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Characterization of protein serine/threonine phosphatase activities in human lung mast cells and basophils

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1 The serine/threonine protein phosphatase (PP) inhibitors, okadaic acid and calyculin, attenuated the IgE-mediated release of histamine from human lung mast cells (HLMC) and basophils in a dose-dependent manner whereas an alternative PP inhibitor, microcystin, was ineffective. Calyculin was more potent than okadaic acid in both cell types. The concentration required to inhibit by 50% (IC_{50}) the release of histamine was 15 (HLMC) and 50 nM (basophils) for calyculin and 200 (HLMC) and 300 nM (basophils) for okadaic acid.

2 Lysates of purified HLMC and basophils dephosphorylated radiolabelled glycogen phosphorylase, a substrate for both PP1 and PP2A. The PP activity in lysates of both cell types was inhibited in a dose-dependent fashion by the PP inhibitors with the following rank order of activity, calyculin (approximate IC_{50}; 0.02–0.1 nM) $\geqslant$ microcystin (0.1 nM) $>$ okadaic acid (70 nM).

3 The PP1-selective inhibitor, inhibitor-2 (I-2), attenuated the dephosphorylation of glycogen phosphorylase in lysates of both HLMC and basophils. I-2 (20 nM) inhibited the glycogen phosphorylase PP activity by $71 \pm 3\%$ and $49 \pm 13\%$ in HLMC and basophil extracts, respectively. There were, approximately, 6 fold greater levels of I-2-sensitive activity in HLMC than in basophils. Qualitatively similar results were obtained with an alternative PP1-selective inhibitor, inhibitor-1 (I-1).

4 Lysates derived from HLMC and basophils dephosphorylated radiolabelled casein which is a PP2A-restricted substrate. HLMC lysates contained, approximately, 2.5 fold higher levels of casein PP activity than basophil lysates.

5 These data indicate that HLMC and basophils both contain PP1 and PP2A. The data suggest that, on a per cell basis, HLMC have higher levels of both PP1 and PP2A. Moreover, the ratio of PP1 to PP2A is higher in HLMC than in basophils.

Keywords: Mast cells; basophils; okadaic acid; phosphatases; dephosphorylation

Introduction

Antigen-mediated aggregation of high affinity IgE receptors on the surface of mast cells and basophils initiates a biochemical cascade leading to the release of mediators which promote an allergic response (Warner & Kroegel, 1994). Although many of the steps in this cascade have yet to be established, studies predominantly in the rat basophil leukaemia (RBL) cell suggest that phosphorylations are important (Benhamou & Siraganian, 1992; Beaven & Metzger, 1993; Hamawy *et al*., 1995). While an emphasis has been placed on protein kinase-mediated phosphorylations in these studies, much less is known about the role that protein phosphatases (PPs) might play in these cells.

The dephosphorylation of serine and threonine residues is catalyzed by four major classes of serine/threonine PP (Cohen, 1989; Cohen & Cohen, 1989; Shenolikar, 1994). These PPs can be classified according to substrate preference, requirement for divalent cations and sensitivity to endogenous inhibitor proteins. Type 1 PPs dephosphorylate the β subunit of phosphorylase kinase and are sensitive to nanomolar concentrations of the endogenous inhibitor proteins, inhibitor-1 (I-1) and inhibitor-2 (I-2). Type 2 PPs dephosphorylate the α subunit of phosphorylase kinase and are insensitive to I-1 and I-2. Whereas the activity of PP2A is unaffected by the presence of divalent cations, PP2B (calcineurin) and PP2C are dependent on Ca^{2+} and Mg^{2+}, respectively. In recent years, several additional PPs have been identified which display some structural and functional homology with PP1 and PP2A (Honkanen *et al*., 1991; Brewis *et al*., 1993; Chen *et al*., 1994). However, in most cells PP1 and PP2A constitute the major contributors to the overall PP activity.

The study and classification of PPs has been aided immeasurably by a number of naturally-occurring compounds which are potent inhibitors of PPs (MacKintosh, 1993; MacKintosh & MacKintosh, 1994). Okadaic acid has been the most widely used of these compounds and inhibits PP2A (IC_{50}; 0.1–1 nM) more potently than PP1 (IC_{50}; 10–100 nM). Microcystin and calyculin are also effective inhibitors and both compounds inhibit PP1 and PP2A equipotently (IC_{50}; 0.1–1 nM). These compounds can inhibit PP2B, but much higher concentrations (IC_{50}; 10 μM) are required. PP2C is insensitive to these compounds.

We and others have previously reported that okadaic acid is an effective inhibitor of mediator release from human lung mast cells (HLMC) and basophils (Botana & MacGlashan, 1993; Peachell & Munday, 1993; Peirce *et al*., 1996, 1997). Moreover, we have reported that extracts of HLMC and basophils contain PP activities which are sensitive to okadaic acid. In the present study, we have attempted to characterize the PP activities present in HLMC and basophils by employing different substrates and by investigating the effects of a variety of PP inhibitors.

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Methods

Buffers

Phosphate buffered saline (PBS) was employed in these studies. PBS contained (mM): NaCl 137; $Na_2HPO_4.12H_2O$ 8; KCl 2.7; KH_2PO_4 1.5. PBS-BSA was PBS which additionally contained: $CaCl_2.2H_2O$ 1 mM; $MgCl_2.6H_2O$ 1 mM; glucose 5.6 mM; bovine serum albumin (BSA) 1 mg ml^{-1}; DNase 15 μg ml^{-1}. PBS-HSA was PBS additionally supplemented with: $CaCl_2.2H_2O$ 1 mM; $MgCl_2.6H_2O$ 1 mM; glucose 5.6 mM; human serum albumin (HSA) 30 μg ml^{-1}. The pH of all PBS buffers was titrated to 7.3.

Hypotonic lysis buffer contained: Tris 50 mM; ethylene diamine tetraacetic acid (EDTA) 1 mM; ethylene glycol-bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.1 mM; dithiothreitol (DTT) 0.5 mM; phenyl methyl sulphonyl fluoride (PMSF) 50 μg ml^{-1}; soybean trypsin inhibitor (SBTI) 50 μg ml^{-1}; leupeptin 5 μg ml^{-1}; aprotinin 5 μg ml^{-1}. The pH was titrated to 8.0.

Preparation of inhibitors and stimuli

Okadaic acid (0.5 mM stock) and calyculin (0.1 mM stock) were prepared in 10% dimethyl sulphoxide (DMSO). Microcystin was prepared as a 0.1 mM stock solution in 10% methanol. Lyophilized 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.

Isolation and purification of human 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 (RT). The upper buffy coat layer was removed, cells were recovered by centrifugation ($120 \times g$, 8 min) and washed twice with PBS. These mixed cell preparations were used in the histamine release experiments.

Purified basophils were prepared by flotation of blood over Percoll density gradients, to generate a basophil-enriched (5–15% purity) fraction, followed by immunomagnetic bead separations according to methods described (Weston *et al.*, 1997). In brief, the basophil-enriched fraction was incubated (1 h) over ice with monoclonal (IgG_{2A}) mouse anti-human IgE and then incubated (30 min) with Dynal magnetic beads coated with a rat anti-mouse IgG_{2A} antibody at a ratio of beads to cells of 4 to 1. The magnetic fraction was harvested, using a Dynal MPC-1 magnet and the magnetically adherent cells counted with Alcian blue to determine basophil purities (Gilbert & Ornstein, 1975). This fraction typically contained $1-3 \times 10^6$ basophils at purities of 91–99%. These purified cells were used to prepare extracts, for use in PP assays.

Isolation and purification of HLMC

Mast cells were isolated from human lung tissue by a modification of the method described by Ali & Pearce (1985). Macroscopically normal tissue from lung resections of patients with carcinoma 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, U.K.) with 0.5–1 l of PBS buffer to remove lung macrophages. The tissue was reconstituted in PBS-BSA (10 ml per g of tissue) containing collagenase la (350 u ml^{-1} 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 HLMC) 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 ($120 \times g$, RT, 8 min), the supernatant discarded and the pellets reconstituted in PBS-BSA (100 ml). The pellet was washed a further two times. HLMC were visualized by microscopy using an Alcian blue stain (Gilbert & Ornstein, 1975). Of the total cells, 3– 13% were mast cells. This method generated $2-9 \times 10^5$ HLMC per g of tissue. HLMC prepared in this manner were used in mediator release experiments.

For PP assays, HLMC were purified further by Percoll density centrifugation followed by immunomagnetic bead separations according to methods described elsewhere (Peirce *et al.*, 1997). The protocol (i.e. incubation times, buffers, cell numbers) for immunomagnetic bead separation was essentially the same as that described for basophils except that a monoclonal (IgG_1) anti-c-kit antibody and Dynal beads coated with rat anti-mouse IgG_1 were employed. These methods generated HLMC purities of 85–99% with cell yields of between $1-3 \times 10^6$ HLMC.

Mediator release

Mediator release experiments were performed in PBS-HSA buffer. HLMC and basophils were incubated with or without PP inhibitors for 2 h before challenge with stimulus. Mediator release was initiated immunologically with an optimal releasing concentration of anti-IgE (1/300, HLMC; 1/3000, basophils). Secretion was allowed to proceed for 20 (HLMC) or 40 (basophils) min at 37°C after which time the cells were pelleted by centrifugation ($160 \times g$, RT, 4 min). Histamine released into the supernatant was determined by the modified (Ennis, 1991) automated fluorometric method of Siraganian (1974). Total histamine content was determined by lysing aliquots of the cells with perchloric acid at a final concentration of acid of 1.6%. Cells incubated in buffer alone served as a measure of spontaneous histamine release which ranged from 2–8% of the total histamine content. 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.

Phosphatase assays

Lysates of purified HLMC preparations, for use in PP assays, were prepared as described elsewhere (Fruman *et al.*, 1992). Purified cells were resuspended in hypotonic lysis buffer (5×10^6 cells in 100 μl) and disrupted by three cycles of freeze-thawing. Following centrifugation ($13{,}000 \times g$, 10 min), supernatants were snap frozen in liquid nitrogen and stored at -80°C for use at a later date. Radiolabelled phosphorylase a was prepared (Resink *et al.*, 1983) from phosphorylase b (5 mg ml^{-1}) by incubation in the presence of phosphorylase kinase (200 u ml^{-1}), $MgCl_2$ (4 mM), $CaCl_2$ (0.1 mM), [γ^{32}P]-ATP (0.2 mM; specific activity $0.75-1.3 \times 10^6$ c.p.m. $nmol^{-1}$) at 37°C for 2 h. Unreacted [γ^{32}P]-ATP was removed by

extensive dialysis (36 h) into 50 mM Tris HCl (pH 7.2 at 4°C), 10% (w:v) glycerol, 0.1 mM EGTA and 1 mM DTT. After dialysis, free ATP represented less than 5% of the total ^{32}P-label. Casein was prepared in essentially the same manner except that the catalytic subunit of cyclic AMP-dependent protein kinase (rather than phosphorylase kinase) was used to phosphorylate the substrate.

PP activities were measured by incubation of the ^{32}P-labelled substrate (casein or phosphorylase) with extracts of purified cells at 37°C in the dialysis buffer in either the absence or the presence of PP inhibitors. PP inhibitors (okadaic acid, microcystin and calyculin) were added to the extracts just prior to the addition of radiolabelled substrate. When I-1 or I-2 was used, cell extracts were incubated for 15 min with the inhibitors before the addition of substrate (Cohen *et al*., 1989). Aliquots of the incubation mixture were removed at 0, 7 and 14 min and added to ice-cold 25% trichloroacetic acid to which BSA (0.3 mg ml^{-1}) was added to aid protein precipitation. After centrifugation (13,000 × *g*, 3 min), duplicate aliquots of the supernatant, containing the released ^{32}P, were added to liquid scintillant (Optiphase, Fisons, Loughborough, U.K.) and counted in a Beckman LS 5000 SE liquid scintillation counter. PP activity in extracts of cells was routinely assayed in a total reaction volume of 80 μl. Cell extracts, containing 5×10^6 cell equivalents per 100 μl, were diluted either 1 in 80 for the assessment of phosphorylase PP activity or 1 in 20 for the assessment of casein PP activity.

Inhibitor-1

I-1 was purified to homogeneity from rabbit skeletal muscle using the procedure of Aitken *et al*. (1982) modified from the original method of Foulkes & Cohen (1979).

Materials

The following were purchased from the sources indicated; DMSO, goat anti-human IgE, Percoll, BSA, casein, HSA, EGTA, ATP, phosphorylase kinase, glycogen phosphorylase b, DTT, aprotinin, PMSF, leupeptin and SBTI (Sigma Chemical Co., Poole, U.K.); EDTA, calcium chloride and magnesium chloride (BDH, Poole, U.K.); microcystin (Gibco BRL, Dundee, U.K.); okadaic acid and calyculin (Alexis Corporation, Nottingham, U.K.); Tris (Bio-Rad, Hemel Hempstead, U.K.); recombinant human I-2 (New England Biolabs, Hertfordshire, U.K.); monoclonal (IgG_1) mouse anti-human c-kit and monoclonal (IgG_{2A}) mouse anti-human IgE (Immunotech, Marseilles, France); magnetic beads coated with rat anti-mouse IgG_1 or IgG_{2A} antibody (Dynal, Wirral, U.K.).

Statistics

Control and drug-treated data sets were compared using Students *t*-test for paired data. Values of $P < 0.05$ were considered statistically significant.

Results

The effects of PP inhibitors on the IgE-mediated release of histamine from HLMC and basophils were determined (Figure 1). In both cell types, calyculin and okadaic acid inhibited histamine release dose-dependently and calyculin was more potent than okadaic acid (Table 1). In contrast, microcystin was completely ineffective as an inhibitor of histamine release in both cell types.

Table 1 Effects of PP inhibitors on histamine release and PP activity

	IC_{50} (nM)			
	HLMC		*Basophils*	
PP inhibitor	*HR*	*PP*	*HR*	*PP*
Okadaic acid	200	70	300	70
Calyculin	15	0.1	50	0.02
Microcystin	inactive	0.1	inactive	0.1

Approximate IC_{50} values for the inhibition by PP inhibitors of (a) histamine release (HR) and (b) PP activity (PP) in extracts of HLMC and basophils. Values have been derived from Figures 1 and 2. Further experimental details can be obtained in the legends to those figures.

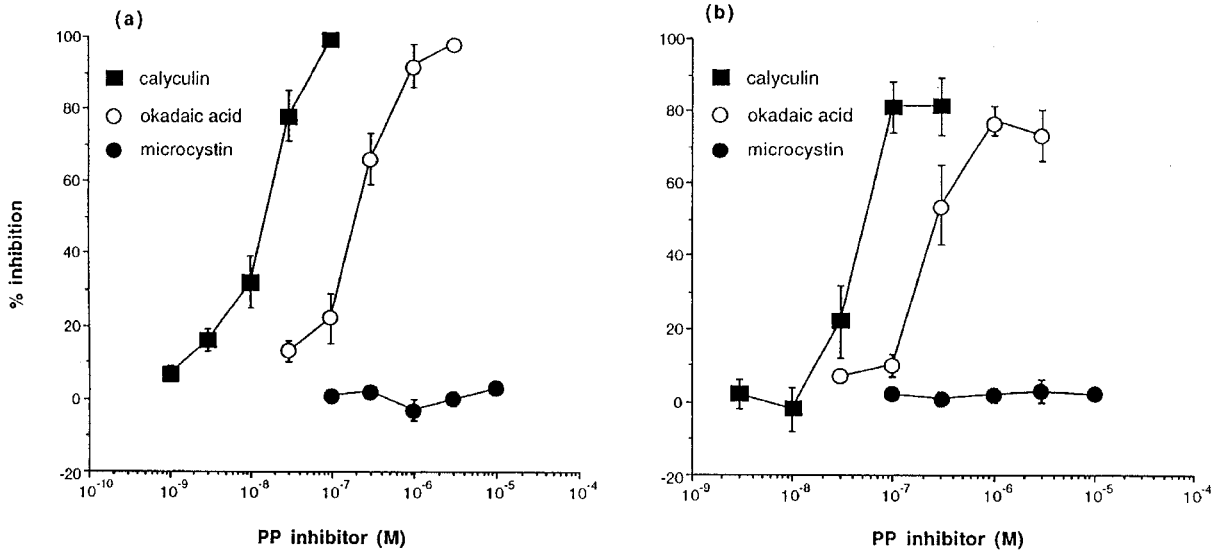

Figure 1 Effect of PP inhibitors on histamine release from HLMC (a) and basophils (b). Cells were incubated for 2 h with a PP inhibitor before challenge with anti-IgE for 20 (HLMC) or 40 (basophils) min. Values are expressed as the % inhibition of the control histamine releases which were 28 ± 4% (HLMC) and 34 ± 5% (basophils). Values are means ± s.e.mean, $n = 4$ (HLMC) and $n = 6$ (basophils).

The effects of these same PP inhibitors on the dephosphorylation of glycogen phosphorylase (a substrate for PP1 and PP2A) by extracts of HLMC and basophils were determined (Figure 2). All three PP inhibitors attenuated the dephosphorylation of glycogen phosphorylase in extracts of both HLMC and basophils and the rank order of potency for the inhibition of calyculin $\geqslant$ microcystin $>$ okadaic acid, held for both cell types (Table 1).

In a further series of experiments the effects of recombinant I-2, a selective inhibitor of PP1, on dephosphorylation of glycogen phosphorylase were determined (Figure 3). Pre-incubation (15 min) with I-2 (20 nM) inhibited the PP activity in HLMC and basophil extracts by $71 \pm 3\%$ and $49 \pm 13\%$, respectively. At this concentration of I-2, PP1 activity should be maximally inhibited (Honkanen *et al.*, 1991). The I-2-sensitive PP activity in HLMC and basophils corresponds to 90 and 15 pmol ^{32}P liberated from glycogen phosphorylase per min per 10^6 cells, respectively. A low concentration (2 nM) of okadaic acid, which is thought to inhibit PP2A selectively (Cohen *et al.*, 1989), inhibited PP activity in HLMC and basophils by $18 \pm 10\%$ and $12 \pm 10\%$, respectively. Combinations of I-2 and okadaic acid led to approximately additive

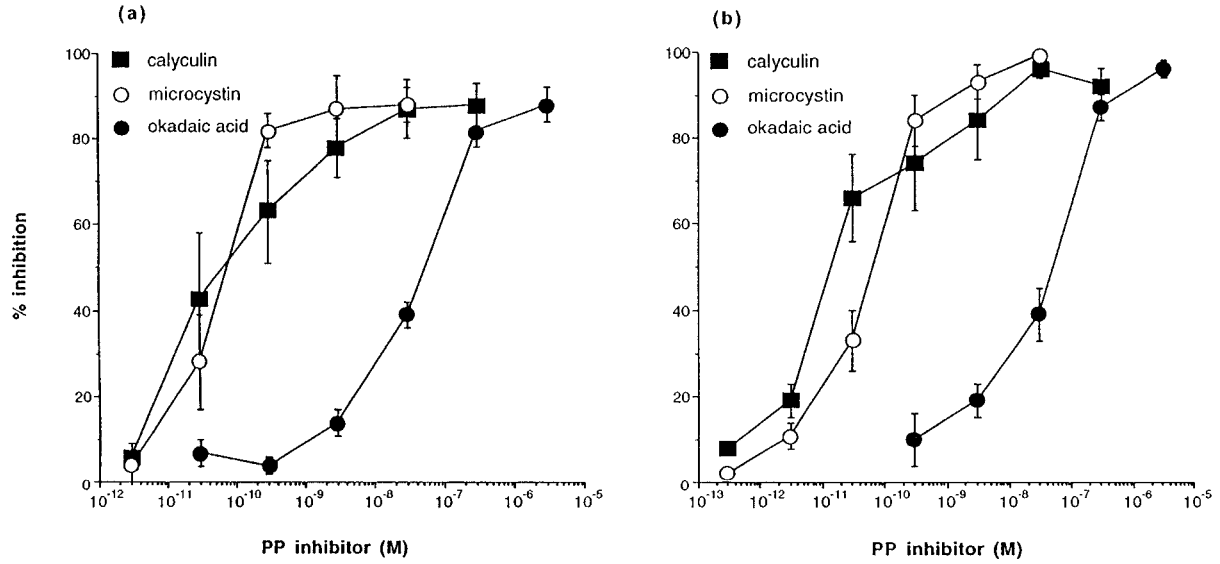

Figure 2 Effect of PP inhibitors on PP activity in extracts of HLMC (a) and basophils (b). Dephosphorylation of glycogen phosphorylase by extracts was followed for 14 min in the absence or presence of increasing concentrations of a PP inhibitor. Results are expressed as the % inhibition of the control PP activities in extracts of HLMC and basophils which were 103 ± 16 and 34 ± 5 pmol of ^{32}P liberated by 1×10^6 cells per min, respectively. Values are means $\pm$ s.e.mean, $n = 4$. HLMC purities were $94 \pm 1\%$ and basophil purities were $97 \pm 1\%$.

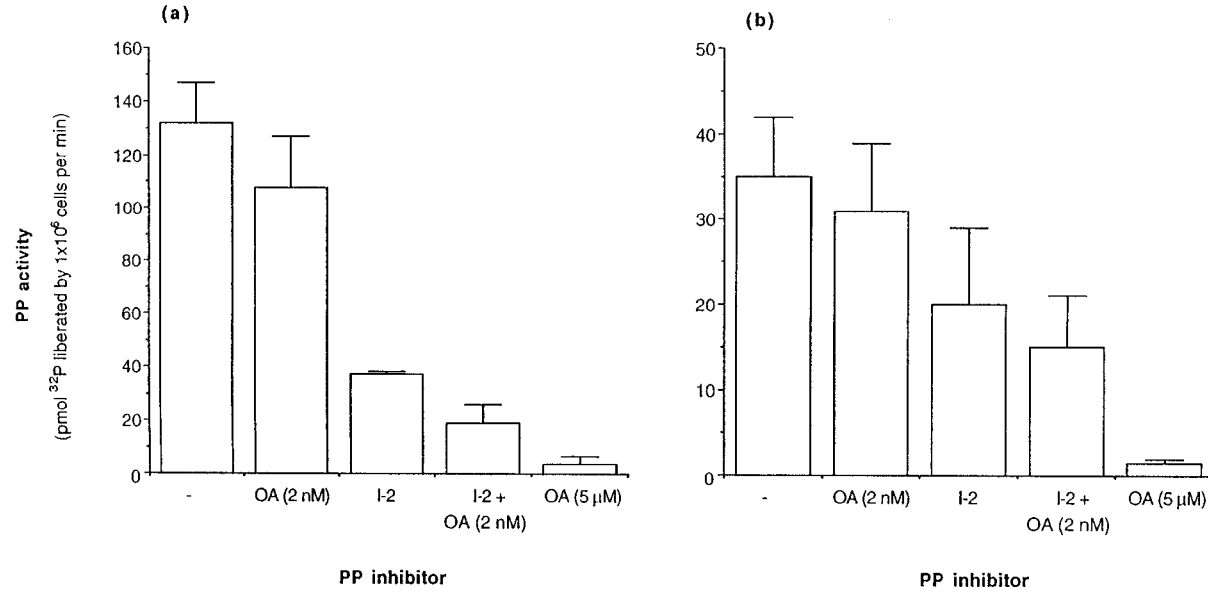

Figure 3 Effect of I-2 on PP activity in extracts of HLMC (a) and basophils (b). Extracts were incubated with either I-2 (20 nM), okadaic acid (OA) or both I-2 and okadaic acid for 15 min and then the dephosphorylation of glycogen phosphorylase followed for 14 min. PP activities are expressed as rates of dephosphorylation. Values are means $\pm$ s.e.mean, $n = 3$. HLMC purities were $93 \pm 2\%$ and basophil purities were $96 \pm 2\%$. All treatments (except those with 2 nM okadaic acid) caused statistically significant ($P < 0.05$) reductions in the PP activity.

levels of inhibition because the PP activity in HLMC and basophils was inhibited by 86±4% and 61±7%, respectively. In these same experiments, a high concentration (5 μM) of okadaic acid, which should block both PP1 and PP2A (Cohen *et al*., 1989), almost completely abolished the PP activity in extracts of either cell type (Figure 3).

In a similar series of experiments, the effects of I-1, partially purified from rabbit skeletal muscle, on the dephosphorylation of glycogen phosphorylase were investigated (Figure 4). I-1 (50 nM) inhibited the PP activity in extracts of HLMC and basophils by 37±4% and 20±9%, respectively. I-1 appeared to be less effective than I-2 at inhibiting phosphorylase PP activity despite being used at a higher concentration which is in keeping with the findings of others (MacKintosh, 1993).

In order to confirm whether HLMC and basophils contain PP2A, studies were performed with an alternative substrate, radiolabelled casein (Figure 5). In the absence of Mg^{2+}, casein is a PP2A-restricted substrate (Agostinis *et al*., 1987). In the presence of EDTA (5 mM), in order to sequester divalent cations, extracts of HLMC and basophils liberated ^{32}P from casein and these PP activities were inhibited by okadaic acid (2 nM) by 81±7% and 90±3% in HLMC and basophils, respectively, but were unaffected by I-2 (20 nM). These data indicate that the okadaic acid-sensitive component of the

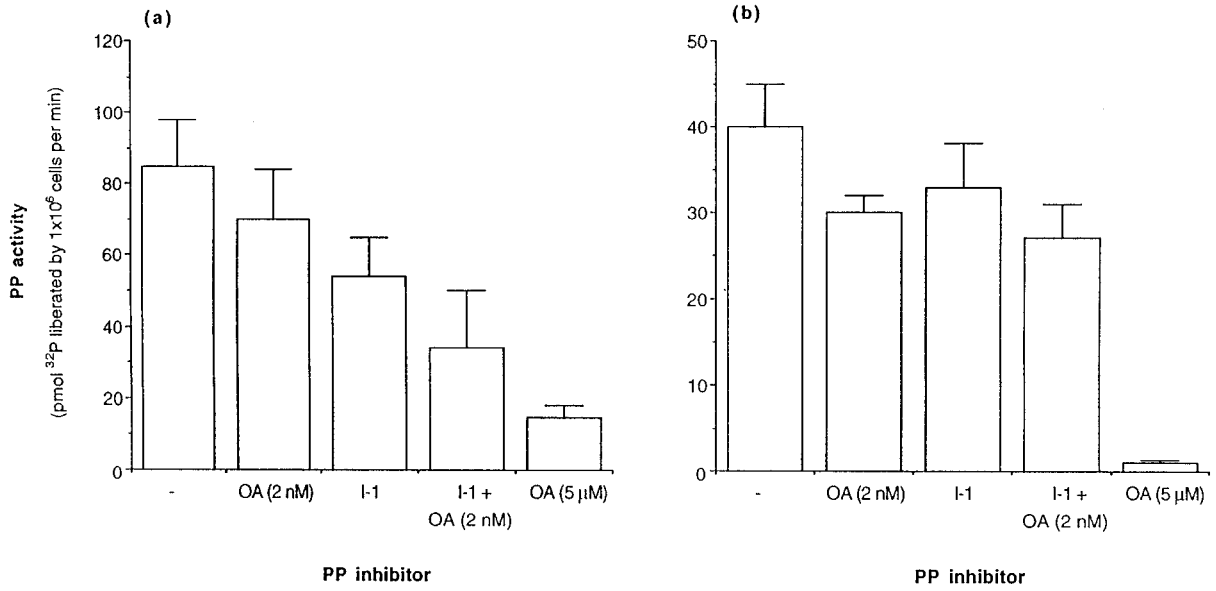

Figure 4 Effect of I-1 on PP activity in extracts of HLMC (a) and basophils (b). Extracts were incubated with either I-1 (50 nM), okadaic acid (OA) or both I-1 and okadaic acid for 15 min and then the dephosphorylation of glycogen phosphorylase followed for 14 min. PP activities are expressed as rates of dephosphorylation. Values are means±s.e.mean, $n=3$. HLMC purities were 95±1% and basophil purities were 98±2%. All treatments (except I-1 in basophils) caused statistically significant ($P<0.05$) reductions in the PP activity.

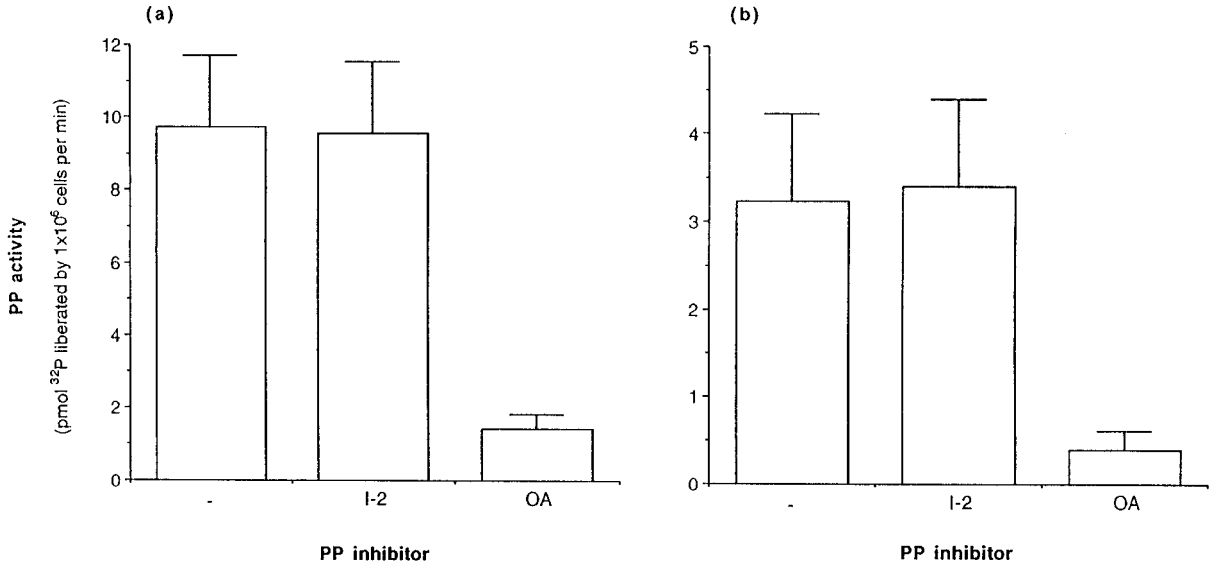

Figure 5 Dephosphorylation of radiolabelled casein by extracts of HLMC (a) and basophils (b). Extracts were incubated with either I-2 (20 nM) or okadaic acid (OA; 2 nM) for 15 min and then the dephosphorylation of casein followed for 14 min. PP activities are expressed as rates of dephosphorylation. Values are means±s.e.mean, $n=6$ (HLMC) and $n=4$ (basophils). HLMC purities were 91±3% and basophil purities were 95±2%. Treatments with okadaic acid caused statistically significant ($P<0.05$) reductions in the PP activity.

casein PP activity in HLMC and basophils corresponds to, approximately, 7.9 and 2.9 pmol of ^{32}P liberated by 1×10^6 cells per min, respectively.

Discussion

A growing body of work has shown that okadaic acid, modulates a wide variety of processes (Cohen *et al*., 1990; MacKintosh & MacKintosh, 1994). An attractive feature of okadaic acid is that it is cell-permeant and this property has been exploited advantageously to establish whether PPs might be involved in regulating cellular processes (Haystead *et al*., 1989; Hardie *et al*., 1991; Hardie, 1993). We, ourselves, have studied okadaic acid, and have shown it to be an effective inhibitor of IgE-mediated histamine release from HLMC (Peachell & Munday, 1993; Peirce *et al*., 1997) and basophils (Peirce *et al*., 1996).

In the present study, the effects of calyculin and microcystin were also investigated. Calyculin was 6 and 10 fold more potent than okadaic acid as an inhibitor of histamine release in basophils and HLMC, respectively. Because calyculin and okadaic acid are equipotent as inhibitors of PP2A but calyculin is more potent than okadaic acid against PP1 (MacKintosh, 1993), these data could suggest that PP1 is the target for the actions of these inhibitors. Conversely, microcystin was completely ineffective as an inhibitor of mediator release from both cell types. Whereas okadaic acid and calyculin are thought to be readily cell permeant, microcystin has been shown to be taken up by some cells (Rutter *et al*., 1991) and not others (Eriksson *et al*., 1990). Reports indicate that a specialized transporter is required to enable the uptake of microcystin (Rutter *et al*., 1991). Presumably, therefore, this transporter is absent in both HLMC and basophils.

The effects of these same compounds were also assessed on the dephosphorylation of glycogen phosphorylase (substrate for PP1 and PP2A) in extracts of HLMC and basophils. The rank order of potency for the inhibition of PP activity was, calyculin (IC_{50}; 0.02 to 0.1 nM) $\geqslant$ microcystin (0.1 nM) $>$ okadaic acid (70 nM). These three inhibitors are approximately equipotent as inhibitors of isolated PP2A (MacKintosh, 1993). In contrast, calyculin and microcystin are about 200 fold more potent than okadaic acid as inhibitors of PP1 (MacKintosh, 1993). That the IC_{50} value for okadaic acid (70 nM) was roughly 700 fold higher than that observed for either microcystin or calyculin strongly suggests that PP1 constitutes part of the phosphorylase PP activities resident in HLMC and basophil extracts. Interestingly, microcystin, which was ineffective in intact cells, was a potent inhibitor of PP activity in broken cell preparations. These findings support the contention that HLMC and basophils are impermeant to microcystin.

Further studies strongly suggested that PP1 is present in both HLMC and basophils because I-2, a selective inhibitor of PP1, reduced the dephosphorylation of glycogen phosphorylase by 71 and 49%, respectively. The concentration (20 nM) of I-2 used in these experiments should achieve maximal inhibition of PP1 (Honkanen *et al*., 1991). Thus, the I-2 sensitive component (90 and 15 pmol of ^{32}P liberated from phosphorylase per min by 1×10^6 HLMC and basophils, respectively) of the total phosphorylase PP activity should correspond to PP1. Based on these considerations, HLMC appear to contain about 6 fold greater levels of PP1 than basophils. In experiments with I-1, phosphorylase PP activity was inhibited in both cell types although it was less effective than I-2 which is in keeping with the findings of others (MacKintosh, 1993). However, although I-2 was less effective than I-2, it displayed a similar trend in that it was more effective at inhibiting PP activity in HLMC than in basophil extracts. These studies with I-1 suggest that HLMC contain about 4 fold higher levels of PP1 than basophils. In these same experiments, a low (2 nM) concentration of okadaic acid, which should inhibit PP2A selectively (Cohen *et al*., 1989), attenuated phosphorylase activity in HLMC and basophils by 18 and 12%, respectively. These data could be interpreted to suggest that both HLMC and basophils contain less PP2A than PP1 although it should be noted that PP1 is about 6 fold more active against phosphorylase than PP2A (Tung *et al*., 1985). Therefore, these studies underestimate the contribution of PP2A to the total PP activity.

When I-2 (20 nM) was employed in combination with okadaic acid (2 nM), 86 and 61% of the phosphorylase PP activity was inhibited in lysates of HLMC and basophils, respectively. Should this combination of inhibitors attenuate most of the PP1 and PP2A present, then these data suggest that in HLMC and basophils about 14 and 39%, respectively, of the phosphorylase PP activity is not due to PP1 or PP2A. The nature of this residual PP activity is unknown although recent studies have identified several novel PPs which are sensitive to okadaic acid (Honkanen *et al*., 1991; Brewis *et al*., 1993; Chen *et al*., 1994). Indeed, the residual PP activity in extracts of basophils and HLMC is abolished by a high concentration of okadiac acid indicating that this residual PP activity is okadaic acid-sensitive. It is possible that a contribution to the residual activity could be made by PP2B because this PP is sensitive to okadaic acid albeit at high concentrations (10 μM). However, this seems unlikely because PP2B requires Ca^{2+} for activity and the PP assay was performed in the presence of EDTA which would be expected to chelate any divalent cations in the system. Should these considerations be correct then a considerably larger proportion of the total phosphorylase PP activity in basophils (39%), than in HLMC (14%), cannot be accounted for by either PP1 or PP2A.

Confirmation that PP2A is present in HLMC and basophils was established employing radiolabelled casein. Both cell types dephosphorylated this substrate which was PP2A-restricted under the assay conditions employed (due to the presence of EDTA to sequester divalent cations). Okadaic acid (2 nM) reduced this PP activity by 80–90% whereas I-2 (PP1-selective) was ineffective. These data support the idea that both HLMC and basophils contain PP2A although HLMC contain about 2.5 fold more PP2A than basophils.

These data indicate that HLMC and basophils both contain PP1 and PP2A. Indeed, preliminary alternative studies of our own indicate that PP2B (Peachell *et al*., 1998) and PP2C (unpublished observations) may also be present in both HLMC and basophils indicating that these cells contain all four of the major classes of serine/threonine PP. However, based on data from the present study, the total PP activity (on a per cell basis) appears to be higher in HLMC than in basophils. Moreover, the ratio of PP1 to PP2A appears to be higher in HLMC than in basophils. Conversely, the ratio of okadaic acid-sensitive but unclassified PP activity to PP1/PP2A appears to be higher in basophils than in HLMC. These findings indicate that the relative contributions of PP1, PP2A and other unknown PPs to the total PP activity differs between HLMC and basophils. Although it is likely that these differences in PP content between HLMC and basophils may be significant, at this time, it is not known how these differences may influence the regulation of the activities of

these cells. Further studies employing a wider range of PP inhibitors may help to unravel some of these issues.

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