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INHIBITION OF MAST CELL SECRETION BY OXIDATION PRODUCTS OF NATURAL POLYAMINES

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Abstract—Mast cells secrete many biologically active compounds upon stimulation by immunoglobulin E (IgE) and specific antigen (Ag), anaphylatoxins, as well as a number of cationic compounds which include drugs, kinins and neuropeptides. The effects of the two naturally occurring polyamines, spermine (SP) and spermidine (SPD), on mast cell secretion were studied because they have been implicated in the modulation of cellular processes, possibly through their cationic charge or the regulation of calcium ions. SP and SPD over the range of 10^{-7} to 10^{-4} M inhibited the release of 5-hydroxytryptamine (5-HT, serotonin) triggered by compound 48/80 (C48/80) in a time- and concentration-dependent manner, as long as at least 2% calf serum (CS) was present. SP also inhibited secretion of both histamine and serotonin stimulated immunologically by using IgE and anti-rat IgE. This inhibition was not accompanied by cytotoxicity. The major available polyamine metabolites tested, N^1-acetyl spermine (N^1-acSP) and N^8-acetyl spermidine (N^8-acSPD), also showed inhibition in the presence of CS, whereas putrescine, N^8,N^1-hexamethylene-bis-acetamide (HMBA) and benzylamine did not. Fetal bovine serum (FBS), as well as human and rat serum, which do not contain polyamine oxidase, did not result in any inhibition with the polyamines tested. Inhibitors of the polyamine oxidase blocked the polyamine effect, indicating that the inhibition of mast cell secretion must derive from aldehydes produced from these polyamines. Addition of the aldehyde inhibitor phenylhydrazine (Ø-HDZ), simultaneously with, but not following the polyamines, blocked their inhibitory effect, further strengthening the involvement of aldehydes. These results indicate that naturally occurring polyamines may regulate mast cell secretion through metabolic products of polyamine oxidase, a similar enzyme of which is also present in human liver, placenta and pregnant serum.

Mast cells are known primarily for their involvement in allergic reactions [1] during which they secrete many biologically active molecules [2] upon stimulation by immunoglobulin E (IgE‡) and specific antigen (Ag). In addition to this immunologic stimulus, mast cells have been shown to secrete in response to various cationic drugs [3] and many peptides [4–8], of which the most powerful appears to be somatostatin [6, 9, 10]. However, analysis of a variety of peptides by number of amino acids and net cationic charge showed that absolute basicity is not sufficient, but the three-dimensional conformation may be more important since the cyclic structure of D-Lys-somatostatin mimics that of the classic mast cell secretagogue compound 48/80 (C48/80) [11].

The naturally occurring polyamines spermine (SP) and spermidine (SPD) are the biosynthetic products of putrescine, which is derived from ornithine by the action of ornithine decarboxylase (ODC), the rate-limiting enzyme [12–14]. Polyamines had been tested previously and were found to have either no effect on mastocytoma cells [15] or to stimulate secretion from mast cells, but at concentrations over 5×10^{-3} M [16]. We tested the effects of these polyamines and some of their analogues on purified rat peritoneal mast cell secretion stimulated by their classic secretagogue, the polycationic C48/80, as well as immunologically.

EXPERIMENTAL PROCEDURES

Materials

SP, N^1-acetyl spermine (N^1-acSP), SPD, N^8-acetyl spermidine (N^8-acSPD), putrescine, N^8,N^1-hexamethylene-bis-acetamide (HMBA), benzylamine, aminoguanidine (AMG), isoniazid, phenylhydrazine (Ø-HDZ) and bovine serum albumin (BSA) were purchased from the Sigma Chemical Co. (St. Louis, MO). Calf serum, (CS), fetal bovine serum, (FBS) and Dulbecco's modified Eagle's medium were purchased from GIBCO (Grand Island, NY), while heat-inactivated CS was purchased from Sigma.

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‡ Abbreviations: IgE, immunoglobulin E; Ag, antigen; AMG, aminoguanidine; BSA, bovine serum albumin; C48/80, compound 48/80; CS, calf serum; FBS, fetal bovine serum; GABA, γ-aminobutyric acid; HMBA, N^8,N^1-hexamethylene-bis-acetamide; 5-HT, 5-hydroxytryptamine (serotonin); LDH, lactic dehydrogenase; N^1-acSP, N^1-acetyl spermine; N^1-acSPD, N^1-acetyl spermidine; N^8-acSPD, N^8-acetyl spermidine; ODC, ornithine decarboxylase; Ø-HDZ, phenylhydrazine; RBL, rat basophilic leukemia; SP, spermine; and SPD, spermidine.

Table 1. Effects of SP and SPD on mast cell secretion

	Serotonin release (% total)			
	Locke's medium		Dulbecco's medium	
Conditions*	Without CS	With CS	Without CS	With CS
Control	3.2 ± 0.6	3.4 ± 1.0	5.0 ± 0.6	4.9 ± 0.4
SP	3.0 ± 0.3	3.7 ± 0.4	5.0 ± 0.9	4.9 ± 1.1
SPD	3.6 ± 0.4	3.5 ± 0.5	5.2 ± 1.1	5.9 ± 0.9
C48/80	56.1 ± 6.9	53.7 ± 5.1	26.5 ± 3.2	22.9 ± 3.6
SP + C48/80	57.7 ± 10.5	3.6 ± 0.6	29.4 ± 4.2	5.4 ± 1.1
SPD + C48/80	55.2 ± 7.4	8.2 ± 1.2	27.2 ± 2.6	5.8 ± 0.9

* Incubation in the presence (10%) or absence of CS was for 30 min at 37° with either SP (10^{-4} M) or SPD (10^{-4} M) alone or followed by C48/80 (0.5 μg/mL) for an additional 10 min. Viability as measured by trypan blue was 90% and LDH release was not different from controls. Values are means ± SD (N = 3).

Table 2. Effect of SP on secretion and viability of mast cells

	Serotonin release (% total)		LDH release (units/L)	
Conditions*	0.5 hr	3 hr	0.5 hr	3 hr
Control	3.2 ± 0.6	5.2 ± 0.6	6.0 ± 1.4	8.9 ± 0.1
SP (10^{-4} M)	3.0 ± 0.3	5.1 ± 1.1	4.5 ± 2.3	7.4 ± 1.2
C48/80 (0.5 μg/mL)	56.1 ± 6.9	51.2 ± 3.4	3.7 ± 4.6	7.3 ± 2.1
SP + C48/80	5.4 ± 1.1	5.7 ± 0.9	0.8 ± 1.3	6.5 ± 1.7

* Mast cells were incubated with SP (10^{-4} M) for 30 min as described under Methods and then stimulated with C48/80 (0.5 μg/mL) for an additional 10 min at 37° either before (0.5-hr time point) or after washing the cells by centrifugation and resuspension in the same volume as before followed by further incubation for 2.5 hr (3-hr time point). [^{3}H]5-HT and LDH release was measured at both time points. Values are means ± SD (N = 3).

Methods

Rat peritoneal mast cells were isolated from male Sprague–Dawley rats, approximately 350 g weight, that were purchased from Taconic, Inc. (Germantown, NY) in HEPES-buffered Locke's solution, pH 7.2 (150 mM NaCl, 5 mM KCl, 5 mM HEPES, 2 mM $CaCl_2$ 1 g dextrose/L and 1 g BSA/1, pH 7.2). Cells were purified (> 90% purity) over 22.5% metrizamide [17] and resuspended in the same buffer. The cells were loaded with [^{3}H]5-hydroxytryptamine [^{3}H]5-HT (15–30 Ci/mmol; New England Nuclear, Boston, MA) to a concentration of 5×10^{-7} M for 1 hr at 37°, were washed twice and then were resuspended in the indicated buffer (4×10^5 cells/mL) and incubated with the compounds to be tested at 37° as indicated. At the end of the incubation, the cells were pelleted by centrifugation at 100 *g* for 5 min. The supernatant was removed, 2% Triton X-100 was added to the pellet to lyse the cells, and both supernatant and pellet were used to measure radioactivity. The release was expressed as the percentage of total [^{3}H]5-HT released calculated as that present in the supernatant over that in the pellet and the supernatant combined. For immunologic stimulation, purified mast cells were incubated for 60 min at 37° with 10 μg of purified rat IgE (Zymed) per 10^6 cells/mL plus 100 μg/mL phosphatidylserine (Sigma) and then washed once by centrifugation and resuspension as described above. Passively sensitized mast cells were challenged with a 1:20 dilution of mouse anti-rat IgE (Zymed) plus the same concentration of phosphatidylserine as before for 30 min at 37°. In these experiments, stimulation with C48/80 was also carried out for 30 min at 37°.

In some experiments, mast cells were incubated in the Dulbecco's modified Eagle's medium (GIBCO) used to culture rat basophilic leukemia (RBL) cells which have been shown to resemble mucosal mast cells [18]. Cell viability was monitored by trypan blue staining, and by the release of lactic dehydrogenase (LDH) [19] using the LD-L kit (Sigma Diagnostics, St. Louis, MO). Polyamine oxidase activity was measured by a spectrophotometric assay [20]; one spectrophotometric unit was defined as the amount of enzyme catalyzing an increase of 0.001 per min in the optical density reading at 250 nm due to the formation of benzaldehyde from benzylamine. Rat serum was

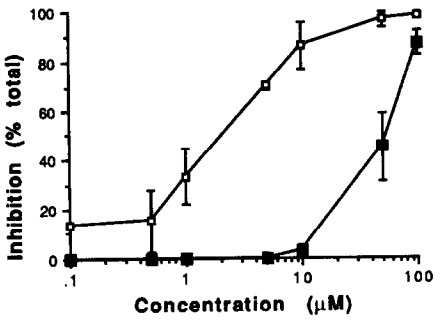

Fig. 1. Concentration–response curve of inhibition of serotonin release from mast cells by SP (□) or SPD (■). Cells were preincubated with SP or SPD for 30 min in Locke's solution +10% CS at 37° before stimulation with 0.5 μg/mL C48/80. Release by C48/80 was 39.59 ± 3.42%. Values are means ± SD (N = 5).

$$\% \text{ Inhibition} = \frac{\text{Release with C48/80 alone after preincubation with SP}}{\text{release with C48/80 alone}} \times 100.$$

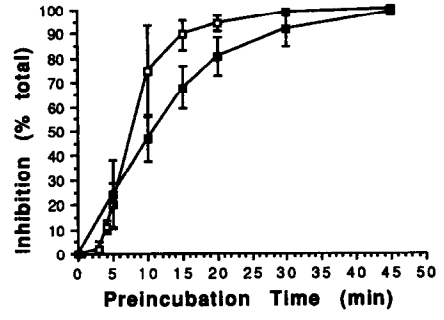

Fig. 2. Time course of inhibition of serotonin release from mast cells by SP (□) or SPD (■). The conditions were the same as those in Fig. 1. Values are means ± SD (N = 5).

prepared from blood collected during decapitation of the rats for cell collection as described above. Human serum was donated from colleagues in the laboratory.

[^{3}H]SP (45 Ci/mmol; New England Nuclear) was incubated with 10^6 mast cells/mL for 60 min and the cells were washed twice with Locke's solution on filter papers with or without excess (10^{-3} M) unlabeled SP. They were then added to 3 mL of scintillation fluid and counted. Alternatively, 10^6 labeled mast cells were homogenized and the proteins precipitated by addition of 10% trichloroacetic acid, following which they were added to scintillation fluid and counted as before.

RESULTS

The effects of SP and SPD on mast cell secretion were first investigated over concentrations ranging from 10^{-7} to 10^{-4} M in Locke's medium. Neither compound (even at 10^{-4} M), during a 30 min preincubation at 37°, had any effect on either basal secretion (less than 5%) or that stimulated by 0.5 μg/mL C48/80 (about 55%) (Table 1). Incubation for longer than 5 hr resulted in significant cytotoxic release as judged by simultaneous LDH release (results not shown). To possibly reduce the cytotoxicity seen with long incubations, these experiments were then repeated with the Dulbecco's modified Eagle's medium supplemented with 10% CS used for culturing RBL cells. Unfortunately, both SP and SPD (10^{-4} M) still caused cytotoxic release if present for longer than 5 hr (results not shown). For incubations of less than 5 hr, neither SP nor SPD (10^{-4} M) had any effect on basal release and there was no effect on serotonin release stimulated by C48/80 (0.5 μg/mL) when Dulbecco's or Locke's was used with CS (Table 1). Serotonin release in response to C48/80 was less when Dulbecco's rather than Locke's medium was used probably because of the somewhat lower pH, the lack of HEPES buffer and the presence of pH color indicator in Dulbecco's which interferred with the serotonin assay. It was apparent that neither Locke's nor Dulbecco's medium without CS had any effect on secretion. Preincubation for 30 min at 37° with either SP or SPD (10^{-4} M) in Locke's or Dulbecco's medium supplemented with 10% CS totally inhibited serotonin release induced by 0.5 μg/mL C48/80 (Table 1).

To check whether the inhibitory effect of SP was washable or whether it was directly altering C48/80, mast cells were incubated with SP (10^{-4} M) for 30 min in Locke's medium plust 4% CS and then the cells were centrifuged for 5 min at 200 *g* and resuspended in Locke's without CS before adding C48/80 (0.5 μg/mL). The extent of the inhibition after the wash was similar (serotonin release 2.8 ± 1.9) to that obtained while SP and CS were still present in the buffer when C48/80 was added (serotonin release 3.1 ± 2.8), as compared to the serotonin release (50.7 ± 3.9) with C48/80 alone.

To establish that this inhibition was not due to any cytotoxic effect of the polyamines, mast cells were preincubated with SP (10^{-4} M) for 30 min at 37°, washed, resuspended in fresh Locke's solution and incubated for an additional 2.5 hr. Mast cell function and viability were determined at both 0.5 and 3 hr by measuring serotonin, as well as LDH release, respectively (Table 2). Under both control and stimulated conditions, serotonin release was comparable at 0.5 and 3 hr, while LDH release remained at control levels at both 0.5 and 3 hr, irrespective of the condition (Table 2). It was, therefore, apparent that the inhibition by SP was not due to a cytotoxic effect.

A concentration–response curve for SP and SPD was, therefore, obtained in Locke's supplemented with 10% CS, following a 30-min preincubation. SP caused 13% inhibition at 10^{-7} M, 70% at 5×10^{-6} M and 100% at 5×10^{-5} M, while SPD had no inhibitory effect with concentrations less than 10^{-5} M and the greatest inhibition of about 85% was achieved at 10^{-4} M (Fig. 1). A time course was performed using 10^{-4} M SP or SPD. Figure 2 shows that as the time of preincubation increased, so did the inhibition which became 100% at 30 min with SP and at about

Table 3. CS requirement for inhibition of mast cell secretion by SP and SPD*

CS concentration (%)	Serotonin release (% total)			Inhibition (% total)	
	C48/80	+SP	+SPD	+SP	+SPD
0.4	45.2 ± 1.3	44.8 ± 0.7	43.9 ± 0.2	0.9	2.8
1	43.1 ± 1.9	36.4 ± 1.2	42.1 ± 0.9	15.5	2.3
2	39.2 ± 1.8	5.9 ± 1.4	20.7 ± 1.2	85.0	47.2
4	35.1 ± 1.7	2.1 ± 0.7	1.7 ± 0.4	94.0	90.2
10	32.3 ± 0.9	0.3 ± 0.9	0.9 ± 1.2	99.1	97.2

* SP or SPD was added at 10^{-4} M for 30 min at 37° in Locke's medium with the CS concentrations shown before stimulation with C48/80 (0.5 μg/mL) for an additional 10 min. Values are means ± SD (N = 3).

Table 4. Effect of SP on mast cell secretion stimulated immunologically or by C48/80

Conditions*	Secretion (% total)	
	Histamine	Serotonin
Control	4.0 ± 0.8	3.1 ± 1.8
Sp	5.2 ± 1.9	9.3 ± 3.5
IgE + anti-rat IgE	33.7 ± 11.0	25.2 ± 8.8
SP + IgE + anti-rat IgE	7.1 ± 3.2	11.7 ± 3.9
C48/80	45.7 ± 7.2	32.1 ± 8.4
SP + C48/80	5.4 ± 2.0	10.0 ± 4.4

* Incubations were carried out in the presence of 4% calf serum (CS) with or without spermine (SP) at 10^{-4} M for 60 min at 37°, followed by either C48/80 (0.5 μg/mL) or immunologically, as described in Methods, for 30 min at 37°. Purified mast cells were incubated for 60 min at 37° with 10 μg of purified rat IgE (Zymed) per 10^6 cells/mL plus 100 μg/mL phosphatidylserine (Sigma) and then the cells were washed once by centrifugation and resuspension. Passively sensitized mast cells were challenged with a 1:20 dilution of mouse anti-rat IgE (Zymed) plus the same concentration of phosphatidylserine as before for 30 min at 37°. Stimulation with C48/80 was also carried out for 30 min at 37°. Values are means ± SD (N = 4)

50 min with SPD. Cell viability after the experimental procedures was always over 95%, as assessed by trypan blue staining, and LDH release average about that of controls (results not shown).

The amount of CS added was then varied to see what concentrations permitted SP or SPD to exert their inhibition. Table 3 shows that 4% was sufficient to permit 94% inhibition with 10^{-4} M SP and about 90% with 10^{-4} M SPD.

The ability of SP to inhibit mast cell secretion was also tested during stimulation with IgE and mouse anti-rat IgE. SP (10^{-4} M) in Locke's solution supplemented with 4% CS inhibited serotonin release by 93.6% and histamine release by 81.9% (Table 4), results comparable with those obtained with non-immunologic stimuli (Table 1). SP had no effect on immunologically induced secretion in the absence of CS (results not shown).

To investigate the possibility that the inhibition seen may be due to some precursor or metabolite of SP or SPD, mast cells were preincubated for 30 min with either putrescine, N^1-acSP, N^8-acSPD or HMBA, all at 10^{-4} M, in Locke's solution with 4% CS before stimulating with 0.5 μg/mL C48/80. Table 2 shows that these molecules used at 10^{-4} M had no effect on their own, while N^8-acSPD caused 46% inhibition and N^1-acSP 100% inhibition (Table 5). Lower concentrations of N^1-acSP and N^8-acSPD showed lower degrees of inhibition (data not shown). HMBA had a marginal inhibitory effect (6.2%) and putrescine had no inhibitory effect at all. Use of these compounds in the absence of CS had no effect on the C48/80-induced release (results not shown). To test whether any aldehyde produced by polyamine oxidase could inhibit mast cell secretion, benzylamine (10^{-4} M), another amine metabolized by the same enzyme, was tested but had no effect on the C48/80-induced secretion (Table 5).

To test if any other component of the serum was necessary for the observed inhibition, 10^{-2} M SP was incubated with 40% CS in the absence of mast cells for 30 min at 37°. Small aliquots of this solution were then added to mast cells so that the final polyamine concentration was 10^{-4} M, but the CS concentration was only 0.4%. The cells were incubated for 10 min at 37° before adding C48/80 (0.5 μg/mL) for another 10 min. Even though the CS concentration was substantially less than the 4% previously shown to be required for almost 100% inhibition (Table 3), the inhibition was nearly total (results not shown), indicating that some inhibitory molecules had been generated during the preincubation.

It appeared that some ingredient of CS was a prerequisite for SP and SPD to exert their inhibitory effect. Serums from other species, therefore, were tested for such an effect. FBS, human and rat serum (all at final concentrations of 10%) had no effect (less than 2% inhibition) on mast cell secretion in the same experiments (results not shown). These serums, therefore, were tested (at a final concentration of 10%) for polyamine oxidase activity, the enzyme that oxidizes SP and SPD to a dialdehyde and monoaldehyde, respectively. Only CS contained enzyme activity (7.3 ± 1.9 units/50 μL), as compared to less than one unit for all the others (Table 6).

Table 5. Effects of some polyamine metabolites and analogues on mast cell secretion

Conditions*	Serotonin release† (% total) Without C48/80	With C48/80	% Inhibition
Control	4.8 ± 1.0	29.8 ± 2.9	
Putrescine	4.1 ± 0.9	32.8 ± 2.6	0.0
N^1-acSP	5.6 ± 0.0	5.7 ± 0.8	99.7
N^8-acSPD	5.3 ± 0.2	18.7 ± 3.4	46.2
HMBA	5.0 ± 0.1	28.5 ± 4.1	6.2
Benzylamine	4.3 ± 1.2	30.5 ± 2.0	0.0

* All compounds were added at 10^{-4} M for 30 min at 37° in Locke's medium with 4% CS before stimulation with C48/80 (0.5 μg/mL) for an additional 10 min. Addition of these compounds in the absence of 4% CS had no effect on C48/80-induced secretion.

† Values are means ± SD (N = 3).

Table 6. Polyamine oxidase activity in various sera

Conditions	Polyamine oxidase (units/50 μL of serum)*
FBS	0.7 ± 0.1
Human serum	0.9 ± 0.2
Rat serum	0.8 ± 0.1
CS	7.3 ± 1.9
CS heated for 2 hr at 56°	7.5 ± 1.4
CS commercially heat-inactivated	6.2 ± 0.9
CS + isoniazid (2×10^{-3} M for 2 hr)	1.4 ± 0.1
CS + AMG (5×10^{-3} M for 2 hr)	2.1 ± 0.1
CS + AMG (5×10^{-3} M for 2 hr)	0.3 ± 0.0

* Fifty microliters is the amount of serum added to each reaction volume (0.5 mL) for a final serum concentration of 10%. Values are means ± SD (N = 4).

To test if any polyamine metabolites were responsible for the inhibitory effect observed, attempts were made to inactivate the polyamine oxidase. Heat treatment of CS for up to 2 hr at 56° did not have any effect on the enzyme activity (Table 6). Alternatively, we used known inhibitors of polyamine oxidase. Treatment of CS with AMG (5×10^{-3} M) for 2 hr totally inhibited (Table 6) the enzyme activity and resulted in abolishing any inhibition of mast cell secretion previously seen with SP (Fig. 3). The same results were obtained (Table 6) when the CS had been preincubated for 2 hr with isoniazid (2×10^{-3} M). AMG and isoniazid at the final concentrations used in the assay (4×10^{-4} and 10^{-4} M, respectively) had no effect on basal mast cell release or that caused by C48/80 (Fig. 3). Inactivation of the aldehydes produced was also tested by the addition of Ø-HDZ which reacts with aldehydes rendering them inactive. Addition of Ø-HDZ (10^{-5} or 10^{-4} M) either 10 or 5 min prior to SP, or added simultaneously with SP (10^{-4} M), abolished the inhibition exerted by SP (Table 7). If Ø-HDZ was added 10 min after SP, it had no effect on the aldehyde-induced inhibition (Table 7).

Incubation of mast cells (10^{-6}/mL) with radiolabeled SP (final concentration of 10^{-6} M) in the presence of 4% CS resulted in label retention by the cells, but this label was substantially reduced by washing with excess unlabeled SP (10^{-5} M). Mast cell proteins precipitated by 10% trichloroacetic acid following similar labeling did not carry any label, indicating that there was no covalent incorporation (results not shown).

DISCUSSION

SP and SPD inhibited C48/80-induced, as well as immunologically stimulated mast cell secretion in a time- and concentration-dependent, noncytoxic manner. This effect occurred only in the presence of CS and could be attributed to the aldehydes produced from SP and SPD by the action of polyamine oxidase, an enzyme found mainly in ruminant sera [21]. Sera with very low polyamine oxidase activity did not support the observed polyamine inhibition of C48/80-induced serotonin release. This enzyme also metabolized the major polyamine monoacetylated derivatives (N^1-acSP, N^1-acSPD and N^8-acSPD), but not the parent compound putrescine, the analog of diacetyl

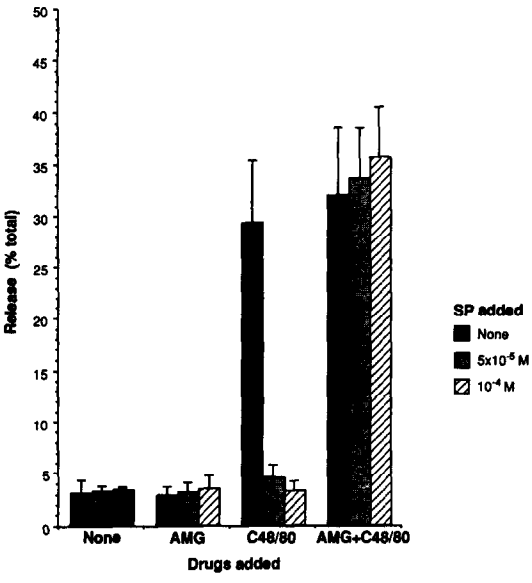

Fig. 3. Effect of AMG on the inhibition of serotonin release from mast cells by SP. CS was treated for 2 hr with 5×10^{-5} M AMG before use. The cells were then incubated for 30 min with either 10^{-4} M or 5×10^{-5} M SP in: (None) CS without any pretreatment; (AMG) CS pretreated with AMG; (C48/80) CS stimulated with 0.5 μg/mL C48/80; and (AMG + C48/80) CS pretreated with AMG, followed by stimulation with 0.5 μg/mL C48/80. Values are means ± SD (N = 5).

Table 7. Effect of Ø-HDZ on SP-induced inhibition of mast cell secretion

Conditions*	Serotonin release (% total)
SP	1.3 ± 1.3
Ø-HDZ	2.3 ± 0.8
C48/80	50.7 ± 3.9
SP→48/80	3.1 ± 2.8
Ø-HZ→SP→ 48/80	52.2 ± 7.6
Ø-HDZ + SP→48/80	48.8 ± 4.8†
SP→Ø-HDZ→48/80	4.8 ± 4.0†

* SP was used at 10^{-4} M, Ø-HDZ at 10^{-5} M and C48/80 at 0.5 μg/mL. The sequence of addition was either SP for 30 min followed by (→) C48/80 for 10 min, or Ø-HDZ added for 10 min before SP, or added simultaneously with (+) SP, or SP followed by (→) Ø-HDZ, followed by (→) C48/80. Values are means ± SD (N = 3) except where noted.

† Average ± range (N = 2).

putrescine, HMBA and the minor putrescine metabolite γ-aminobutyric acid (GABA) [22]. However, only N^1-acSP and N^8-acSPD were available commercially; consequently, N^1-acSPD could not be tested. Likewise, pure polyamine aldehydes or polyamine-derived polycations were not available commercially and could not be tested in the absence of CS.

Moreover, aldehydes derived from other amines (e.g. benzylamine) metabolized by the same enzyme did not have the same effect as those derived from SP and SPD. One must conclude, therefore, that the inhibition of mast cell secretion seen must be specific for the aldehydes of the naturally occurring polyamines and not a general aldehyde effect or a non-specific effect on the plasma membrane. The reaction catalyzed by polyamine oxidase resulted in the formation of the aldehydes mentioned, as well as ammonia and hydrogen peroxide. These latter products do not seem to play a role in the observed inhibition, since benzylamine, which is converted to benzylaldehyde with similar concentrations of the same by-products as when SP or SPD is used, did not cause any inhibition.

The aldehydes formed by the reaction of SP or SPD with polyamine oxidase must bind to the cells and cause the observed effects. Washing of the cells and resuspension in a polyamine and CS-free medium did not abolish the inhibition nor did subsequent addition of the aldehyde inhibitor Ø-HDZ. Incubation of mast cells with a high concentration of polyamines and CS for 30 min followed by washing and observation for up to 3 hr did not result in any cytotoxicity which may have arisen from a slow action of any incorporated polyamines. The continuous presence of a high concentration of polyamines and CS for over 5 hr resulted in cytotoxicity as judged by LDH release. A reversible, non-cytotoxic inhibition of lymphocyte proliferation has also been reported when polyamine oxidase-derived polycations are added early [23]. In another study, polyamine-induced inhibition of uridine incorporation and protein synthesis in thymic leukocytes was reversible if polyamines were washed within 4 hr [24]. These results support ours showing that the aldehydes produced did not have any cytotoxic effects on mast cells unless the incubation was longer than 5 hr.

Seemingly, there is a high affinity of these aldehydes for some surface components of the mast cell which could be some receptor(s) or regulatory protein. An uptake mechanism for SP and/or SPD has been documented for some secretory cells such as platelets [25], neuroblastoma cells [26] and B lymphocytes, but not T lymphocytes [27]. Mast cells do not seem to have such an active uptake mechanism, even though polyamine aldehydes must bind to the surface of mast cells as shown by the inability of washing to remove the inhibition. Polyamines have been shown to incorporate into proteins through a transglutaminase reaction [28, 29], and covalently bound polyamines have been implicated in the regulation of signal transduction [30, 31]. However, our preliminary observations indicate that polyamines probably do not act through covalent incorporation into mast cell proteins.

Polyamines have been shown to inhibit functional characteristics of many cultured cells [32–35], but there is controversy as to whether such inhibition depends entirely on the presence of ruminant sera [36–38]. In particular, polyamines have been shown to inhibit gastric acid secretion [39–41], platelet aggregation [42], possibly secretion from polymorphonuclear leukocytes [43], as well as exocytosis

in sea urchin eggs [44]. Moreover, there is evidence that polyamines may participate in the regulation of insulin [45, 46] and neurotransmitter secretion [47]. Polyamines, the concentration of which is high in malignant cells [12–14], may also be the reason why breast carcinoma-associated mast cells are resistant to the secretory action of C48/80 [48].

SP and SPD may affect free intracellular calcium ion levels [49] which are critical in stimulus–secretion coupling [1]. In fact, a number of recent publications clearly indicate that SP and SPD could regulate calcium ion levels [50–53]. In addition, polyamines appear to inhibit protein kinase C [41], an enzyme implicated in stimulus–secretion coupling [54] and also in mast cell secretion [55]. Polyamines have also been implicated in the regulation of protein phosphorylation [56], and the inhibition of mast cell secretion by disodium cromoglycate (cromolyn) has been associated with increased phosphorylation of one specific protein [57, 58].

The ability of polyamine metabolites to inhibit mast cell secretion, of which a preliminary account has already been reported [59], may be of therapeutic importance in other pathophysiological conditions where mast cells appear to be involved, such as migraine headaches [60] and multiple sclerosis [61]. Diamine oxidase has also been isolated from mammalian liver [62], and was shown to generate the same end products as those found in ruminant serum, and is also present in human endometrium [63] and placenta [64]. Moreover, polyamine interaction with human pregnancy serum inhibits lymphocyte transformation [65], and high polyamine oxidase activity has been detected in human pregnant serum [66], as well as human and bovine milk [67]. This information suggests that aldehydes generated from naturally occurring polyamines and/or polycations produced from them may be involved in the physiologic down-regulation of the immune system during pregnancy. It is noteworthy that allergic and other autoimmune syndromes improve considerably during pregnancy [68, 69], and such syndromes as multiple sclerosis and interstitial cystitis have been associated recently with mast cell activation [70, 71].

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