

Regulation of immunoglobulin E-mediated secretion by protein phosphatases in human basophils and mast cells of skin and lung

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Abstract

A wide range of serine/threonine protein phosphatase (PP) inhibitors were studied for effects on the immunoglobulin E (IgE)-mediated release of histamine from human lung mast cells, human skin mast cells and basophils. Okadaic acid (OA) inhibited the release of histamine from all three cell types in a concentration-dependent manner. Two structural analogues of okadaic acid, okadaol and okadaone, known to be less active than the parent molecule as inhibitors of PP, were less active than okadaic acid as inhibitors of histamine release in these three cell types. A number of PP inhibitors, showing differences in selectivity for PP1 and PP2A, were also evaluated. Calyculin, which is roughly equipotent as a PP1 and PP2A inhibitor, attenuated the release of histamine from all three cell types. Similarly, tautomycin (TAU), which shows greater selectivity for PP1 over PP2A, was also effective at inhibiting histamine release in all three cell types. In contrast, fostriecin, which is very much more potent as an inhibitor of PP2A over PP1, was ineffective as an inhibitor in all three cell types. These data indicate that the regulation of mediator release by PPs is similar in lung mast cells, skin mast cells and basophils. Moreover, the data suggest that PP1 is important in the control of cellular activity. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Mast cell; Basophil; Protein phosphatase; Okadaic acid; Tautomycin; Fostriecin

1. Introduction

The mast cell has long been recognised as central to the mediation of allergic reactions (Warner and Kroegel, 1994). Immunoglobulin E (IgE)-triggered activation of mast cells by allergen can lead to the release of a wide variety of autacoids, such as histamine and sulphur-containing leukotrienes, with pro-inflammatory and spasmogenic properties (Schleimer et al., 1984). The basophil may also be activated in an IgE-dependent manner and may contribute to late phase responses in allergic conditions (Schleimer et al., 1984). Despite similarities between mast cells and basophils, a large body of evidence has shown that these cell types express structural and functional differences (Warner and Kroegel, 1994). Moreover, mast cells do not constitute a homogeneous population as mast cells isolated from different sites can show substantial differences in

content and variable responsivity to both stimuli and inhibitors (Pearce, 1983; Lowman et al., 1988).

Because of the important role that mast cells and basophils play in allergy, considerable effort has been invested in trying to understand processes that modulate the activity of these cells. As with other cells, evidence has accumulated that phosphorylation/dephosphorylation events are crucial to regulating mast cells and basophils (Paolini et al., 1991; Benhamou and Siraganian, 1992; Beaven and Metzger, 1993; Hamawy et al., 1995). Although the effects of protein kinases have been studied quite intensively, less is known about the role that protein phosphatases play.

Four major classes of serine/threonine protein phosphatase (PP) have been identified, these being PP1, PP2A, PP2B and PP2C. These PPs constitute discrete gene products and can be discriminated functionally because requirements for activation, susceptibility to inhibitors and substrate specificities differ (Cohen, 1989; Cohen and Cohen, 1989; Shenolikar, 1994). Indeed, a growing number of naturally occurring, cell-permeant PP inhibitors are becoming available, and these can be used to probe the impor-

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tance of PPs in intact cells (Haystead et al., 1989; Cohen et al., 1990; Hardie et al., 1991; MacKintosh and MacKintosh, 1994). For example, cyclosporin is known to inhibit PP2B (Liu et al., 1991), okadaic acid (OA) inhibits both PP2A and PP1 but is about 10- to 100-fold more potent against PP2A (Cohen et al., 1989), and calyculin is roughly equipotent as an inhibitor of PP1 and PP2A (Ishihara et al., 1989).

We have previously reported that okadaic acid is an effective inhibitor of mediator release from human lung mast cells and basophils, suggesting that PP1 and/or PP2A may regulate the activity of these cells (Peachell and Munday, 1993; Peirce et al., 1996, 1997, 1998). Moreover, cyclosporin is known to inhibit mediator release from lung mast cells and basophils, suggesting that PP2B may also regulate cellular activity (Triggiani et al., 1989; Cirillo et al., 1990). In the present study, we have extended the range of PP inhibitors, to include tautomycin (TAU) (more potent at PP1 than PP2A) and fostriecin (very much more potent at PP2A than PP1), to try to gain a better understanding of the role that PPs play in lung mast cells and basophils. Moreover, we have also investigated the effects of a range of PP inhibitors on mediator release from the human skin mast cell so as to determine whether mast cells derived from different sites show functional heterogeneity to these inhibitors.

2. Materials and methods

2.1. Buffers

Phosphate buffered saline (PBS) contained (mM): NaCl 137, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 8, KCl 2.7, KH_2PO_4 1.5. PBS-BSA was PBS which additionally contained: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1 mM, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1 mM, glucose 5.6 mM, bovine serum albumin 1 mg/ml, DNase 15 $\mu\text{g}/\text{ml}$. PBS-HSA was PBS additionally supplemented with: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1 mM, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1 mM, glucose 5.6 mM, human serum albumin 30 $\mu\text{g}/\text{ml}$. The pH of all PBS buffers was titrated to 7.3.

2.2. Preparation of compounds

Okadaic acid, okadaol, okadaone (all 0.5 mM stocks), calyculin-A (0.1 mM stock) and microcystin-LR (0.1 mM stock) were prepared in 10% dimethyl sulphoxide. Tautomycin (1 mM stock) and fostriecin (1 mM stock) were made up as stock solutions in 100% and 10% methanol, respectively. Lyophilised polyclonal goat anti-human IgE antibody was reconstituted in distilled water. All stock solutions were stored at -20°C with the exception of anti-IgE which was stored at 4°C . The drugs were diluted to the desired concentration in buffer just prior to use. Vehicles, at the concentrations used in experiments, had no effects on control activities.

2.3. Isolation of basophils

Mixed leukocyte preparations were obtained from whole blood by dextran sedimentation. Briefly, 50 ml of venous blood was mixed with 12.5 ml of 6% dextran and 5 ml of 100 mM EDTA, then allowed to sediment for 90 min at room temperature. The upper buffy coat layer was removed, cells were recovered by centrifugation ($120 \times g$, 8 min) and washed twice with PBS. These mixed leukocyte preparations were used in the histamine release experiments.

2.4. Isolation of human lung mast cells

Macroscopically normal lung tissue from resections was used in this study. Most of the patients were undergoing surgery for carcinoma. The male to female split was 70% to 30%, and 90% of the patients were white caucasians. Mast cells were isolated from human lung tissue by a modification of the method described by Ali and Pearce (1985). Lung tissue was stripped of its pleura and chopped vigorously for 15 min with scissors in a small volume of PBS buffer. The chopped tissue was washed over a nylon mesh (100 μm pore size; Cadisch and Sons, London, UK) with 0.5–1 l of PBS buffer to remove lung macrophages. The tissue was reconstituted in PBS-BSA (10 ml/g of tissue) containing collagenase Ia (350 U/ml of PBS-BSA) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37°C . The supernatant (containing some mast cells) was separated from the tissue by filtration over nylon mesh. The collagenase-treated tissue was then reconstituted in a small volume of PBS-BSA buffer and disrupted mechanically with a syringe. The disrupted tissue was then washed over nylon gauze with PBS-BSA (300–600 ml). The pooled filtrates were sedimented ($120 \times g$, room temperature, 8 min), the supernatant discarded and the pellets reconstituted in PBS-BSA (100 ml). The pellet was washed two times further. Lung mast cells were visualized by microscopy using an alcian blue stain (Gilbert and Ornstein, 1975). Mast cells prepared by these methods were used in mediator release experiments.

2.5. Isolation of human skin mast cells

Human skin mast cells were generated from foreskins obtained from surgical circumcisions of infants. The tissue was sliced into small fragments with scalpels and chopped finely with scissors and washed over nylon gauze with RPMI1640 buffer supplemented with foetal calf serum (2%). The chopped tissue was incubated (2 h) in RPMI1640 (20 ml) supplemented with collagenase Ia (350 Units/ml), DNase (0.2 mg/ml), bovine serum albumin (25 mg/ml) and foetal calf serum (2%) and agitated by using a water-driven magnetic stirrer immersed in a water bath set at 37°C . After the incubation, the tissue was aspirated with a

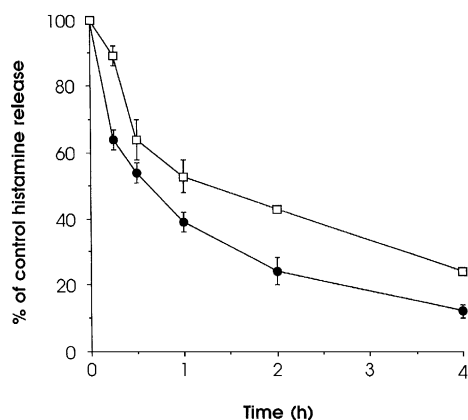


Fig. 1. Kinetics of inhibition. Human lung mast cells were incubated without or with okadaic acid (circles; 1 μ M) or tautomycin (squares; 3 μ M) for time periods as indicated before challenge with anti-IgE for histamine release. Results are expressed as a percentage of the control histamine release which was $30 \pm 2\%$. Values are means \pm S.E.M. for 10 experiments. All incubation times with both drugs led to statistically significant ($P < 0.05$) reductions in histamine release.

syringe in a small volume of the digestion incubate and the disrupted tissue then washed over nylon gauze with the remaining digestion incubate and a further 50 ml of fresh RPMI1640 buffer. The filtrate was sedimented ($120 \times g$, room temperature, 8 min), the supernatant was discarded, and the pellets were washed in PBS–HSA (50 ml) and sedimented. The washing step with PBS–HSA and sedimentation step was repeated. Skin mast cells prepared in this manner were used in histamine release experiments.

2.6. Mediator release

Histamine release from lung mast cells, skin mast cells and basophils was initiated immunologically with a maximal releasing concentration of anti-IgE (1:300, mast cells; 1:1000, basophils). Secretion was allowed to proceed for 25 min (mast cells) or 45 min (basophils) at 37 $^{\circ}$ C, after which time the cells were pelleted by centrifugation ($400 \times g$, room temperature, 3 min). Histamine released into the supernatant was determined by a modification (Ennis,

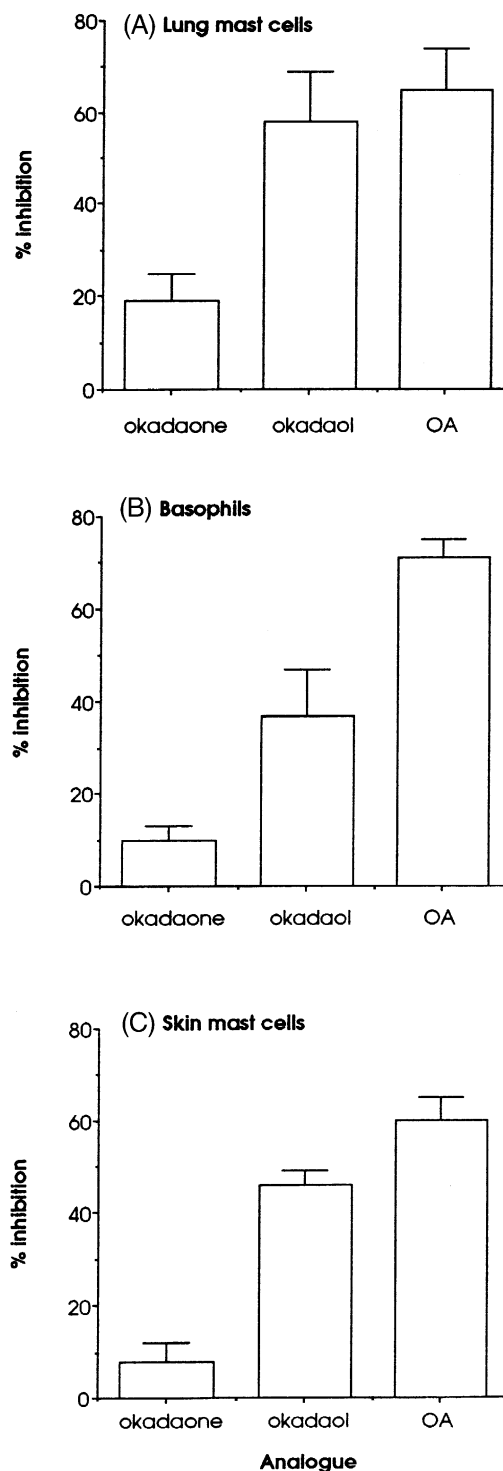


Fig. 2. Effects of okadaic acid (OA, 10 μ M), okadaol (10 μ M) and okadaone (10 μ M) on histamine release. Cells were incubated (2 h) without or with okadaic acid or an analogue before challenge with anti-IgE. Results are expressed as the % inhibition of the control histamine releases which were $30 \pm 3\%$ (lung mast cells), $36 \pm 3\%$ (basophils) and $31 \pm 7\%$ (skin mast cells). Values are means \pm S.E.M. for nine (lung mast cells), eight (basophils) and four (skin mast cells) experiments. All treatments caused statistically significant ($P < 0.05$) levels of inhibition except okadaone in skin mast cells.

Table 1
Time-dependence of inhibition of histamine release

Time (h)	% inhibition					
	Lung mast cells		Basophils		Skin mast cells	
	TAU	OA	TAU	OA	TAU	OA
0.5	42 \pm 5	48 \pm 3	57 \pm 5	55 \pm 5	24 \pm 5	34 \pm 9
4	77 \pm 4	89 \pm 1	73 \pm 4	91 \pm 2	81 \pm 3	83 \pm 4
18	82 \pm 9	92 \pm 7	81 \pm 8	95 \pm 2	72 \pm 7	75 \pm 8

Cells were incubated for the time periods indicated with either tautomycin (TAU, 3 μ M) or okadaic acid (OA, 1 μ M) before activation with anti-IgE. Data are expressed as the % inhibition of the control histamine releases which were $30 \pm 2\%$ (lung mast cells), $26 \pm 5\%$ (basophils) and $16 \pm 2\%$ (skin mast cells). Values are means \pm S.E.M. from 4 to 14 experiments.

Table 2

IC₅₀ values for the inhibition of PP1 and PP2A by PP inhibitors

	IC ₅₀			
	Okadaic acid	Calyculin	Tautomycin	Fostriecin
PP2A	0.1–1	0.3	30	3.2
PP1	10	0.3	0.5	131 ^a

All values are in nM except the value indicated by (^a) which is in μ M. Values have been taken from the following references: Cohen et al. (1989), Ishihara et al. (1989), Takai et al. (1995) and Walsh et al. (1997) for okadaic acid, calyculin, tautomycin and fostriecin, respectively.

1991) of the automated fluorometric method of Siraganian (1974). When PP inhibitors were employed, cells were first incubated with a given PP inhibitor, for time periods as indicated in the text, at 37 °C before the addition of anti-IgE, and then samples were processed as indicated above. Total histamine content was determined by lysing aliquots of the cells with 1.6% perchloric acid. Cells incubated in buffer alone served as a measure of spontaneous histamine release (< 6%). Histamine release was thus expressed as a percentage of the total histamine content after subtracting the spontaneous histamine release. All experiments were performed in duplicate.

2.7. Materials

The following were purchased from the sources indicated; anti-human IgE, bovine serum albumin, collagenase, DNase, dimethyl sulphoxide, foetal calf serum, fostriecin, human serum albumin (all from Sigma, Poole, UK); RPMI1640 (Gibco BRL, Dundee, UK); okadaic acid, okadaol, okadaone, calyculin, microcystin, tautomycin (Alexis, Nottingham, UK).

2.8. Data analysis

All experiments were performed in duplicate and results were expressed as means \pm S.E.M. IC₅₀ values represent the concentrations of PP inhibitors that inhibit enzymatic activity and the release of histamine by 50%. The effects of PP inhibitors were analysed for significance using Student's *t*-test for paired data. Values of *P* < 0.05 were taken as significant.

3. Results

We have previously reported that okadaic acid inhibits IgE-dependent histamine release from human lung mast cells and basophils in a time-dependent manner (Peachell and Munday, 1993; Peirce et al., 1996). In the present study, the time-dependence of inhibition for okadaic acid (1 μ M) and an alternative PP inhibitor, tautomycin (3 μ M), was evaluated in lung mast cells, basophils and skin mast cells. In lung mast cells, the onset of inhibition was

slower with tautomycin and both compounds showed roughly comparable kinetics of inhibition with half-maximal inhibition at around 30 min and maximal inhibition at 2 to 4 h (Fig. 1, Table 1). A similar time-dependence for

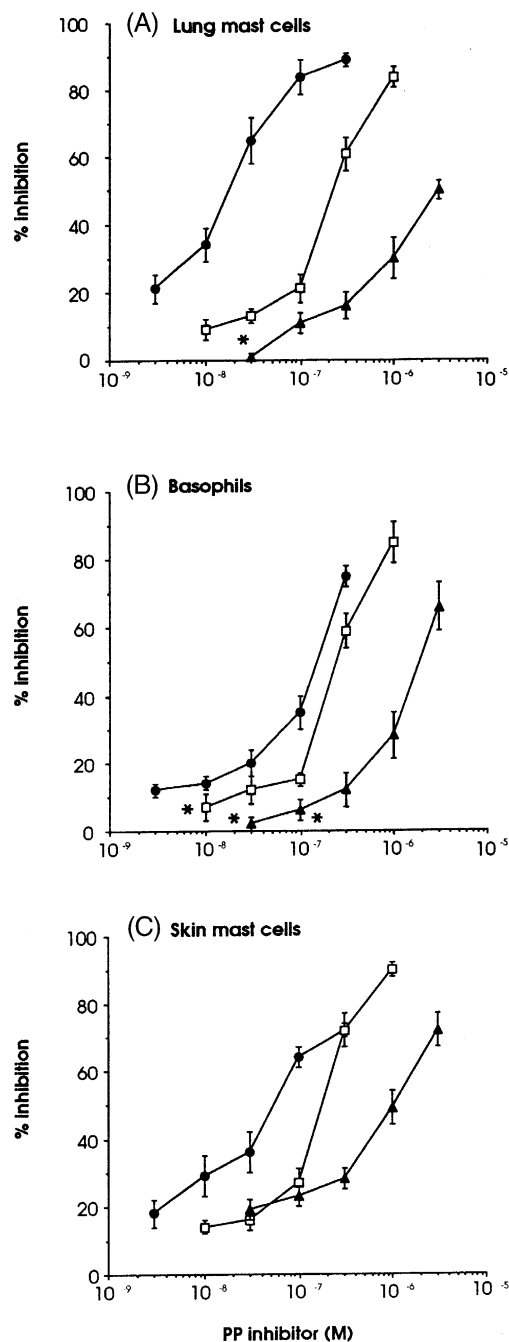


Fig. 3. Effects of calyculin, okadaic acid and tautomycin on histamine release. Cells were incubated (2 h) without or with calyculin (circles), okadaic acid (squares) or tautomycin (triangles) before challenge with anti-IgE. Results are expressed as the % inhibition of the control histamine releases which were $32 \pm 4\%$ (lung mast cells), $33 \pm 5\%$ (basophils) and $23 \pm 2\%$ (skin mast cells). Values are means \pm S.E.M. for 8–12 (lung mast cells), 6–8 (basophils) and 8–11 (skin mast cells) experiments. Treatments which did not cause statistically significant (*P* < 0.05) levels of inhibition are indicated by an asterisk (*).

inhibition by these compounds was observed in both basophils and skin mast cells although basophils succumbed sooner to the effects of PP inhibitors than skin mast cells (Table 1). Unless otherwise indicated, a 2 h incubation of cells with PP inhibitors was employed in all further experiments.

The effects of two analogues of okadaic acid, okadaol and okadaone, known to be less active as PP inhibitors than okadaic acid (Nishiwaki et al., 1990), were evaluated on the stimulated release of histamine from lung mast cells, basophils and skin mast cells (Fig. 2). The rank order of activity for inhibition was okadaic acid > okadaol > okadaone for all three cell types.

The effects of alternative PP inhibitors, tautomycin and calyculin, as well as okadaic acid, were investigated. Tautomycin is more potent against PP1 than PP2A, okadaic acid is about 10-fold more potent against PP2A than PP1, and calyculin is roughly equipotent as an inhibitor of PP1 and PP2A (see Table 2 for IC_{50} values of these compounds as inhibitors of PP1 and PP2A). All three compounds inhibited IgE-dependent histamine release in a concentration-dependent manner from lung mast cells, basophils and skin mast cells (Fig. 3). The rank order of activity for inhibition was calyculin > okadaic acid > tautomycin in all three cell types, although there were quantitative differences in the relative potencies of these compounds dependent on the cell type (Table 3). In particular, whereas okadaic acid showed similar potency in all three cell types, calyculin and tautomycin displayed greater variability in potency.

The effects of an alternative PP inhibitor, fostriecin, were also studied. Fostriecin, which is much more potent at PP2A than PP1 (see Table 2), was ineffective as an inhibitor in all three cell types (Table 4). Indeed, after overnight incubation (24 h), fostriecin (1 μ M) still failed to inhibit the IgE-mediated release of histamine from lung mast cells (data not shown, $n = 3$). Microcystin, which is equipotent at PP1 and PP2A, but which is known to enter cells only if a selective transporter is present (Eriksson et al., 1990; Rutter et al., 1991), was relatively ineffective as

Table 4

Effect of fostriecin and microcystin on histamine release

	% inhibition		
	Lung mast cells	Basophils	Skin mast cells
Fostriecin	5 \pm 9	– 1 \pm 3	14 \pm 4
Microcystin	14 \pm 3 ^a	3 \pm 4	21 \pm 3 ^a
Okadaic acid	89 \pm 6 ^a	79 \pm 5 ^a	93 \pm 2 ^a

Cells were incubated (2 h) with a PP inhibitor (3 μ M) before challenge with anti-IgE. Results are expressed as the % inhibition of the control histamine releases which were 31 \pm 4% (lung mast cells), 29 \pm 4% (basophils) and 20 \pm 6% (skin mast cells). Values are means \pm S.E.M. from four (lung mast cells), five (basophils) and three (skin mast cells) experiments.

^aDenotes statistically significant ($P < 0.05$) levels of inhibition.

an inhibitor of histamine release in all three cell types studied (Table 4).

4. Discussion

A large body of literature exists showing that mast cells isolated from different species can differ functionally (Pearce, 1983). Moreover, mast cells derived from different sites within the same species can also differ appreciably in response to stimuli and inhibitors (Pearce, 1983; Lowman et al., 1988). As a corollary, the basophil, which is often considered to be the blood-borne counterpart of the mast cell such that it expresses high affinity IgE receptors and releases histamine following activation, can also display differences in response to agents (Warner and Kroegel, 1994). The aim of the present study was to assess the responses of human lung mast cells (a mucosal mast cell), human skin mast cells (a connective tissue mast cell) and basophils to a panel of PP inhibitors and, thereby, to try to establish whether modulation of a particular PP(s) may affect cell function.

The prototypic cell-permeant PP inhibitor, okadaic acid, attenuated the stimulated release of histamine from lung mast cells, skin mast cells and basophils in a concentration-dependent manner. Similar findings have been reported by ourselves and others in basophils and lung mast cells (Botana and MacGlashan, 1993; Peachell and Munday, 1993; Peirce et al., 1996, 1997, 1998). These data suggest that inhibition of PPs in mast cells and basophils leads to inhibition of release. Lengthy incubations (≥ 2 h) of all cell types with okadaic acid were needed to obtain optimal inhibitory effects. This may indicate that either access of okadaic acid to intracellular targets and/or the consequences of PP inhibition on cell function may take some time. Analogues of okadaic acid, okadaol and okadaone, known to be less effective as inhibitors of PPs than okadaic acid (Nishiwaki et al., 1990), were less effective as inhibitors of histamine release in all cell types. These data further suggest that these compounds are acting as PP inhibitors in these cell types.

Table 3

 IC_{50} values for the inhibition of histamine release by PP inhibitors

	IC_{50} (μ M)		
	Lung mast cells	Basophils	Skin mast cells
Calyculin	0.02 \pm 0.004	0.13 \pm 0.02	0.05 \pm 0.007
Okadaic acid	0.29 \pm 0.04	0.26 \pm 0.03	0.21 \pm 0.03
Tautomycin	3.0 ^a	2.4 \pm 0.6	1.3 \pm 0.3

IC_{50} values were calculated from the individual experiments that were used to generate Fig. 3 and are means \pm S.E.M. Further experimental details can be found in the legend to that figure.

^aThe IC_{50} value for tautomycin is approximate because, in about half of the experiments, inhibition of histamine release by tautomycin was less than 50% at the highest concentration (3 μ M) of inhibitor employed.

Okadaic acid is about 10-fold more potent against PP2A than PP1 (Cohen et al., 1989) such that studies with okadaic acid alone would not be able to establish which of these PPs may be targeted by the inhibitor to modulate the response of a cell. Calyculin, which is about as potent as okadaic acid as an inhibitor of PP2A but some 100-fold more potent than okadaic acid as an inhibitor of PP1 (Ishihara et al., 1989), was also studied and was found to be more potent than okadaic acid as an inhibitor of histamine release in all three cell types. These data could suggest that the main PP, which these inhibitors target to modulate release, is PP1, although effects on PP2A cannot be discounted. An alternative PP inhibitor, tautomycin, which is more potent against PP1 than PP2A (Takai et al., 1995), was also effective at attenuating histamine release from all three cell types. Again, these findings point to a prominent role for PP1 in these cells especially as alternative studies indicate that treatment of cells with tautomycin inhibits PP1 selectively (Favre et al., 1997; Yan and Mumby, 1999). The rank order of potency of calyculin > okadaic acid > tautomycin was identical for all three cell types suggesting similarities in regulation by PPs of mast cells and basophils. That tautomycin was the least potent at inhibiting histamine release may be a little surprising if PP1 is involved in regulating release because tautomycin is more potent than okadaic acid as an inhibitor of isolated PP1 (see Table 2). However, studies in alternative cell systems have shown that calyculin enters cells most easily compared to both okadaic acid and tautomycin, whereas tautomycin is the least permeant (Favre et al., 1997). Hence, the potency order observed in the present study may have been influenced by the incubation time used (2 h), and data from the present work (Fig. 1) show that tautomycin displays relatively slower kinetics of inhibition compared to okadaic acid.

Further studies were performed with fostriecin, a PP inhibitor which is very much more potent at PP2A than PP1 (Walsh et al., 1997; Hastie and Cohen, 1998). Indeed, at the extracellular concentration (3 μ M) of fostriecin employed, and given the IC₅₀s for inhibition by this compound of PP1 and PP2A (see Table 3), it would seem that fostriecin would be able to inhibit PP2A at this concentration but would have no effect on PP1. Alternative studies have employed fostriecin to inhibit PP2A selectively in intact cells (Chiang et al., 2001). In the present study, fostriecin was found to be ineffective as an inhibitor of histamine release in all three cell systems even after overnight incubation of cells with the inhibitor. These data with fostriecin suggest that inhibition of PP2A does not affect release in mast cells and basophils and further implicate PP1 as the major PP regulating secretory activity.

As a generalisation, these findings indicate that inhibition of PP1 inhibits histamine release in lung mast cells, skin mast cells and basophils, and that the regulation of secretion by PPs is similar in all three cell types. Despite

this overall impression, it should be noted that there are quantitative differences in the effects of PP inhibitors on these cell types. For example, the potency of calyculin appears to be quite variable among the cell types studied. Moreover, okadaol appears to inhibit histamine release, relative to okadaic acid, to a greater extent in lung and skin mast cells than in basophils. These quantitative differences could reflect relative differences in PP content or the relative importance of PPs in the regulation of secretory activity in a given cell. Previous studies of our own have shown that human lung mast cells contain about 6-fold more PP1 and 2-fold more PP2A than basophils on a per cell basis (Peirce et al., 1998). Moreover, basophils contain substantially more okadaic acid-sensitive PP activity which cannot be accounted for by PP1 or PP2A. Indeed, certain alternative PPs that have been described more recently are known to be sensitive to okadaic acid (Honkanen et al., 1991; Brewis et al., 1993; Chen et al., 1994). Thus, the possibility that alternative PPs, other than PP1, may also be involved in regulating the cells cannot be excluded.

In summary, the present paper has shown that PP inhibitors inhibit histamine release from lung mast cells, skin mast cells and basophils. This suggests that PPs can regulate secretion in these cells. That the effects on secretion of a wide panel of PP inhibitors are similar in all three cell types indicates similarities in regulation. Moreover, that tautomycin (PP1-selective) inhibits histamine release, whereas fostriecin (highly PP2A-selective) does not, suggests that PP1 is important in regulating the activity of lung mast cells, skin mast cells and basophils.

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