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Abstract

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KEYWORDS: histamine release, rat peritoneal mast cells, mouse IgE, phosphatidylserine.

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ANTIGEN-INDUCED AND NON-ANTIGEN-INDUCED HISTAMINE RELEASE FROM RAT MAST CELLS SENSITIZED WITH MOUSE ANTISERUM

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Abstract. Marked IgE-mediated histamine release from rat mast cells sensitized *in vitro* with mouse antiserum occurs in the presence of added Ca^{++} and phosphatidylserine (PS), although a considerable degree of antigen-induced histamine release which may utilize intracellular or cell-bound calcium is also observed. The decay in the responsiveness to Ca^{++} of the sensitized cells stimulated by antigen in Ca^{++} -free medium in the presence of PS is relatively slow, and maximum release is produced by Ca^{++} added 1 min after antigen. Histamine release also occurs when Ca^{++} is added after PS in the absence of antigen to the sensitized cells suspended in Ca^{++} -free medium. Unlike the antigen-induced release, the intensity of this non-antigen-induced release varies depending on both mast-cell and antiserum pools. A heat-labile factor(s), which is different from antigen-specific IgE antibody and is also contained in normal mouse serum, is involved in this reaction. In the antigen-nondependent (PS + Ca^{++})-induced release, no decay in the responsiveness to Ca^{++} is observed after PS addition. Both the antigen-induced and non-antigen-induced release are completed fairly rapidly and are dependent on temperature, pH and energy.

Key words : histamine release, rat peritoneal mast cells, mouse IgE, phosphatidylserine.

Intraperitoneal injections of minute amounts of protein together with aluminium hydroxide gel induce persistent formation of a high titer of reagin in some strains of mice (1). The mouse reagin can elicit heterologous passive cutaneous anaphylaxis (PCA) in rats, while IgG₁, another type of mouse homocytotropic antibody, can not (2). Partially purified mouse IgE antibody sensitizes rat peritoneal mast cells *in vitro* and the IgE molecules from the two species are structurally and functionally similar (3,4).

IgE antibody is also produced in rats when they are sensitized with protein antigen using *Bordetella pertussis* vaccine as an adjuvant (5,6). However, using this method, it is often difficult to obtain IgE antibody of a titer high enough for adequate sensitization *in vitro* of mast cells of the same species. Mast cells sensitized *in vitro* may be more suitable for the study of the mode of action of drugs on anaphylactic histamine release than the mast cells obtained from actively sensitized animals, because it is easier to obtain histamine release of the desired intensity by *in vitro* sensitization. Therefore, it seems that mouse IgE antibody may be of

great applicative value for immunopharmacological studies.

The present paper describes the fundamental characteristics of histamine release from rat peritoneal mast cells passively sensitized with mouse IgE antibody *in vitro*.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats of 11-18 weeks of age, weighing 300-450 g obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan), were used. Male BALB/c mice 8-12 weeks of age obtained from the same source or from the Mouse Colony of Okayama University Medical School were also used. The room temperature was kept at 23-26°C.

Chemicals. Ovalbumin (5 x crystalline, OA) was purchased from ICN Pharmaceuticals (Cleveland, O.); rabbit anti-mouse IgG (Lot No. 702) from Medical and Biological Laboratories (Nagoya, Japan); Sepharose-4B from Pharmacia Fine Chemicals (Uppsala, Sweden); bovine serum albumin (fraction V) from Armour Pharmaceutical Co. (Kankakee, Ill.); 2,4-dinitrophenol from Ishizu Pharmaceutical Co. (Osaka, Japan); and phosphatidyl serine (PS) from Nakarai Chemicals (Kyoto, Japan) and also a pure PS preparation, which gives one spot by thin layer chromatography on silica gel G using a solvent system of chloroform-methanol-7N ammonia mixture (60:35:5 v/v), from PL Biochemicals (Milwaukee, Wis.).

Preparation of antisera. Mice were immunized by intraperitoneally injecting 0.2 ml of 0.9% saline containing 1 µg OA and 1 mg Al(OH)₃ gel, twice at an interval of 4 weeks, as described by Levine and his colleagues (1,7). The animals were bled from the carotid artery 7 days after a booster injection. The blood collected from 20-40 mice was pooled, diluted with two volumes of Hanks' balanced salt solution (NaCl 137 mM, KCl 5.36 mM, MgSO₄ 0.811 mM, Na₂HPO₄ 0.337 mM, KH₂PO₄ 0.440 mM, NaHCO₃ 4.17 mM, CaCl₂ 1.26 mM, MgCl₂ 0.492 mM, glucose 5.55 mM; HBSS) and centrifuged. The resulting five fold dilution of antiserum was stored at -20°C. The mouse antisera elicited strong heterologous PCA reactions in the dorsal skin of the rat, giving a threshold reaction (a blue spot of 5 mm in diameter) at 640-1280 fold dilution.

Preparation of Sepharose-OA complex and adsorption of antibody. Sepharose-OA complex was prepared according to the methods of Axén *et al.* (8) and Weinstein *et al.* (9). The complex obtained was finally washed with HBSS and stored at 4°C. Four ml of a five fold dilution of mouse anti-OA serum and 1.7 g wet weight of Sepharose-OA gel were put in a glass tube and gently mixed for 5 min at room temperature. The mixture was filtered under mild suction and the filtrate was obtained.

Preparation of PS suspension. Unless otherwise indicated, PS preparations from Nakarai Chemicals were used. Aliquots of PS stock solution (in chloroform) were put in teflon homogenizer. The solvent was evaporated under nitrogen stream and PS was suspended in appropriate media.

Sensitization of rat mast cells. Rat peritoneal cells containing 3-7 % of mast cells were obtained by peritoneal lavage with HBSS and used without further purification. Usually 2-4 rats were used for one experiment and the peritoneal cells obtained were pooled. The cells containing $1.0 - 1.5 \times 10^6$ mast cells were usually suspended in 1 ml of a fivefold dilution of mouse antiserum in a polypropylene tube. The suspensions were incubated for 60 min at 37°C with intermittent gentle shaking. In the following procedures, the suspen-

ding media contained 0.05 % bovine serum albumin.

Antigen-induced histamine release from mast cells suspended in Ca^{++} -containing medium. After incubation in mouse antiserum, the cells were washed twice by suspending in a sufficient volume of HBSS and centrifuging at $150 \times g$ for 5 min at room temperature. The cells were put in glass centrifuge tubes so that each tube contained $1.0 - 1.5 \times 10^5$ mast cells. Each cell suspension was kept for 2 min at 37°C and PS was added. After 3-min incubation with PS, OA was added. Usually the incubation was continued for a further 5 min at 37°C . Then each tube was immediately transferred to an ice bath and centrifuged at $350 \times g$ for 10 min at 0°C . Both the supernatant and the precipitate were assayed for histamine content. All determinations of the histamine release were performed in duplicate. The final reaction volume was 1.0 ml. The final concentration of OA was $20 \mu\text{g/ml}$. Unless otherwise specified, the final concentration of PS was $30 \mu\text{g/ml}$. PS and OA were suspended or dissolved in HBSS at ten times the final concentrations and 0.1 ml of each was added to the cell suspension.

Antigen-induced histamine release from mast cells suspended in Ca^{++} -free medium. After being washed twice with " Ca^{++} -free" HBSS (HBSS without added Ca^{++}), the sensitized cells were resuspended in similar fresh medium. Ca^{++} was added before or after antigen challenge, so that its final concentration became equal to the concentration in ordinary HBSS (1.26 mM). The other procedures were the same as described for the release in ordinary HBSS.

Non-antigen-induced histamine release. The procedures were the same as described above except that antigen was not added.

Effect of pH. The sensitized cells were suspended in Ca^{++} -free buffered solutions with the pH in the range of 5.6-8.1 (NaCl 154 mM, KCl 2.7 mM, $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ buffer 6.7 mM, glucose 5.55 mM and 0.05 % bovine serum albumin). PS, CaCl_2 (the final concentration of 0.90 mM) and OA were successively added to each cell suspension at intervals of 4 min.

Histamine assay. The histamine content of each sample was determined fluorometrically. The extraction procedures with organic solvents were omitted as described by Loeffler *et al.* (10). The fluorophore was formed as described by Shore *et al.* (11), except that 2.0 M citric acid instead of 3.0 N HCl was used as the acidifying agent according to Anton and Sayre (12).

The percentage of histamine release was calculated using the following equation: histamine release (%) = (histamine content of the supernatant) $\times$ 100 / [(histamine content of the supernatant) + (histamine content of the precipitate)].

RESULTS

Antigen-Induced Histamine Release

Characteristics of mast-cell-sensitizing substance in antisera. From the sensitized rat mast cells, OA released about 70 % of cellular histamine in the presence of PS and Ca^{++} (Table 1). The IgG antibodies in the mouse antiserum were absorbed with rabbit anti-mouse IgG of a quantity sufficient to eliminate the activity to induce 2-h homologous PCA. Both this procedure and repeated washing with fresh HBSS of the sensitized mast cells had little influence on the intensity of the antigen-induced histamine release. The mast-cell sensitizing activity of the antiserum almost disappeared after heating at 56°C for 60 min (Table 1).

Effects of Ca⁺⁺ and phosphatidylserine. The addition of antigen alone to the sensitized cells induced a considerable degree of histamine release in the absence of added Ca⁺⁺ (Table 2). The histamine release was only slightly enhanced when Ca⁺⁺ was added prior to antigen. PS had no significant effect on the intensity of

TABLE 1. EFFECT OF HEATING OF ANTISERUM ON ANTIGEN-INDUCED AND NON-ANTIGEN-INDUCED HISTAMINE RELEASE^a

Exp.	Antiserum	% Histamine release			
		Ordinary HBSS		Ca ⁺⁺ -free HBSS	
		PS + OA	PS	PS + Ca ⁺⁺	PS
(a)	Nonheated	72.7±5.3	23.2±7.1	39.7±10.2	12.0±2.1
(b)	Heated	15.2±2.9 ^b	4.1±0.8	9.1± 2.0	7.8±0.5

^a, Anti-OA serum was heated at 56°C for 60 min. Mast cells were incubated with nonheated and heated antisera from the same pool. The serum-treated cells were resuspended in ordinary or Ca⁺⁺-free HBSS. OA or Ca⁺⁺ was added 3 min after PS. All values are the means ± S.E.M. of three experiments on different pools of cells. When the cells treated with nonheated antiserum were resuspended in ordinary HBSS, histamine release was 2.3 ± 0.2% of the total (n = 3). Almost the same degree of release was observed, when they were resuspended in Ca⁺⁺-free HBSS and Ca⁺⁺ alone was added. With heated antiserum similar results were obtained in such tests.

^b, P < 0.01 as compared with the value in (a) by Student's *t*-test.

TABLE 2. ENHANCEMENT BY PS OF ANTIGEN-INDUCED AND NON-ANTIGEN-INDUCED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS SENSITIZED *IN VITRO*^a

Exp.	Inducers and sequence of addition	% Histamine release ^b	P Compared with
(a)	OA	21.0±2.2 (9)	(e) < 0.001
(b)	Ca ⁺⁺ + OA	25.6±3.8 (4)	(a) N.S. ^c ; (f) < 0.02
(c)	PS + OA	26.7±2.0 (14)	(a) N.S.; (g) < 0.001
(d)	PS + Ca ⁺⁺ + OA	48.4±3.7 (11)	(a),(c),(h) < 0.001; (b) < 0.005
(e)	None	5.0±0.5 (10)	
(f)	Ca ⁺⁺	5.5±0.7 (4)	(e) N.S.
(g)	PS	9.6±1.6 (13)	(e) < 0.02
(h)	PS + Ca ⁺⁺	16.9±3.8 (11)	(e), (f) < 0.02; (g) N.S.

^a, Sensitized cells were resuspended in Ca⁺⁺-free HBSS. PS (final concentration of 3 µg/ml), Ca⁺⁺ and OA were added in the sequence described. The time interval between each addition was 3 min.

^b, All values are the means ± S.E.M. of the numbers of experiments shown in parentheses on different pools of cells.

^c, Not significant.

net antigen-induced histamine release in the absence of added Ca^{++} . However, in the presence of added Ca^{++} , PS markedly enhanced the antigen-induced release. The enhancement by PS of the net antigen-induced release reached a maximum at a PS concentration of $10 \mu\text{g/ml}$. When tested in the absence of added Ca^{++} , the incubation of the cells with EDTA (5 mM) for 30 min at 37°C had no effect on the antigen-induced release irrespective of the presence or absence of PS.

Time course. Antigen-induced histamine release in the presence of PS and Ca^{++} was completed within 1 min of the start of reaction at 37°C (Fig. 1).

Effect of temperature and pH. Histamine release in the presence of antigen was dependent on temperature and pH with the temperature optimum around 37°C and the pH optimum in the range of 7.0-8.0 (Fig. 2&3).

Effect of 2,4-dinitrophenol. 2,4-Dinitrophenol (0.1 mM), an inhibitor of oxidative phosphorylation, almost completely inhibited the antigen-induced histamine release when glucose was omitted from the medium. However, in the presence of glucose (5.55 mM), no inhibition was observed.

Dependence on the time interval between antigen stimulation and Ca^{++} addition. When Ca^{++} was added to the cell suspensions at various times after antigen stimulation in the presence of PS, maximum histamine release was induced by Ca^{++} added 1

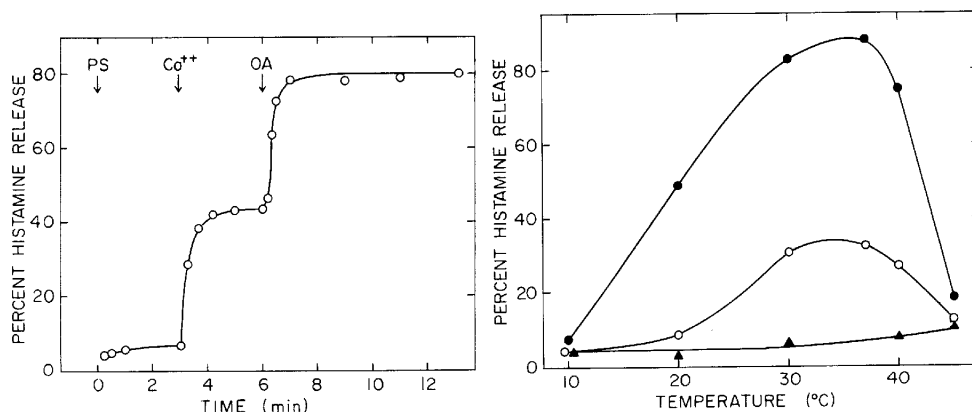


Fig. 1. (left) Time course of histamine release. PS, Ca^{++} and OA were added at 0, 3 and 6 min, respectively. At various times after the addition of PS, the reaction was stopped by adding 4 ml of ice-cold Ca^{++} -free HBSS to each reaction mixture. Each tube was immediately transferred to an ice bath. When none was added, histamine release was 3.8% of the total. Results from a single experiment.

Fig. 2. (right) Effect of temperature on the (PS + Ca^{++} + OA)-, (PS + Ca^{++})- and Ca^{++} -induced histamine release. The sensitized cells suspended in Ca^{++} -free HBSS were preincubated for 3 min at various temperatures in the range of 10 - 45°C . PS, OA and Ca^{++} , maintained at the same temperature as each cell suspension, were successively added to the cells suspended in Ca^{++} -free HBSS at intervals of 3 min. PS + Ca^{++} + OA (●), PS + Ca^{++} (○) and Ca^{++} (▲). Results from a single experiment.

min later. Thereafter, the intensity of release declined slowly with the delay of Ca^{++} addition (Fig. 4).

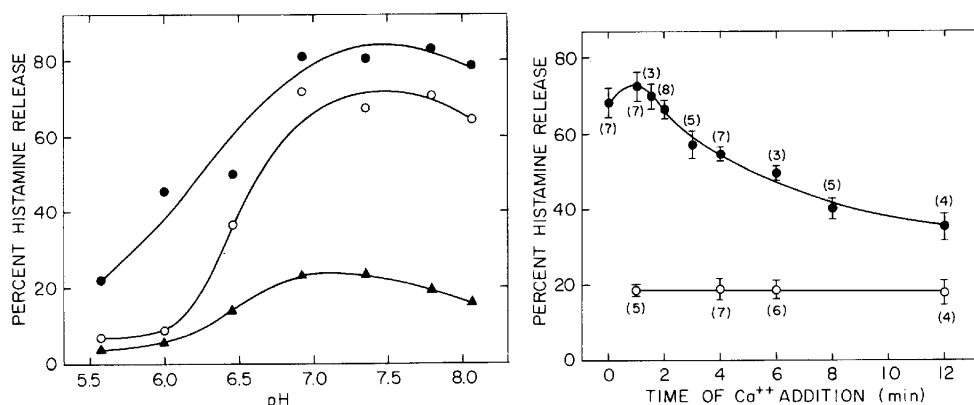


Fig. 3. (left) Effect of pH on the (PS + Ca^{++} + OA)-, (PS + Ca^{++})- and Ca^{++} -induced histamine release. For the experimental procedures see *Materials and Methods*. PS + Ca^{++} + OA (●), PS + Ca^{++} (○) and Ca^{++} (▲). The pH of the reaction medium had a slight influence on the histamine assay and this effect was compensated for by using standards of corresponding pH. Results from a single experiment.

Fig. 4. (right) Dependence of anaphylactic histamine release on the time interval between antigen stimulation and Ca^{++} addition. Sensitized cells were stimulated by OA in Ca^{++} -free HBSS in the presence of PS. OA was added at zero time and Ca^{++} was added at different intervals after OA. Parallel experiments were conducted in which PS and Ca^{++} were added without antigen stimulation. In these experiments PS was added at zero time. The (PS + OA)-induced histamine release was $19.6 \pm 0.9\%$ of the total (mean $\pm$ S.E.M., $n = 8$). PS + OA + Ca^{++} (●) and PS + Ca^{++} (○). The number of experiments on different pools of cells contributing to each point is shown in parentheses and the vertical bars represent S.E.M.

Non-Antigen-Induced Histamine Release

The sensitized mast cells released histamine in response to PS and Ca^{++} even in the absence of antigen (Table 2). A similar reaction was also elicited from cells which had been incubated with normal mouse serum instead of mouse anti-OA serum. Sensitized mast cells were used in the following experiments to study the (PS + Ca^{++})-induced release reaction. Highly pure PS preparation (PL Biochemicals) evoked essentially the same reaction as elicited by less pure preparation (Nakarai Chemicals), though less prominent.

Effects of Ca^{++} and phosphatidylserine. The antigen-nondependent (PS + Ca^{++})-induced release was evident when Ca^{++} was added after PS (Table 2). The intensity of the release induced by Ca^{++} and PS added in that sequence was little different from the intensity of the release in the presence of PS alone (Table 3).

Histamine Release from Mast Cells

TABLE 3. DEPENDENCE OF (PS + Ca⁺⁺)-INDUCED HISTAMINE RELEASE ON THE SEQUENCE OF ADDITION OF THE INDUCERS^a

Exp.	Medium	Inducers and sequence of addition	% Histamine release ^b	P Compared with
(a)	Ordinary HBSS	None	3.2±0.4 (4)	
(b)	"	PS	16.0±2.2 (11)	(a) < 0.001; (d), (g) N.S. ^c
(c)	Ca ⁺⁺ -free HBSS	None	4.2±0.4 (12)	
(d)	"	PS	12.3±1.6 (5)	(c) < 0.01
(e)	"	Ca ⁺⁺	4.4±0.3 (14)	(c) N.S.
(f)	"	PS + Ca ⁺⁺	40.2±2.4 (19)	(b),(c),(d),(e),(g) < 0.001
(g)	"	Ca ⁺⁺ + PS	10.8±1.9 (4)	(c),(e) < 0.05; (d) N.S.

^a, Sensitized cells were resuspended in ordinary or Ca⁺⁺-free HBSS. PS and Ca⁺⁺ were added in the sequence described at an interval of 3 min.

^b, All values are the means ± S.E.M. of the numbers of experiments shown in parentheses on different pools of cells.

^c, Not significant.

In the (PS + Ca⁺⁺)-induced release, no decay of the responsiveness to Ca⁺⁺ was observed after the addition of PS (Fig. 4).

The intensity of the (PS + Ca⁺⁺)-induced histamine release increased with an increase in PS concentration (Fig. 5). When the release was tested using sensitized mast cells that were isolated on a concentrated solution of bovine serum albumin (13), the results obtained were similar to those with mixed peritoneal cells.

Variation depending on mast-cell and antiserum pools. The intensity of the non-antigen-induced histamine release varied markedly depending on the mast-cell and antiserum pools, while that of the release in the presence of antigen was fairly constant irrespective of the differences in the cell and antiserum pools (Fig. 6).

Time Course. The Ca⁺⁺-induced histamine release in the presence of PS was completed within 1 min of the start of the reaction at 37°C (Fig. 1).

Effect of temperature and pH. The (PS + Ca⁺⁺)-induced histamine release was dependent on temperature (optimum: 30-37°C) and pH (optimum: 7.0-8.0) (Fig. 2 & 3).

Effect of 2,4-dinitrophenol. The (PS + Ca⁺⁺)-induced release was almost completely inhibited by 2,4-dinitrophenol (0.1 mM) in the absence of glucose but the inhibition was counteracted by glucose (5.55 mM).

Factor(s) in antisera involved in non-antigen-induced release. The heating of anti-OA serum destroyed the activity in eliciting the (PS + Ca⁺⁺)-induced histamine release (Table 1). The activity remained almost unchanged, even when the antigen-specific antibody involved in the antigen-induced release was almost completely eliminated from the serum by immunoadsorption with Sepharose-OA (Table 4).

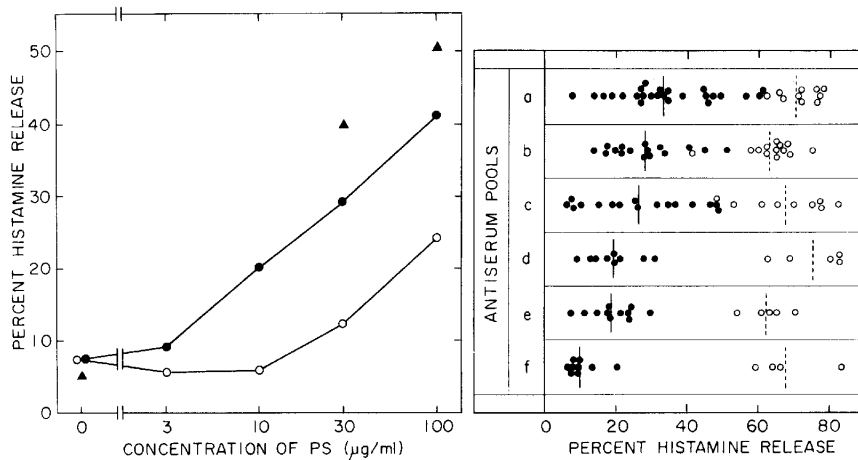


Fig. 5. (*left*) Relationship between concentration of PS and antigen-nondependent (PS + Ca²⁺)-induced histamine release from sensitized mast cells. Sensitized mixed peritoneal cells from a single pool were suspended in Ca²⁺-free (●) or ordinary (○) HBSS. PS was added to all cell suspensions but Ca²⁺ was added only to the suspensions in Ca²⁺-free HBSS 3 min after PS. The (PS + Ca²⁺)-induced histamine release from mast cells isolated, sensitized and suspended in Ca²⁺-free HBSS (▲). Results from a single experiment.

Fig. 6. (*right*) Variation of the intensity of the (PS + Ca²⁺)- and (PS + Ca²⁺ + OA)-induced histamine release from sensitized mast cells. PS, Ca²⁺ and OA were successively added to cell suspensions in Ca²⁺-free HBSS at intervals of about 3 min. Each value represents the intensity of histamine release as to individual cell pools. PS + Ca²⁺ + OA (○) and PS + Ca²⁺ (●). The broken and solid vertical bars show the means of the (PS + Ca²⁺ + OA)- and (PS + Ca²⁺)-induced release, respectively. Histamine release following the addition of Ca²⁺ alone was $4.8 \pm 0.3\%$ of the total (mean $\pm$ S.E.M. of 52 experiments using all antiserum pools).

TABLE 4. EFFECT OF IMMUNOADSORPTION OF ANTIBODIES ON ANTIGEN-INDUCED AND NON-ANTIGEN-INDUCED HISTAMINE RELEASE^a

Exp.	Adsorption	% Histamine release ^b			
		PS + Ca ²⁺ + OA	Ca ²⁺ + OA	PS + Ca ²⁺	Ca ²⁺
(a)	Sepharose, once	73.9 $\pm$ 0.9	13.4 $\pm$ 1.8	23.6 $\pm$ 5.7	4.7 $\pm$ 0.8
(b)	Sepharose, twice	72.8 $\pm$ 3.1	15.0 $\pm$ 2.9	21.6 $\pm$ 6.7	4.3 $\pm$ 0.5
(c)	Sepharose-OA, once	22.9 $\pm$ 5.0 ^c	4.5 $\pm$ 0.3 ^d	21.4 $\pm$ 5.6	3.7 $\pm$ 0.9
(d)	Sepharose-OA, twice	20.0 $\pm$ 6.8 ^e	5.1 $\pm$ 0.9 ^d	20.0 $\pm$ 6.8	4.0 $\pm$ 0.5

^a, Anti-OA antibodies in antiserum were adsorbed with Sepharose-OA. The same pool of antiserum treated with Sepharose served as controls. Mast cells incubated with these antisera were resuspended in Ca²⁺-free HBSS and inducers of histamine release were added. Ca²⁺ was added 3 min after PS simultaneously with OA or alone.

^b, All values are the means $\pm$ S.E.M. of three experiments on different pools of cells.

^c, $P < 0.01$ as compared with the value in (a).

^d, $P < 0.05$ as compared with the corresponding values in (a) and (b).

^e, $P < 0.005$ as compared with the value in (b).

DISCUSSION

In view of the effects on antigen-induced histamine release of repeated washing of sensitized mast cells, the heat-treatment of antiserum and the absorption of IgG₁ with the rabbit anti-mouse IgG, it is clear that the substance which sensitized rat mast cells *in vitro* and elicited this reaction belongs in the IgE class of immunoglobulins.

The antigen-induced histamine release from rat mast cells passively sensitized with heterologous IgE antibody showed temperature-, pH- and energy-dependency similar to that of the anaphylactic mast-cell disruption utilizing homologous IgE antibody (14). The time course of this heterologous reaction was fairly rapid at 37°C.

The anaphylactic histamine release from mast cells obtained from actively sensitized rats requires Ca⁺⁺ in the reaction medium (15) and is enhanced by PS (16), although in Ca⁺⁺-free media antigen induces some degree of histamine release which is not inhibited by EDTA (15). In the present experiments, however, antigen induced a considerable degree of histamine release in the absence of added Ca⁺⁺. EDTA and the addition of Ca⁺⁺ alone did not affect such a reaction to any significant extent. As has been postulated for compound 48/80-induced histamine release (17), it appears that the cell-bound Ca⁺⁺ or the calcium in intracellular reservoirs is utilized for this reaction which is not dependent on extracellular Ca⁺⁺. PS did not substantially enhance the antigen-induced histamine release in the absence of external Ca⁺⁺ and a marked potentiation by PS was observed when Ca⁺⁺ was present. A similar requirement for the extracellular Ca⁺⁺ in the PS-induced potentiation of anaphylactic histamine release has been observed, using peritoneal mast cells from actively sensitized rats (16).

By determining ⁴⁵Ca uptake by mast cells from actively sensitized rats, it has been suggested that the antigen-stimulation causes rapid opening of the calcium gate in the mast-cell membrane which is followed by slowly-occurring closure (18). The response to Ca⁺⁺ of the homologously sensitized mast cells which have been activated by antigen-challenge declines with time and the decay is slowed down by 10 µg/ml of PS (19). In the present experiments, the decay in the presence of 30 µg/ml of PS occurred more slowly. The fact that the maximum histamine release occurred when Ca⁺⁺ was added 1 min after antigen suggests that the amount of calcium which entered through the open calcium channels during a short period, e.g. of the order of several seconds, determines the degree of the histamine release. It also seems probable that the process of calcium gate opening persists until approximately 1 min after the antigen application in the heterologous passive anaphylaxis in rat mast cells.

It is clear from the following facts that the antigen-non-dependent (PS + Ca⁺⁺)-induced histamine release, like the antigen-induced release, is not a result of cell injury: 1) The reaction was temperature-, pH- and energy-dependent. 2) It required Ca⁺⁺ in the medium and was inhibited by disodium cromoglycate and

theophylline (20), which have been known to interfere with Ca^{++} influx into mast cells (18, 21). The observed action of the pure PS preparation seems to exclude the possibility that lysoderivatives of PS contaminating the PS preparation evoked the (PS + Ca^{++})-induced release. Indeed, lyso-PS causes histamine release at concentrations over 10^{-5} M only by a cytolytic mechanism (22). However, lyso-PS stimulates histamine secretion from concanavalin A-treated mast cells at far less concentrations than PS. Potentiation by PS of ligand-induced histamine release may involve its conversion to lyso-PS by a mast cell phospholipase A (22).

Immunoabsorption with Sepharose-OA failed to eliminate from the antiserum the heat-labile factor(s) which makes rat mast cells sensitive to the action of PS and Ca^{++} . Therefore, the factor(s) is different from anti-OA IgE protein and is probably the same substance(s) as contained in the normal mouse serum. It has recently been found that in the presence of mouse plasma lyso-PS stimulates histamine secretion from mouse mast cells. Heat-labile components bound to a plasma macroglobulin seem to be involved in this reaction (23). Consequently, it appears that rat mast cells pretreated with such components in mouse serum released histamine in response to Ca^{++} and PS, which might have been converted to lyso-PS, or PS plus contaminating lyso-PS.

Dextran-induced histamine release from rat mast cells also requires Ca^{++} and PS (24, 25). The present results are similar to the observations on the dextran-induced histamine release (19) in that the intensity of release markedly varies depending on the sequence of addition of PS and Ca^{++} , as well as in that no decay is observed in the responsiveness to Ca^{++} after the addition of PS. Mouse serum components may play a dextran-like role in the (PS + Ca^{++})-induced release.

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