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DIFFERENCES IN REQUIREMENTS AND ACTIONS OF VARIOUS HISTAMINE-RELEASING AGENTS

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Abstract—Histamine release induced by dextran, concanavalin A (con A), antigen, anti-IgE, the ionophore A23187, somatostatin, compound 48/80, polylysine, and protamine was studied in Sprague–Dawley rat mast cells. Release by dextran, con A, antigen, and anti-IgE was highly dependent on addition of phosphatidyl serine (PS). The same agents plus A23187 were highly dependent on exogenous Ca^{2+}, but only dextran was completely dependent. The other (basic) agents showed little or no dependence on exogenous PS or Ca^{2+}. Addition of 0.1 mM Mn^{2+} with the A23187 (in the presence of 1.0 mM Ca^{2+}), which probably markedly reduced the level of the A23187–Ca complex, did not greatly reduce release by the A23187. Treatment of the cells with neuraminidase increased release by con A, A23187, and antigen; did not greatly affect release by dextran; and definitely decreased release by 48/80, polylysine, and protamine. Each agent desensitized the cells to a higher concentration of the same agent, and most also reduced release by other agents. A23187 and protamine sometimes sensitized to each other, however, and dextran and antigen regularly sensitized to A23187. The observations indicate that the different agents did not act in an identical manner.

A number of diverse agents are capable of releasing histamine in a noncytotoxic manner from mast cells, and may leave the cells desensitized to subsequent application of the same or another agent [1–4]. The cell reactions induced by the different agents are similar in many respects, but differences have been noted [4–11]. In the present study, using Sprague–Dawley rat peritoneal mast cells, we compared the effects of omitting Ca^{2+} and phosphatidyl serine (PS), respectively, from the complete medium, and of penetrating the cells with neuraminidase, on histamine release by eight different agents. In addition, we assessed the ability of each agent to desensitize the cells to itself, and to other agents. The differences in requirements and actions of the different releasing agents, which we observed, are described and discussed.

METHODS AND MATERIALS

Peritoneal mast cells were obtained from large male Sprague–Dawley rats as previously described [12, 13]. In experiments using specific antigen (egg albumin) or rabbit anti-rat-IgE serum as the histamine releaser, the donor rats had been immunized with the antigen and pertussis vaccine, which was shown to induce production of IgE anti-egg albumin antibody [3].* The normal cell medium, from which Ca^{2+} was omitted in some experiments, was Krebs–Ringer phosphate, pH 7.0, containing 1.0 mM Ca^{2+}, no Mg^{2+}, and 1 mg/ml of crystalline bovine serum albumin. In addition, a final concentration of 10 μg/ml of PS was added at the time of adding the histamine-releasing agent, unless otherwise noted, and the normal medium to which PS had been added is referred to as the complete medium.

In each experiment, replicate 2.5-ml samples of the cell suspension containing about 10^5 mast cells with 2 μg histamine [13] were pipetted into siliconized tubes, and incubated with the appropriate media for 10 min. To induce histamine release, one of the releasing agents in 1.0 ml of the same medium in which the cells were suspended, with or without PS, was added to the respective tubes. Control samples were treated in the same manner, except that the releasing agent was omitted. The cells were incubated for 15 min at 25°, during which time release by all of the agents was determined to be essentially complete. The tubes were then placed in ice, and centrifuged in the cold. The supernatant solutions were decanted, and histamine in solutions and cells, respectively, was quantified fluorometrically [13, 15].

Levels of release varied from one experiment to another, as shown by the standard deviation values. This inevitably occurs because of the use of cells from different rats. There was little variation in release from replicate samples of the same cell preparations [13], however, and replicate samples were used in each experiment to compare effects of the different conditions (as already noted). Statistical comparisons of release under different conditions (i.e. P value determinations) were then made from percentage changes in paired release values: $t = \bar{d}\,(s_d/\sqrt{n})$, P from tables. Despite the different levels of release in different experiments, effects of various conditions in the different experiments were remarkably consistent.

In the experiments with neuraminidase, half of the peritoneal cells from two rats was suspended in 0.6 ml of the normal buffer containing neuraminidase (250

* Although evidence has been presented that histamine release by concanavalin A is dependent on the presence of IgE antibody on the mast cells [14], we obtained similar degrees of release by concanavalin A from mast cells of the following groups of Sprague–Dawley rats: (a) germ free, (b) conventional and (c) immunized with egg albumin and pertussis vaccine. The anti-rat-IgE antibody, however, also produced similar degrees of release from cells of the three groups.

units/ml, *V. cholerae*, CalBiochem grade B), and incubated at 37° for 30 min. The neuraminidase had been dialyzed against the normal buffer. The activity of the enzyme under these conditions may be inferred from other publications [16, 17]. The remaining half of the cells were similarly incubated with the normal buffer without the enzyme. The respective cells were then resuspended in normal buffer and treated as previously described with the releasing agents, together with PS unless noted to the contrary.

In the desensitization experiments with a single releasing agent, cells were exposed (for 15 min at 25°) first to a low concentration (concentration 1) of the agent, and then (without removing the original medium) to a higher concentration (concentration 2) of the same agent. If the agent was PS dependent, PS was added to the cells with the first addition. An experiment was made as shown by the following example:

Cell sample	First addition	Second addition	Histamine release (%)
A	Agent I to concn 1	Buffer	10
B	Buffer	Agent I to concn 2	50
C	Agent I to concn 1	Agent I to concn 2	30

Release in tube C should have equalled that in tube B, unless exposing the cells to concentration 1 decreased the additional release produced by increasing the concentration (of the same agent) to concentration 2. The percentage reduction in additional release that occurred in tube C [(50 − 30)/(50 − 10) = 50 per cent, in the sample shown] was taken as the degree of desensitization of the agent against itself.

To assess the ability of one agent to change release by a second agent, experiments were made in the same manner, except that additions of agent II in relatively high concentration were substituted for the second additions of agent I. The degree of change in agent II released caused by agent I was then determined by comparing release by agent II when added after agent I (in per cent of the histamine remaining in the cells after incubation with agent I) with per cent release by agent II when not preceded by agent I. Degree of release was sensitive to small changes in concentration of some of the agents, and varied with different pools of cells. Consequently, release values in a few experiments were such that the effect of agent I on release by agent II could not be reliably assessed (agent I released no histamine, or agent II released less than 5% histamine, or agent I released more histamine than was usually releasable by agent II), and these experiments were excluded.

Another method of carrying out desensitization experiments, applicable only to PS-dependent agents and employing only relatively high concentrations of the agents, consisted of comparing release by an agent followed in 15 min by PS, with release by the same agent added together with PS; or by comparing release by agent II plus PS added 15 min after agent I, with release by agent II plus PS preceded by addition of only buffer. This method was used in a few experiments with results similar to those obtained with the usual method.

Dextran, average mol. wt 2×10^6, was obtained from Pharmacia Fine Chemicals, and phosphatidyl serine and egg albumin (recrystallized five times) were obtained from nutritional Biochemicals Corp. The rabbit anti-rat-IgE antiserum, which gave a slight precipitin line against normal rat α_1-globulin but none against any rat immunoglobulin except IgE, was a gift from Dr. Henry Metzger of NIH. Concanavalin A was a product of Miles Laboratory, Inc. The ionophore A23187 was a gift from Eli Lilly & Co.; it was dissolved in dimethylsulfoxide (DMSO), from which working solutions were made in the buffer. Final levels of DMSO (less than 0.01 per cent) were without effect on histamine release. Compound 48/80 was obtained from Burrows Welcome Co., and the protamine sulfate (salmon), grade 1, and poly-L-lysine, mol. wt 15,000–50,000 were obtained, from Sigma Chemical Co. Somatostatin and a second preparation of con A were purchased from CalBiochem.

RESULTS

Agent concentrations. Histamine release changed from slight to near maximal over the following concentration ranges of the different releasing agents: dextran 10^0 to 10^3 μg/ml [15], concanavalin A (con A) 1–10 μg/ml, antigen 10^{-2} to 10^0 μg/ml [3], anti-IgE serum 1/3200 to 1/400, A23187 0.05 to 0.2 μg/ml, 48/80 0.1 to 1.0 μg/ml, protamine 1–5 μg/ml, polylysine 1–5 μg/ml, and somatostatin 0.5 to 10 μg/ml. Some variation occurred from one experiment to another. During one period, for obscure reasons, we had much difficulty inducing the formation of IgE antibody against the antigen, and higher than usual concentrations of antigen were necessary to produce significant histamine release. The upper ranges of the dose–response curves were relatively flat, as usual, so that the values given for near maximal concentration are somewhat arbitrary. Low and high concentrations of the agents for use in the desensitization experiments to be presented later were selected from the upper and lower portions of the ranges shown above. Since somatostatin has not heretofore been recognized as a histamine-releasing agent, the dose–response curve obtained with this agent is shown in Fig. 1.

Dependence of histamine release on PS and Ca^{2+}. The releasing agents could be divided into several groups, based on the dependence of release on PS and Ca^{2+} in the medium, as shown in Table 1. Dextran stood alone in being highly dependent on PS and completely dependent on Ca^{2+}. Con A, antigen, and anti-IgE were likewise highly dependent on PS, but less completely dependent than dextran on exogenous Ca^{2+}. The ionophore A23187 was unique in being slightly (if at all) affected by the presence of PS, but largely dependent on Ca^{2+}. The basic releasers 48/80, protamine, and polylysine differed from the agents already discussed in that they did not require exogenous PS or Ca^{2+}; in fact the release was often increased by omitting PS, particularly when the level of release in the complete medium was markedly submaximal. The relationship of release by protamine to PS level in one experiment is shown in

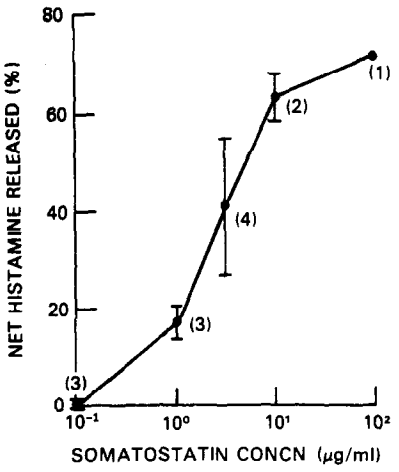

Fig. 1. Histamine release by somatostatin. PS (10 μg/ml) was added with the agent to the cells in the normal medium containing 1.0 mM Ca^{2+}. The number of experiments represented by each point is shown in parentheses, and the standard deviation by the vertical line.

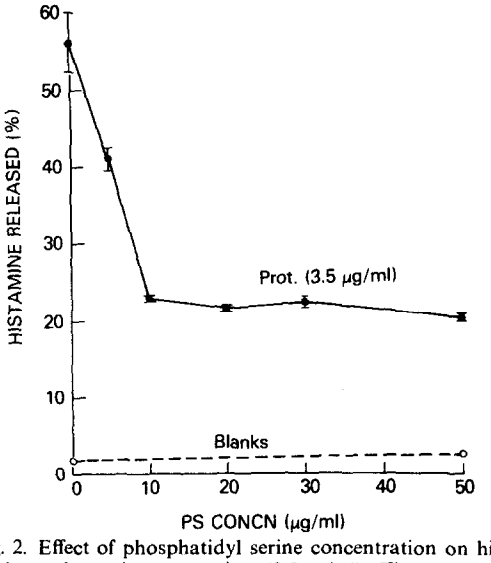

Fig. 2. Effect of phosphatidyl serine concentration on histamine release by protamine (3.5 μg/ml). The releasing agent together with various amounts of PS was added to the cells in normal medium containing 1.0 mM Ca^{2+}. Results with duplicate cell samples are shown.

Fig. 2. In experiments to be discussed later, the basic agents were often employed without PS. This did not significantly influence the relative effects of other conditions on histamine release by these agents, as will be shown. Histamine release by somatostatin, which probably also acts by virtue of its basic amino acid residues, was also largely independent of Ca^{2+} and PS in the medium.

Omission of both PS and Ca^{2+} in experiments with the various releasing agents had about the same effect as omitting the most active of the two factors. Inclusion of heparin, 15 units/ml, in the medium strongly inhibited release by protamine and polylysine, but had little effect on the activity of the other agents.

EDTA was not employed in conjunction with the Ca-free media in the experiments referred to above. However, the residual release produced by antigen, con A, anti-IgE and A23187 in the absence of exogenous Ca^{2+} was usually not abolished by suspending the cells in 1.0 mM EDTA solution for 10 min before adding the agent also prepared in 1.0 mM EDTA solution (Table 2). Also, the high degree of release induced by the basic agents in the absence of exogenous Ca^{2+} was not reduced by the inclusion of EDTA in the medium (Table 2).

In an attempt to determine whether the ionophore A23187 must exist in the form of the Ca complex in order to induce release, or whether it simply requires the presence of Ca^{2+} in the medium, as do dextran, con A, antigen, and anti-IgE, we tested the effect on release by the ionophore (0.2 μg/ml with 1.0 mM Ca^{2+}) of adding the ionophore (to the cells) in an $MnCl_2$ solution sufficient to give a final concentration of 0.1 mM Mn^{2+}. Mn^{2+} is bound by the ionophore in preference to Ca^{2+} [18]. Use of Mn^{2+} in four experiments reduced release with the ionophore

Table 1. Effect of omitting Ca^{2+} and PS on histamine release by various agents

Releasing agent (μg/ml)	No. of experiments	Release in different media (net ± S. D.)*		
		Complete (%)†	Less PS (%)	Less Ca^{2+}† (%)
Dextran, 10^3	9	59 ± 18	12 ± 15‡	−2 ± 1‡
Concanavalin A, 10	16	37 ± 17	2 ± 3‡	8 ± 6‡
Antigen, 10^{-1}	5	30 ± 17	3 ± 4‡	15 ± 14‡
Anti-IgE (1/400)	6	29 ± 11	2 ± 3‡	15 ± 8‡
A23187, 0.18 ± 0.04	11	68 ± 25	64 ± 29§	20 ± 16‡
Somatostatin, 3	3	44 ± 15	53 ± 19‖	37 ± 10‖
48/80, 1	5	50 ± 18	76 ± 13¶	37 ± 7‖
Protamine, 3.5, 5	7	36 ± 13	47 ± 18¶	31 ± 9‖
Polylysine, 5	6	53 ± 23	70 ± 13‖	34 ± 7‖

* Replicate cell samples were used throughout each experiment, and comparisons of release under different conditions (P value determinations) are based on percentage change between paired release values. One of the media was omitted in a few experiments.

† Normal buffer, with 10 μg PS added with the releasing agent.

‡ Release decreased, $P \leq 0.01$, compared with that in complete medium.

§ Release decreased, $0.05 < P < 0.10$.

‖ Release not significantly changed, $P > 0.20$.

¶ Release increased, $0.10 < P < 0.20$.

Table 2. Effect of EDTA on histamine release in complete medium minus Ca^{2+}*

Releasing agent (μg/ml)	No. of experiments	Histamine release (net ± S. D.)† Without EDTA (%)	With EDTA‡ (%)
Concanavalin A, 10	4	4 ± 3	2 ± 2
Antigen, 10^{-1}	2	9 ± 10	7 ± 8
Anti-IgE (1/400)	3	10 ± 7	7 ± 5
A23187, 0.12, 0.15	4	9 ± 4	8 ± 4
48/80, 1	2(2)§	31 ± 6 (48 ± 6)§	35 ± 0 (51 ± 5)§
Protamine, 3.5, 5	2(4)	31 ± 1 (42 ± 15)	33 ± 3 (43 ± 14)
Polylysine, 3.5, 5	2(3)	37 ± 3 (47 ± 3)	43 ± 1 (49 ± 3)

* Release in the complete medium (not shown) by the non-basic agents was somewhat less than the corresponding values shown in Table 1.

† Net release (over that in the identical medium without the releasing agent) occurred in every experiment, except for a single one with con A in the presence of EDTA. Replicate cell samples were used throughout each experiment.

‡ The cells were suspended in 1.0 mM EDTA for 10 min before the agent (prepared in 1.0 mM EDTA) was added.

§ The data in parentheses were obtained without using PS.

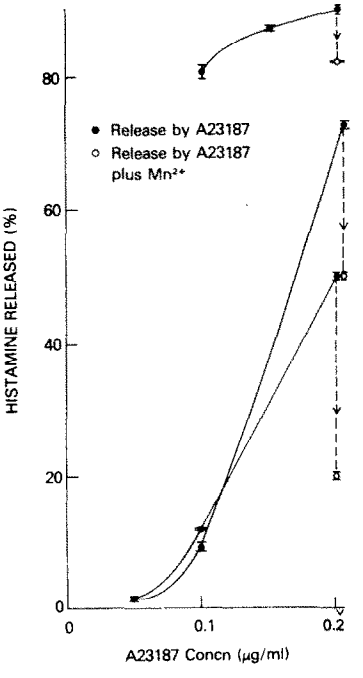

Fig. 3. Effect of 0.1 mM Mn^{2+} on histamine release by 0.2 μg/ml of A23187, shown by the vertical dashed lines with arrows (three experiments). Cells and agent were each prepared in normal medium containing 1.0 mM Ca^{2+}, and PS and Mn^{2+} (when employed) were premixed with the agent before its addition to the cells. Release values obtained with lower concentrations of the ionophore in each experiment are also shown. In each individual experiment, the identical A23187 solution was added to the cells with and without Mn^{2+}, and was used to prepare the more dilute solutions. Results with duplicate cell samples are shown. Medium blanks (without the agent A23187) of 1.4% histamine release without Mn^{2+} and 0.9% with Mn^{2+} have not been subtracted. The high-release experiment was made earlier than the other two, and with a different preparation of the ionophore.

by 33 ± 21 per cent, compared with values obtained without Mn^{2+}; however, Mn^{2+} also caused a similar (28 ± 7 per cent) reduction in release by dextran. As shown in Fig. 3 (three experiments), adding Mn^{2+} was less effective in reducing ionophore-induced histamine release than was halving the ionophore concentration, whereas inclusion of the Mn^{2+} must have reduced the concentration of the ionophore–Ca complex to much lower levels [18].

Effect of neuraminidase. Treatment of the mast cells with neuraminidase affected the subsequent release of histamine by the various agents in a dissimilar manner, as shown in Table 3. Release by con A, A23187, and antigen was increased, whereas release by the basic agents 48/80, protamine, and polylysine was significantly decreased. Release by dextran was less clearly reduced. The level of release by the non-basic agents was relatively low, due to the 30-min initial incubation and other manipulations to which both control and enzyme-treated cells were subjected. The manipulations had less effect on release by the basic agents. The level of release probably did not influence the direction of the change in release produced by neuraminidase, however, since using PS decreased release by the basic agents, but increased the release-depressing effect of the neuraminidase treatment. The precise conditions of the treatment with neuraminidase appeared to be important in determining the results, as treatment of the cells with the enzyme at lower pH values and in the presence of higher levels of Ca^{2+} gave results that differed somewhat from those presented.

Cell desensitization. In the case of each releasing agent studied, exposure of the cells to a low level of the agent consistently decreased the release produced by a higher concentration of the same agent (Table 4). The degree of desensitization produced by polylysine and by 48/80 appeared to be less than that induced by the other agents.

Exposure of the cells to a relatively low level of one releasing agent usually, but not in all cases, de-

Table 3. Effect of preincubation of cells with neuraminidase on histamine release by various agents

Releasing agent (μg/ml)	No. of experiments	Histamine release (net ± S. D.) Control (%)	Treated (%)
Concanavalin A, 10	5	8 ± 3	14 ± 5*
A23187, 0.2	5	9 ± 9	20 ± 12*
Antigen, 1	3	13 ± 12	17 ± 13*
Dextran, 10^3	6	10 ± 5	9 ± 5†
Polylysine, 5	2(7)‡	13 ± 3 (58 ± 12)	4 ± 2 (27 ± 19)§
48/80, 1	2(7)	21 ± 5 (72 ± 7)	6 ± 1 (57 ± 11)§
Protamine, 5	2(7)	38 ± 14 (52 ± 12)	12 ± 4 (29 ± 16)§

* Release increased, P = 0.004, 0.01, 0.06, respectively, based on percentage change between paired release values with replicate cell samples.

† Release decreased, P = 0.07.

‡ Values in parentheses refer to experiments in which PS was omitted.

§ Release decreased, P < 0.003; P values are not given for data derived from only two experiments.

Table 4. Desensitization of cells by various agents to a higher concentration of the same agent

Releasing agent*	No. of experiments	Degree of desensitization (%)
Dextran	4	69 ± 16
Concanavalin A	4	86 ± 9
Antigen	3	62 ± 3
A23187	7	54 ± 26
A23187†	5	61 ± 15
48/80†	5	26 ± 18
Protamine‡	7	68 ± 15
Polylysine‡	3	26 ± 16

* Concentrations of agents as described in text.

† PS not used.

‡ PS used in some experiments; results with and without PS were similar.

Table 5. Effect of pre-addition of agent I on histamine release by agent II

Agents I and II*	No. of experiments	Change in release by agent II (%)
Con A, Ag	3	−90 ± 8†
A23187, Ag	4	−77 ± 12
A23187, Dext	4	−74 ± 6
Dext, Ag	4	−56 ± 23
Ag, Dext	4	−50 ± 26
Con A, Dext	9	−42 ± 20
Ag, con A	4	−35 ± 15
Prot, Ag	6	−35 ± 26
Polyl, Prot‡	3	−31 ± 20
Dext, con A	7	−24 ± 13
Prot, Dext	4	−21 ± 17
Dext, Prot	7	−10 ± 9
Ag, Prot	7	− 5 ± 6
Prot, Polyl‡	2	−29 ± 1§
	1	+ 8§
A23187, Prot‡	3	−58 ± 8§
	2	+23 ± 2§
Prot, A23187‡	2	−30 ± 20§
	5	+69 ± 55§
Dext, A23187	5	+32 ± 46
Ag, A23187	5	+32 ± 33

* Concentrations as described in text. Agent II was added to the medium containing agent I, without washing the cells.

† Mean ± S. D.

‡ PS was used with all combinations except these.

§ In all experiments with the same agent combination, changes in release were in the same direction or virtually nil, with these exceptions.

creased the release produced by the subsequent addition to the cell medium of a second agent in relatively high concentration, as shown in Table 5. When A23187 was followed by protamine and vice versa, increased release by the second agent occurred in some experiments. The changes in both directions were definite, and the reason for the divergent results was not evident. Finally, small additions of dextran or antigen to the cells increased the release produced by subsequent addition of A23187, in accord with previous observations with antigen and A23187 [9, 10].

DISCUSSION

In this study we found that various agents differed markedly in their dependence on exogenous PS and Ca^{2+} in causing histamine release from Sprague–Dawley rat mast cells. The results with PS are in general agreement with those of others [6, 7, 19], to the limited extent that common agents were used in the respective studies. Dextran alone in the present study was completely dependent on exogenous Ca^{2+} We, like others [20, 21], found that some release by antigen occurred in the absence of Ca^{2+}, but our observation of significant residual release by A23187 in the absence of exogenous Ca^{2+} is apparently at variance with observations of a majority of investigators [9, 10, 19, 22]. The variance might be explained, however, by differences in the experimental conditions (as noted below). In accord with our present observations, others have found 48/80 to be largely independent of exogenous Ca^{2+} in its action [23, 24], but comparable studies apparently have not previously

been made with protamine or polylysine, and somatostatin has not previously been described as a histamine-releasing agent. In our earlier studies of release by dextran, it appeared that both Ca^{2+} and PS might act (in part) at superficial cell sites [13], perhaps as part of the dextran receptor. The association between dextran and the receptor must be very loose, however, as we (in unpublished preliminary experiments with fluoroscein-labeled dextran) have been unable to demonstrate binding of dextran by the mast cells. It appears that the basic agents (which require neither PS nor Ca^{2+} in the medium) and the ionophore (which does not require PS) are able to bypass certain steps required for release by the other agents, as previously suggested for the ionophore from different evidence [9, 10].

It is possible that anionic PS decreases the activity of the cationic releasers by forming complexes with them. This depressant effect of PS was variable, however, and the results shown in Fig. 2 are difficult to explain on the basis of complex formation. Anionic heparin completely inhibited release by protamine and polylysine, but not that by 48/80 or somatostatin. It would be of interest to know whether heparin inhibits histamine release by leukocyte cationic protein [8] and whether the heparin-binding cationic apolipoprotein [25] releases histamine, which might be of physiological significance in local areas.

Despite previous contrary evidence that has already been discussed, the idea seems to be prevalent that histamine release is dependent on an influx of Ca^{2+} into the cells from the medium. In the present study, we obtained some release with various agents in Ca-free medium containing EDTA, and release by the basic agents in such medium was of high degree and not greatly decreased from that in normal or complete medium containing Ca^{2+}. EDTA was used in our experiments to eliminate residual Ca^{2+} in the medium. Our experiments with 48/80 and EDTA cannot be compared with those of Douglas [26], in which the cells were incubated in 2.0 mM EDTA for 3 hr at 37°, and in which intracellular Ca^{2+} stores undoubtedly were more effectively exhausted. If Ca^{2+} is required for histamine release, as seems almost certain, our results support the concept that intracellular mobilization of Ca^{2+} as well as influx of Ca^{2+} from the medium [26, 27] may play a role in release by some or all of the agents.

Certain effects of the ionophore A23187, including the release of histamine [22], have been attributed to the transfer of Ca^{2+} into the cells by the ionophore, acting in the form of the Ca complex. However, the failure of Mn^{2+} (which would be expected to displace Ca^{2+} from the complex) to abolish histamine release by the ionophore, as observed here, suggests the possibility that the ionophore may be able to induce release in the presence of Ca^{2+}, even when it is not in the form of the Ca complex. The ionophore may simply resemble certain other histamine releasers, which are not known to form Ca complexes, but which require Ca^{2+} in the medium for release. Histamine release by antigen, for example, is increased by Ca^{2+} in the medium, and has also been shown to be accompanied by an influx of Ca^{2+} into the cells [22]. It is unlikely that Mn^{2+} substitutes for Ca^{2+} in supporting release by the ionophore as it fails to substitute for Ca^{2+} in release by dextran [13].

Altering the cell surfaces by digestion with neuraminidase, like omitting Ca^{2+} and PS from the medium, affected histamine release by the various agents differently. Under the conditions of our experiments, it might be concluded that receptors for con A, A23187, and antigen were unmasked by the enzyme, whereas receptors for the cationic agents (perhaps negatively charged sialic acid residues) were removed, leading to a decrease in release. Another possibility is that release by agents dependent on exogenous Ca^{2+} was increased due to an increase in Ca exchangeability produced by the enzyme [28].

Exposure of the cells to any one of the releasing agents desensitized them to the same agent, as had previously been observed with dextran and antigen [2, 3, 29], and with certain other agents [1, 4]. Experiments on desensitization by one agent to another agent are difficult to interpret, because interactions of the two agents cannot be ruled out, even if the cells are washed between exposures (which they were not here), and also because release of some histamine (by the first agent) may influence the degree of subsequent release, even without causing true desensitization. It appeared from our experiments, which must be considered exploratory rather than definitive, that most of the agents desensitized to one another, in somewhat varying degree. Protamine and A23187 sometimes sensitized to one another, however, and dextran and antigen regularly sensitized the cells to A23187.

Thus, it appears that the different histamine-releasing agents did not act in an identical manner to produce their effects. The present observations expand and elaborate the thesis of other investigators [4, 9, 10], indicating that different releasing agents may act on different receptors, or that some require certain steps not required by others.

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