast cell ^{45}Ca influx induced by compound 48/80 and its inhibition by disodium cromoglycate*

Disodium cromoglycate (mM)	^{45}Ca activity (cpm 10^6 mast cells)			
	Compound 48/80 (μg/ml)			
	0	0·1	0·2	1·0
0	1971 ± 238	2400 ± 100	2718 ± 200	3754 ± 300
0·05	1628 ± 100	2168 ± 50	2300 ± 120	3017 ± 90
0·25	1640 ± 193	2100 ± 100	2251 ± 100	2370 ± 40
0·5	1600 ± 56	2000 ± 150	2041 ± 225	2357 ± 200

* Cells were purified as described in the text. At time zero 5 μl ^{45}Ca (1·25 μCi, carrier free) was added to a 2-ml suspension of mast cells in phosphate-buffered salt solution, pH 6·8. At 1 min either cromoglycate in buffer or buffer alone (50 μl) was added to the tubes followed immediately by compound 48/80 (2 μl) at the concentrations specified in the table. The incubation was continued for 5 min at 37°. All determinations are averages of four experiments performed in duplicate. Determination of radioactivity and further experimental details are described in the text.

cell calcium ion levels, a dose-dependent inhibition of ^{45}Ca uptake into the fibroblasts was demonstrated (Fig. 1).

The data in Table 1 clearly demonstrate that disodium cromoglycate, added to mast cell suspensions immediately before the addition of compound 48/80, decreases accumulation of cellular ^{45}Ca. The effect of 0·5 mM disodium cromoglycate was not significantly different from the decrease in ^{45}Ca accumulation seen when cells were pretreated with 0·25 mM cromoglycate. However, addition of 0·25 mM sodium cromoglycate as compared with 0·05 mM more effectively decreased ^{45}Ca accumulation caused by the addition of 1 μg/ml of compound 48/80. All of the drug concentrations tested were partially effective in preventing increased cellular ^{45}Ca after treatment with compound 48/80, but at the lowest concentration (0·05 mM disodium cromoglycate/ml), 1 μg of 48/80 per ml still caused a significant accumulation of cellular ^{45}Ca.

To establish the relationship between ^{45}Ca influx and histamine release, it was necessary to measure histamine release under the present experimental conditions. The effect of both compound 48/80 and disodium cromoglycate on the histamine content of the mast cells was highly consistent. More variability was observed between control cell suspensions. The data of Fig. 2 indicate that only 0·2 and 1·0 μg of 48/80 per ml produced significant histamine release. This correlates with the ^{45}Ca influx data presented in Table 1. In the absence of compound 48/80, histamine release was decreased below control levels by cromoglycate. From the data of Fig. 2 it is evident that 0·25 mM disodium cromoglycate inhibited histamine release caused by 1·0 μg of 48/80 per ml, approximately 40 per cent. A lower concentration, 0·05 mM cromoglycate, inhibited release 20 per cent. Referring to Table 1 and the values obtained for ^{45}Ca influx at these concentrations of cromoglycate in the presence of 1 μg of 48/80 per ml, it can be seen that when the control values are subtracted, the percentage inhibition of ^{45}Ca influx by cromoglycate is the same as its inhibition of histamine release.

To study the effect of disodium cromoglycate on the external mast cell surface, the technique of whole cell electrophoresis was utilized. In saline–sorbitol, female rat peritoneal mast cells were found to have an electrophoretic mobility of

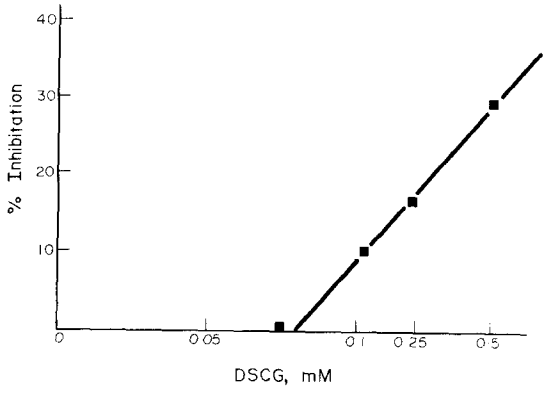

Fig. 1. Inhibition of 3T3 fibroblast ^{45}Ca uptake by disodium cromoglycate (DSCG). Cromoglycate (25 μl) was added to 1·0-ml incubations containing approximately 10^6 cells. At 1·0 min, 2·0 μl ^{45}Ca was added to each tube and the incubation was continued for 5 min. Values are averages of three determinations performed in duplicate.

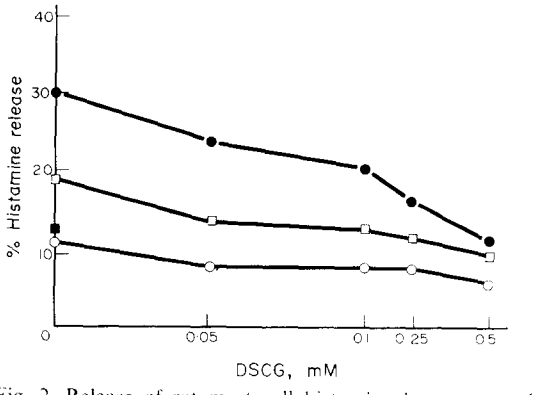

Fig. 2. Release of rat mast cell histamine by compound 48/80 and inhibition of release by disodium cromoglycate. Experiments were performed as described in Methods. Values are percentages of total cell histamine obtained by boiling the cells, and they have not been corrected for control release. Each point represents an average of three experiments performed in duplicate. Key: (○ ○) no 48/80 (■ ■) 0·1 μg; (□ □) 0·2 μg; and (● ●) 1 μg of 48/80 per ml.

Table 2. Effect of disodium cromoglycate on the electrophoretic mobility of rat mast cells*

Disodium cromoglycate (mM)	Electrophoretic mobility ($\mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$)
0 (control)	$-1{\cdot}74 \pm 0{\cdot}07$
0·1	$-1{\cdot}94 \pm 0{\cdot}08$
1·0	$-2{\cdot}30 \pm 0{\cdot}07$

* Mobilities were measured in 0·145 M NaCl, 0·6 mM $NaHCO_3$ (pH 7·2) at 25°. Experiments were performed as described in the text. Data are means of four experiments ± 1 S.D.

$-3{\cdot}25 \pm 0{\cdot}15\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$. This indicates that the cells have a high negative surface charge. In all cases a unimodal pattern of the observed mobilities was found, indicating that the mast cells were a homogeneous population. In the whole cell electrophoresis system, human erythrocytes had a mobility of $-2{\cdot}80\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$, and rat erythrocytes had an electrophoretic mobility of $-3{\cdot}05\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$. The value reported by Heard and Seaman [16] for human erythrocytes was $-2{\cdot}78\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$. The effect of disodium cromoglycate on the mobility of mast cells was studied by adding the drug to the cells suspended in saline (pH 7·2) just prior to electrophoresis. The ionic strength of the solution was kept constant. As seen in Table 2, the mobility of mast cells in saline, as expected, is much less than in the low ionic strength saline–sorbitol. However, as observed microscopically, the cells appeared to be more stable, particularly after drug addition, in saline. The addition of disodium cromoglycate to mast cells suspended in physiological saline increased their electrophoretic mobility in a dose-dependent manner (Table 2). The same dose-related increases in electrophoretic mobility after drug addition were found if the cells were washed twice with fresh saline minus drug prior to mobility measurements. Disodium cromoglycate at 0·1 mM, which increased the mobility of mast cells, had no effect on rat erythrocytes suspended in saline–sorbitol (Table 3). At a concentration of 1 mM or greater, cromoglycate decreased the electrophoretic mobility of rat erythrocytes in a dose-dependent manner (Table 3).

To further elucidate the nature of the mast cell surface change caused by cromoglycate, the electrokinetic properties of the mast cell surface were studied. The cells were treated with increasing concentrations of *C. perfringens* neuraminidase, which selectively removes terminal *N*-acetylneuraminic acid residues from surface membrane glycoconjugates. The electrophoretic mobility of mast cells, treated with 40 μg/ml of neuraminidase for 30 min and measured in saline–sorbitol, was reduced from $-3{\cdot}25$ to $-2{\cdot}25\ \mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$. The decrease in electrophoretic mobility is not a result of nonspecific absorption of the enzyme, since boiled neuraminidase had no effect. A curve of mobility versus pH indicated that the half maximum mobility of the cells occurred at a bulk solution pH of 4·3. The surface pK is calculated from this value, using a correction factor [19] which takes the higher H^+ ion concentration at the surface into consideration. The calculated surface pK is approximately 3·5. Perhaps more significant is the fact that, when the isoelectric point (zero mobility) of the mast cell membrane is reached, it becomes positively charged, indicating the presence of cationic groups on the membrane surface. Disodium cromoglycate is negatively charged. If it were to interact ionically with a positively charged surface group, the effect might be to increase negativity and electrophoretic mobility. Since it has been reported that calcium is present on the mast cell membrane [6] and since an effect of disodium cromoglycate on mast cell calcium influx had been demonstrated, we investigated the possibility that the drug might bind Ca^{2+}. Using the techniques of gel filtration and the murexide test for free Ca^{2+}, no binding of cromoglycate to Ca^{2+} over a wide range of ion concentrations (1 μM to 10 mM) was detected. EDTA at the same concentration as the drug was used as a control.

Table 3. Effect of disodium cromoglycate on the electrophoretic mobility of rat erythrocytes*

Disodium cromoglycate (mM)	Electrophoretic mobility ($\mu m \cdot s^{-1} \cdot V^{-1} \cdot cm$)
0 (control)	$-3{\cdot}05 \pm 0{\cdot}4$
0·1	$-3{\cdot}04 \pm 0{\cdot}10$
1·0	$-2{\cdot}80 \pm 0{\cdot}08$
2·0	$-2{\cdot}49 \pm 0{\cdot}06$

* Mobilities were measured in 0·0145 M NaCl, 4·5% sorbitol, 0·6 mM $NaHCO_3$, pH 7·2, at 25°. Experiments were performed as described in the text. Data are means ± 1 S.D. of four experiments.

DISCUSSION

The results presented demonstrate that compound 48/80 causes a dose-dependent influx of calcium ions when added to a suspension of rat peritoneal mast cells. It has previously been shown that histamine release from mast cells can be stimulated by increasing cellular Ca^{2+} levels [10] and that increased amounts of calcium are associated with sensitized rat peritoneal mast cells upon addition of antigen [11]. The concentration range of compound 48/80 used in these experiments (0·1 to 1·0 μg/ml) has been shown to produce a dose-dependent histamine release from rat mast cells [3]. Although compound 48/80 was not as effective under our experimental conditions, it did produce a dose-related loss of mast cell histamine in the range of 0·1 to 1·0 μg of 48/80 per ml. The increased levels of cellular Ca^{2+} detected here were directly related to the amount of compound 48/80 added. This finding is in agreement with the dose-dependent nature of histamine release by compound 48/80. The close correlation between the concentration of the histamine-releasing stimulus, Ca^{2+} influx and the amount of mast cell histamine released suggests a cause-and-effect relationship between influx of Ca^{2+} and release of histamine.

The main purpose of these studies was to elucidate the mechanism whereby disodium cromoglycate prevents histamine loss from mast cells in response to a releasing stimulus. The data presented show that disodium cromoglycate prevents the influx of Ca^{2+}

triggered by addition of compound 48/80. Previous reports [2, 3] on the efficacy of disodium cromoglycate have indicated that the drug will not completely prevent histamine release from rat mast cells by compound 48/80 *in vitro*. At the highest concentration of cromoglycate (0·5 mM) studied here, the influx of Ca^{2+} into compound 48/80-treated cells could not be completely prevented. The drug was more effective at preventing Ca^{2+} influx at high concentrations. It was less effective against a stronger histamine-releasing stimulus, increased amounts of compound 48/80. These data indicate a strong correlation between the effect of disodium cromoglycate on Ca^{2+} influx and its inhibition of histamine release by compound 48/80. Incubation of rat peritoneal mast cells with only disodium cromoglycate decreased calcium levels below control cell values. This suggested that inhibition of Ca^{2+} influx by the drug is a primary effect of disodium cromoglycate rather than a consequence of the drug's inhibition of histamine release. Since disodium cromoglycate prevents Ca^{2+} uptake by 3T3 fibroblasts, it appears that the effect of the drug on Ca^{2+} influx is not specific for mast cells or limited to histamine-releasing conditions.

The sequence of events leading to histamine release from mast cells has been extensively studied. The first event is thought to be a membrane alteration resulting from the combination of antigen or compound 48/80 with the mast cell surface. Disodium cromoglycate does not prevent the antigen–antibody reaction [5], and it is thought to prevent histamine release by inhibition of a step subsequent to the initial binding of the releasing stimulus to the mast cell membrane [4, 5]. Ca^{2+} is necessary for the activation of mast cells by dextran, which triggers histamine release [20]. It has been suggested that the antigen antibody stimulus opens calcium "gates" in the membrane, permitting an influx of Ca^{2+} [8]. If calcium is not present when the stimulus is given, no histamine is released and the "gates" close so that the cells are desensitized to subsequent stimuli.

It seems probable that part of the ^{45}Ca uptake measured here represents exchange diffusion, while the remainder is the result of an active process. In accordance with the previously described theory of mast cell histamine release [8], the drug might be preventing calcium "gates" from opening or it might compete with Ca^{2+} ions for entry into the cell. Since we have shown that cromoglycate does not bind Ca^{2+}, this cannot be the explanation for the effect of the drug on Ca^{2+} influx. After activation of the cells by antigen, intracellular granules which contain histamine complexed to a protein–heparin matrix become exposed to the extracellular milieu by a process which does not necessarily involve loss of granules from the cells [12, 21]. Lagunoff [12] has described invaginations of the mast cell membrane which interiorize extracellular fluid, thereby bringing granules in contact with extracellular fluid without actually propelling them to the surface of the cell. Histamine release is thought to occur by a simple cation exchange of sodium ions for histamine [22]. The role of elevated cellular calcium in the final series of events leading to histamine release has not been determined.

The alteration of the electrokinetic properties of the mast cell surface by disodium cromoglycate suggests that the effect of the drug on Ca^{2+} influx is due to a membrane alteration. Inhibition of cyclic AMP phosphodiesterase by cromoglycate has been demonstrated [23]. It is possible that inhibition of Ca^{2+} influx by cromoglycate may be partially or totally related to increased mast cell cyclic AMP. This is the first characterization of the electrokinetic properties of the rat mast cell surface membrane. The titration curve of the mast cell surface indicated the presence of positively charged groups, and the presence of Ca^{2+} on the mast cell surface has previously been demonstrated [6]. Using two highly sensitive techniques, no binding of disodium cromoglycate to calcium could be detected. Since neuraminidase reduced mast cell mobility approximately 30 per cent, it was apparent that sialic acid contributed significantly to the high negative charge at the mast cell surface. Sialic acid is also concentrated on the surface of erythrocytes [24]. Disodium cromoglycate altered the electrophoretic mobility of erythrocytes less effectively and in a different manner from its effect on mast cells. Since the drug's alteration of mast cell mobility was specific, it seemed unlikely that it was modifying sialic acid groups on the cell surface. It would not be necessary for disodium cromoglycate to bind to the mast cell surface for cell mobility to be increased. Perturbations of the membrane could result in different charged groups being expressed at the hydrodynamic plane of shear, the surface layer where mobility is measured.

The data presented here demonstrate that a histamine-releasing stimulus causes a dose-dependent influx of Ca^{2+} ions into mast cells which can be prevented by disodium cromoglycate, a drug used to prevent histamine release in the treatment of asthma. The data suggest that the drug modifies the mast cell membrane. It is tempting to speculate that this modification is a stabilization of the cell membrane which prevents the increase in Ca^{2+} permeability accompanying antigen stimulation.

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