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Studies on Histamine-Retaining Granules Obtained from Isolated Rat Mast Cells

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Abstract

Histamine-retaining granules were isolated from rat mast cells after sonication in either sucrose or Ficoll-Hypaque media. The preparations obtained were compared in regard to recovery and spontaneous loss of histamine. The effect of agents known to release histamine from intact rat mast cells (antigen, compound 48/80, decylamine, the ionophores A23187 and X537A as well as ATP) was studied on the granules. Antigen and compound 48/80 did not release histamine. Decylamine and X537A induced a pronounced release independent of the presence of divalent cations. ATP caused a small, but significant release, which showed an absolute requirement for magnesium. A23187 released histamine only in the presence of either calcium or magnesium, and this release was unaffected by certain agents known to inhibit histamine release from intact rat mast cells. The results seem to exclude the possibility that agents known to induce release of histamine from intact rat mast cells by a calcium- and energy-dependent process would exert this action through a direct effect on intracellularly localized granules.

Introduction

The function of most secretory systems is dependent on the presence of calcium ions and the activity of calcium as a secretagogue has been closely correlated with the cellular production of metabolic energy (for reference see [1]). Calcium has been suggested to act as a link in the stimulus-secretion coupling [2, 3], but the actual mechanism by which calcium and metabolic energy couple stimulation to secretion in the release processes has not yet been elucidated.

Adenosine-5′-triphosphate (ATP) has been shown to initiate the release of biologically active substances from various isolated granular preparations by a mechanism which depends on the presence of divalent cations, e.g. noradrenaline from chromaffin granules of the adrenal medulla [4], vasopressin from granules from the neurohypophysis [5, 6], and amylase from zymogen granules from the parotid gland [7, 8]. In addition, it has been shown that the content of insulin of beta-granules is reduced after exposure to ATP [20].

Considering that intact rat mast cells are stimulated by ATP to release histamine by an energy-dependent process which is strictly calcium-dependent [9] it was of interest to extend the studies regarding this effect to isolated histamine-containing granules. The present investigation reports the results of such experiments as well as the action of compound 48/80, the ionophores A23187 and X537A, decylamine, and divalent cations on such granules. In addition, the effect of antigen was investigated on granules obtained from mast cells of actively sensitized rats.

Methods and materials

Mast cells from Wistar rats (200–250 g) were collected from the abdominal and thoracic cavities and isolated on Ficoll as previously described [10]. In experiments concerning the effect of antigen on granules, cells were obtained from rats actively sensitized to horse serum [10]. The cells were washed twice with buffered salt solution (BSS) containing NaCl (131 m*M*), KCl (2.4 m*M*), Tris buffer (10 m*M*, pH 7.0 at 37 °C), and human serum albumin (0.5 mg/ml). In some experiments the Tris buffer was substituted by Sørensen phosphate buffer (6.7 m*M*, pH 7.0). The cells were washed a third time with a sucrose solution (0.34 *M*, adjusted to pH 7.0 with Tris base) containing human serum albumin (1 mg/ml). Thereafter they were resuspended in sucrose in a cell concentration of about 10^6/ml. The cell suspension cooled on ice was sonicated with a MSE 100 watt ultrasonic disintegrator set at 4 microns amplitude for

15 seconds. The procedure for isolation of granules is similar to that used by ANDERSON et al. [11].

The sonicated suspension was centrifuged (36 *g* for 5 minutes at room temperature) and the granule-containing supernatant was recentrifuged (3000 *g* for 20 minutes at room temperature). The sucrose supernatant was discarded and the granules resuspended in BSS in half the sucrose volume. After 5–10 minutes at room temperature to allow cation exchange of the histamine of membrane-free granular material, the suspension was again centrifuged (3000 *g* for 15 minutes at room temperature). The supernatant was eventually saved for analysis and the granules resuspended in the same volume of BSS.

Alternatively the granules were prepared in a solution containing Ficoll (6.4% w/v), Hypaque (10.5% w/v), and human serum albumin (1 mg/ml) instead of the sucrose solution. The cell suspension was then sonicated at 4 microns amplitude for 30 seconds and diluted 1:5 with BSS prior to the centrifugation step. Decrease of the density by dilution was necessary for optimal recovery of granules after centrifugation. Experience showed that better granule preparations were obtained by the use of freshly prepared solutions. Consequently, both sucrose and Ficoll-Hypaque solutions were prepared once a week and stored at 4 °C.

The incubation procedures and the determination of histamine release were essentially identical to the methods used for studying histamine release from intact mast cells [10] with the one exception that after incubation the tubes were centrifuged at 2300 *g* for 15 minutes at 4 °C.

The composition of the incubation medium and the experimental conditions are described in the legends to the figures. Unless otherwise stated, the spontaneous histamine release has been deducted from all values presented and the values given in the text represent mean ± S.E.M. Student's *t*-test or *t*-test for paired data were used for statistical evaluation of the results.

All chemicals were of analytical grade and all solutions were made up by redistilled water. Human serum albumin was kindly supplied by AB Kabi, Stockholm (Sweden), compound 48/80 by AB Leo, Helsingborg (Sweden), the ionophore A23187 by Lilly Research Centre Ltd, Windlesham (England), and the ionophore X537A by Hoffmann-La Roche, Basel (Switzerland). Phenylglyoxal hydrate and diisopropyl fluorophosphate (DFP) were obtained from Fluka AG, Buchs (Switzerland) and phenylmethylsulfonylfluoride (PMSF) from Calbiochem, Los Angeles (USA). N-ethyl-maleimide (NEM), antimycin A and the various nucleotides were obtained from Sigma Chemical Corp., St. Louis (USA). Stock solutions of ATP (disodium salt, about 100 m*M*) were neutralized by the addition of NaOH and assayed spectrophotometrically. The stock solution was stored at −20 °C.

Results

Recovery and spontaneous release of histamine

The recovery of histamine-containing granules was estimated by comparing the histamine content of isolated mast cells prior to sonication with the values found in the final granule suspension. In 9 experiments using Ficoll-Hypaque solution a recovery of 42 ± 1.8% (range 33–50%) was found. Similar values were obtained when comparing the recovery of granules isolated from the same pool of mast cells using Ficoll-Hypaque and sucrose.

The spontaneous histamine release from granules isolated from the same pool of mast cells was smaller for Ficoll-Hypaque granules as compared to sucrose granules ($n = 4$, $p < 0.05$). However, due to large variations, no statistical difference was evident when values from 11 individual experiments were compared (28.6 ± 3.8% for sucrose granules, 27.5 ± 3.4% for Ficoll-Hypaque granules after incubation for 10 minutes at 37 °C). The presence of calcium and magnesium (3 m*M*) in the BSS did not reduce, but if anything, tended to increase the loss of histamine. Again, in the presence of 3 m*M* $MgCl_2$, the spontaneous histamine release was lower for Ficoll-Hypaque granules than for sucrose granules, when granules isolated from the same pool of mast cells were compared ($n = 4$, $p < 0.01$), whereas no statistical difference was observed between the values from 15 individual experiments (36.8 ± 3.9% for sucrose granules, 31.3 ± 2.4% for Ficoll-Hypaque granules after incubation for 10 minutes at 37 °C).

The presence of $CaCl_2$ (10 m*M*) increased the spontaneous histamine release from 25.6 ± 3.7% to 50.8 ± 6.9% ($n = 5$, $p < 0.01$) and $MgCl_2$ (10 m*M*) caused an increase from 30.7 ± 3.4% to 51.5 ± 3.6% ($n = 8$, $p < 0.001$). Cadmium (3 m*M*), berylium (5 m*M*), and zinc (5 m*M*) induced histamine release from the granules, whereas barium (3 m*M*) and strontium (10 m*M*) were without effect (Table 1).

When the ratio between the concentrations of NaCl and KCl in the BSS was reversed, the spontaneous histamine release almost doubled. A similar effect was noted in some experiments by the presence of PS (50 μg/ml). In addition, a graded increase of the loss of histamine from the granules was observed when the pH of the BSS was decreased below 6. 95% of the histamine was released at pH 4.

Adenosine-5′-triphosphate (ATP)

ATP induced a small, but significant dose-dependent release of histamine in the presence of magnesium (3 m*M*) (Fig. 1). Maximal release was observed at an ATP concentration of

Table 1
The influence of divalent cations on the spontaneous release of histamine and on ionophore-induced release from sucrose granules. The granules were incubated for 10 minutes at 37 °C in BSS in the presence of the ions and the ionophore A23187 (5×10^{-6} *M*). No correction has been made for spontaneous release. Mean ± S.E.M. of 5 experiments (1a) and 3 experiments (1b).

	Divalent cation	A23187	
		0	5×10^{-6} *M*
1a	$MgCl_2$ (3 m*M*)	46.0 ± 5.0	83.6 ± 2.3
	$CdCl_2$ (3 m*M*)	88.1 ± 2.0	73.3 ± 2.7
	$BeCl_2$ (5 m*M*)	71.6 ± 2.3	74.1 ± 3.0
	$ZnSO_4$ (5 m*M*)	74.8 ± 4.5	60.1 ± 4.6
1b	0	28.6 ± 3.3	22.2 ± 3.2
	$BaCl_2$ (3 m*M*)		21.9 ± 3.6
	$SrCl_2$ (10 m*M*)		29.2 ± 2.9
	$MgCl_2$ (3 m*M*)		78.0 ± 2.6

Table 2
Influence of ATP (2×10^{-4} *M*) on histamine release from Ficoll-Hypaque granules incubated for 30 minutes at 37 °C in the absence and presence of $MgCl_2$ (3 m*M*). No correction has been made for spontaneous release. Mean ± S.E.M. of 5 experiments.

Divalent cation	ATP	
	0	2×10^{-4} *M*
0	27.7 ± 3.0[a]	28.2 ± 3.0[c]
$MgCl_2$ (3 m*M*)	32.6 ± 3.8[b]	41.3 ± 2.6[d]

t-test for paired data: [a-b] $p < 0.05$; [c-d] $p < 0.001$; [b-d] $p < 0.01$.

2.5×10^{-4} *M*, and higher concentrations tended to give a smaller release.

Histamine release induced by ATP showed an absolute requirement for the presence of magnesium (Table 2), and optimal effect was observed with 3 m*M* of the ion. Higher concentrations tended to decrease the release. Calcium could not substitute for magnesium. Granules exposed to ATP (2×10^{-4} *M*) in the presence of calcium (10^{-6}—5×10^{-3} *M*) did not respond with a release which differed from the spontaneous release. The histamine release induced by ATP (2×10^{-4} *M*) and magnesium (3 m*M*) was unaffected by low concentrations of calcium, whereas concentrations above 10^{-4} *M* acted inhibitorily.

In four experiments the time course for the histamine release induced by ATP (10^{-4} *M*) and magnesium (3 m*M*) was studied. After 5 minutes 1.5 ± 1.7% was released, after 10 minutes 5.0 ± 1.7%, and after 30 minutes 9.4 ± 1.2%.

Since the release induced by ATP and magnesium never exceeded 14% under the conditions described, various factors were investigated as to their possible activating action on the release. Reversed ratio between sodium and potassium, reduction of the pH, phosphatidylserine (2–50 μg/ml), cyclic AMP, dibutyryl

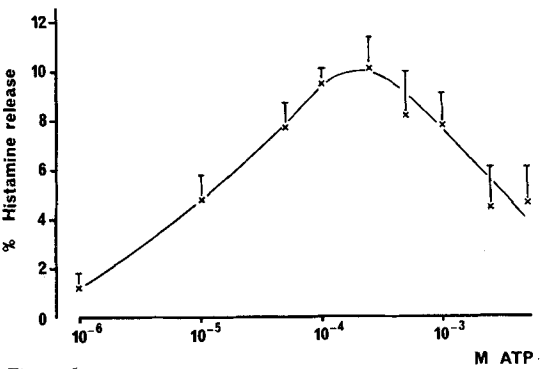

Figure 1
Histamine release from Ficoll-Hypaque granules induced by different concentrations of ATP in the presence of $MgCl_2$ (3 m*M*). The granules were incubated for 30 minutes at 37 °C. Mean ± S.E.M. of 4 experiments.

cyclic AMP, cyclic GMP, dibutyryl cyclic GMP, ADP, AMP, and pyrophosphate (1 m*M* of each) did not enhance the release induced by ATP and magnesium. The nucleotides tested did not release histamine by themselves in the presence of magnesium.

Since ISHIDA et al. [7] found that a protein-like tissue factor was needed for ATP to induce the release of amylase from zymogen granules of the parotid gland, different fractions of sonicated mast cells were added to the incubation medium. This did not enhance histamine release from the granules induced by ATP and magnesium, neither did the presence of washed mast cells which had released histamine by previous exposure to A23187 and calcium.

Decylamine and the ionophore X537A

Both agents released substantial amounts of histamine from the granular preparations irrespective of the presence of calcium or magnesium. Decylamine (20 μg/ml) released 82.6 ± 8.5% of the total histamine content (56.0 ± 8.9% after deduction of spontaneous release, $n = 4$). X537A (10^{-5} *M*) virtually depleted both sucrose and Ficoll-Hypaque granules for histamine, irrespective of the presence of magnesium (3 m*M*) (Table 3).

The ionophore A23187

A23187 induced a dose-dependent release of histamine. In the presence of magnesium (3 m*M*) (Fig. 2) optimal release was observed above 5×10^{-6} *M* of the ionophore. The release from Ficoll-Hypaque granules and sucrose granules obtained from the same pool of mast cells was identical (Table 3). However, due to a lower spontaneous release from Ficoll-Hypaque granules, the specific release was higher in this preparation.

The release showed an absolute requirement for the presence of either calcium or magnesium and each ion functioned equally well for the release (Fig. 3). Strontium (10 m*M*),

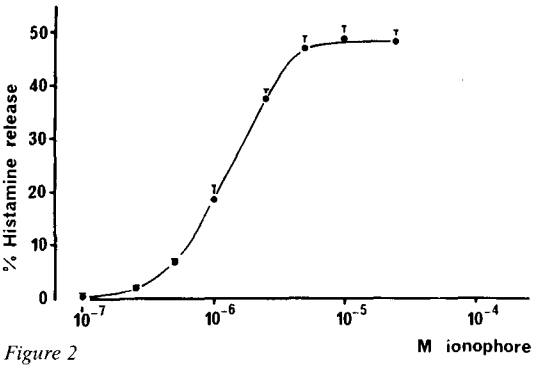

Figure 2
Histamine release from sucrose granules induced by different concentrations of the ionophore A23187 in the presence of $MgCl_2$ (3 m*M*). The granules were incubated for 10 minutes at 37 °C. Mean ± S.E.M. of 5 experiments.

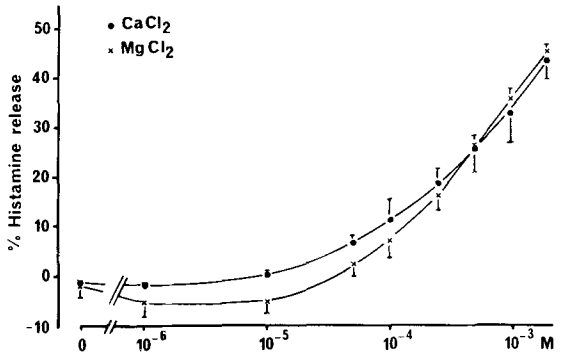

Figure 3
The influence of $CaCl_2$ and $MgCl_2$ on histamine release from sucrose granules induced by the ionophore A23187 (5×10^{-6}*M*). The granules were incubated for 10 minutes at 37 °C. Mean ± S.E.M. of 4 ($CaCl_2$) and 5 ($MgCl_2$) experiments.

Table 3
Influence of $MgCl_2$ (3 m*M*) on histamine release induced by the ionophores A23187 (5×10^{-6} *M*) and X537A (10^{-5} *M*) from granules isolated from the same pool of mast cells in either sucrose or Ficoll-Hypaque. The granules were incubated for 10 minutes at 37 °C. No correction has been made for spontaneous release. Mean ± S.E.M. of 3 experiments.

Ionophore	Sucrose granules $MgCl_2$		Ficoll-Hypaque granules $MgCl_2$	
	0	3 m*M*	0	3 m*M*
0	38.3 ± 10.6	41.8 ± 7.4	18.9 ± 5.9	23.1 ± 5.9
A23187	31.6 ± 12.0	81.2 ± 3.2	24.5 ± 5.7	80.9 ± 3.5
X537A	96.7 ± 2.6	98.4 ± 1.7	98.1 ± 0.3	97.2 ± 0.6

barium (3 m*M*), cadmium (3 m*M*), berylium (5 m*M*), and zinc (5 m*M*) could not substitute for calcium or magnesium. The release induced by the latter ions themselves was not enhanced by the presence of the ionophore (Table 1).

The presence of lanthanum (10^{-6} and 10^{-5} *M*) did not significantly influence the release induced by A23187 in the presence of either 1 m*M* of calcium or magnesium. Lanthanum (10^{-4} *M*), however, acted markedly inhibitorily [72.1 ± 4.7% inhibition in the presence of calcium (1 m*M*), 75.8 ± 5.3% inhibition in the presence of magnesium (1 m*M*), $n = 4$]. Phosphatidylserine (10 μg/ml) did not influence the release induced by the ionophore in the presence of calcium or magnesium (1 m*M*), whereas PS (50 μg/ml) in some experiments acted inhibitorily.

The histamine release induced by A23187 (5×10^{-6} *M*) in the presence of calcium (3 m*M*) was completed within 20 minutes with half maximal release occurring within 1–2 minutes (Fig. 4), and the time course was identical for the release in the presence of magnesium (3 m*M*).

NEM (10^{-3} *M*) did not inhibit the release by the ionophore in the presence of calcium, neither did antimycin A (10^{-6} *M*), or DFP (10^{-2} *M*) (Table 4, a and b). On the other hand, phenylglyoxal (10^{-3} *M*) and PMSF (10^{-3} *M*) acted markedly inhibitorily on the release and this effect was significantly counteracted ($p < 0.01$) by increasing the concentration of calcium from 1 to 3 m*M* (Table 4, c).

Antigen and compound 48/80

Neither compound 48/80 (0.6 μg/ml) nor antigen (dialyzed horse serum, 0.75% [v/v]) induced a significant release of histamine from granules incubated in BSS containing magnesium ($1\text{–}3 \times 10^{-3}$ *M*) and/or calcium ($10^{-6} - 10^{-3}$ *M*). In fact, the spontaneous histamine release decreased in the presence of 48/80,

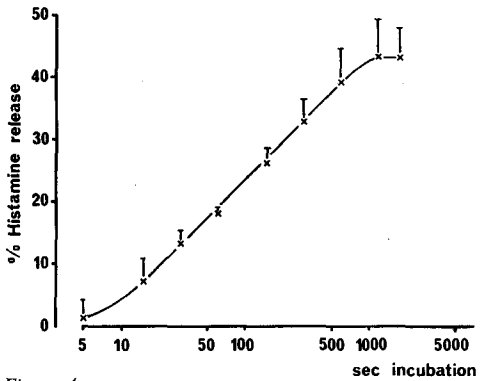

Figure 4
Time course for histamine release from sucrose granules induced by the ionophore A23187 (5×10^{-6} *M*) in the presence of $CaCl_2$ (3 m*M*). The granules were incubated at 37 °C in 0.5 ml of BSS. The incubation was terminated by placing the tubes in an icebath and the simultaneous addition of 1.5 ml ice-cold BSS. Mean ± S.E.M. of 4 experiments.

Table 4
Inhibition of histamine release from sucrose granules induced by the ionophore A23187 (5×10^{-6} *M*) in the presence of $CaCl_2$ (1 and 3 m*M*). The granules were preincubated with the agents in calcium-containing BSS for 5 minutes at 37 °C prior to the addition of ionophore. The incubation was continued for 10 minutes at 37 °C. Mean ± S.E.M. is presented.

		$CaCl_2$	
		1 m*M*	3 m*M*
4a		$n = 3$	$n = 3$
	0	41.4 ± 10.1	47.1 ± 11.1
	Antimycin A 10^{-6} *M*	41.5 ± 8.8	48.6 ± 10.5
	NEM 10^{-3} *M*	50.4 ± 8.7	53.9 ± 9.7
4b		$n = 5$	$n = 3$
	0	35.6 ± 2.1	39.5 ± 2.2
	DFP 10^{-2} *M*	30.3 ± 2.0	39.5 ± 3.8
4c		$n = 4$	$n = 4$
	0	47.3 ± 9.3	52.6 ± 9.6
	PMSF 10^{-3} *M*	11.4 ± 6.4	24.7 ± 5.6
	Phenylgly. 10^{-3} *M*	14.4 ± 7.5	30.0 ± 6.3

so that $5.8 \pm 1.5\%$ ($n = 6$, $p < 0.05$) less of the total histamine content was released. Antigen in two experiments released 2.0 and 5.3%, respectively.

Discussion

Histamine is ionically linked to the matrix of mast cell granules and is instantaneously released from its binding sites through a simple cation exchange when free access to the extracellular fluid is established [12]. Granules surrounded by a perigranular membrane retain their histamine and a substantial fraction of such granules can be obtained after mild sonication of isolated rat mast cells [11]. The granular preparations studied in the present investigation were obtained by procedures similar to those used by ANDERSON et al. [11], and since contaminating membrane-free granules were depleted of histamine by washing with BSS, the histamine release observed can be ascribed to derive from intact granules. The spontaneous loss of histamine from granular preparations obtained from the same pool of mast cells was found to be smaller when Ficoll-Hypaque was used in the isolation procedure as compared to sucrose. This might be ascribed to differences in mechanical fragility, since no increase of this loss was observed during incubation of the granules up to 30 minutes.

The exchange of histamine from the granular matrix of intact mast cells occurs as a consequence of the stimulation of the plasma membrane by mast cell-active agents. Certain agents (antigen, compound 48/80, ATP and A23187) depend on calcium and metabolic energy for the coupling of stimulation to secretion [13]. These coupling factors are involved in an exocytosis-vacuolization process [14–16], by which the granular matrix will communicate with cations of the surrounding medium. Other agents (decylamine and X537A) act probably through a direct membrane-destructive action on the cell completely independent of such coupling factors [17, 18]. Although less recognized, the possibility exists that histamine release induced by mast cell-active agents might occur as a consequence of a direct action on the granules within the cell. Therefore, information regarding the effectiveness of these agents to release histamine from isolated histamine-retaining granules are of importance.

It is evident from the present results that the protection that the perigranular membrane constitutes to the matrix can be markedly altered by some mast cell-active agents, but not by others. The release of histamine induced by decylamine and X537A is consistent with the view that these agents exert a direct destructive action on the membranes, which makes cations available for exchange. In agreement with the action of these agents on intact mast cells their effect on the granules occurred independent of the presence of divalent cations. On the other hand, compound 48/80 and antigen did not release histamine from the granules, which excludes the possibility of a direct effect of these agents on granules within intact mast cells.

ATP is an effective releaser of histamine from intact rat mast cells as well as of biogenic compounds from various granular preparations. In spite of great variation in the experimental procedures in search of a possible missing cofactor, ATP was comparatively ineffective to release histamine from isolated mast cell granules. The release did not exceed 14% of the total histamine content in any single experiment and showed an absolute requirement for the presence of magnesium. Calcium could not substitute for magnesium, but in fact, acted inhibitorily. This action of the ions is the reverse of that found for histamine release induced by ATP from intact rat mast cells [9, 19]. Based on the present results neither extracellular nor metabolically derived ATP are likely to be involved in the release of histamine from intact rat mast cells through a direct action on the granules.

The release induced by ATP of biogenic compounds from various granular preparations differs regarding the influence of calcium and magnesium. Both ions are needed for the release of amylase from zymogen granules [7]. Magnesium is needed for the release of catecholamines from granules of the adrenal medulla as well as for the release of vasopressin from granules obtained from the posterior pituitary gland, and calcium cannot substitute for magnesium [4–6]. The release of histamine from mast cell granules was specifically induced by ATP and no other nucleotide investigated was effective. This is in agreement with the findings concerning the release of catecholamines from adrenal medullary granules [4]. In contrast, vasopressin as well as insulin were found to be released from granular preparations by ADP and AMP as well [6, 20]. At present, therefore, no unifying mechanism can be put forward to

explain the release exerted by ATP on the various granular preparations.

In agreement with the effect of the ionophore X537A, A23187 was found to induce pronounced release of histamine from isolated mast cell granules. In contrast, however, the release from the granules induced by A23187 depended on the presence of either calcium or magnesium. Neither strontium nor barium could substitute for these ions, which might be explained by the fact that strontium and barium have a comparatively lower affinity for A23187 [21]. The action of A23187 on intact rat mast cells also required the presence of divalent cations. The specificity, however, was greater since, in this preparation, only calcium could function and magnesium, strontium, and barium were completely ineffective [22]. Among various agents shown to inhibit the release of histamine from intact rat mast cells when exposed to A23187 and calcium [23] phenylglyoxal (an amino group blocking agent), and PMSF (an esterase inhibitor) were also found to block the release from isolated granules. In contrast, neither antimycin A (an inhibitor of oxidative phosphorylation), NEM (an inhibitor of sulfhydryl groups), nor DFP (another esterase inhibitor) influenced the release.

Stimulation of intact mast cells by certain releasing agents including A23187 seems to be dependent on calcium as an important trigger of an energy-dependent exocytosis-vacuolization process. The development of communication between the surrounding medium and the granular matrix allows a passive exchange of cations for histamine. On the other hand, stimulation of isolated mast cell granules by A23187 is triggered by magnesium as well as by calcium and does not seem to be energy-dependent. The similar affinity for calcium and magnesium [21] might explain the function of the ionophore as a carrier of either of these ions across the perigranular membrane whereby cations become available for exchange with histamine. Another possibility that cannot be excluded is that the ionophore, when complexed with calcium and magnesium, would primarily change the permeability of the perigranular membrane for monovalent cations in the incubation medium. The observed differences as to the specificity of divalent cations and the action of various inhibitors do not suggest a common mechanism for the release of histamine induced by A23187 from intact rat mast cells and isolated granules.

In conclusion, the present results do not support the possibility that calcium- and energy-dependent releasing agents like antigen, compound 48/80, ATP, and the ionophore A23187 induce histamine release from intact rat mast cells by a primary action on intracellularly localized granules.

Acknowledgments

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