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Effect of okadaic acid on immunologic and non-immunologic histamine release in rat mast cells

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Abstract—We have studied the effect of the protein phosphatase (PP) inhibitor, okadaic acid (OKA) on histamine release elicited by immunologic and non-immunologic stimuli in peritoneal and pleural rat mast cells. When cells were stimulated with antigen (egg albumin), OKA strongly inhibited histamine release. This finding suggests that PP1 and PP2A substrates mediate immunoglobulin E- (IgE) dependent secretion in mast cells. In contrast, after non-immunologic activation of mast cells with different drugs, such as the neuropeptide growth hormone releasing factor (GRF) and the cytostatic agents Adriamycin, navelbine and mitoxantrone, there is no effect of OKA on histamine secretion. These results indicate that IgE-dependent secretion is mediated by substrates of PP1 and PP2A, whereas following non-immunological stimuli, they activate pathways that lead to protein phosphorylation; these proteins are not substrates of PP1 and PP2A.

Key words: okadaic acid, mast cells, histamine release, GRF, compound 48/80, Adriamycin, navelbine, mitoxantrone

Exocytosis is a complex process regulated by several signal transduction pathways which generate the selective phosphorylation of unknown substrates [1, 2]. A useful cellular model to study exocytosis is the rat mast cell, which releases granules as a result of immunological and non-immunological activation [3].

The exocytotic process activated by antigen starts with the cross-linking of cell membrane-immunoglobulin E (IgE*) receptors, while non-immunological activation is a less well known process which is usually mediated by direct activation of G-proteins [4], both types of stimulus activate specific kinases. The covalent phosphorylations induced by protein kinases are reversed by the action of phosphatases (PP), balancing the effects of protein kinases and thus regulating the cellular response [1].

In order to study the role of protein phosphorylation in mast cell stimulation, we utilized the toxin OKA, a fatty acid obtained from the sponge *Halichondria okadai* [5], that specifically inhibits PP1 and PP2A, two of the four major PP that dephosphorylate serine–threonine residues. This division of PP in type 1 and type 2 is based on whether they dephosphorylate the β or α subunit, respectively, of the enzyme phosphorylase kinase [6]. The inhibition of both PP is concentration dependent. Within the nanomolar range, PP2A is inhibited, whereas at 1 μM OKA totally blocks both phosphatases [7]. At higher concentrations (5 μM) it is also inhibited PP2B, and in contrast OKA does not affect PP2C and other PP even at higher concentrations. Furthermore no protein kinases are inhibited by OKA [8].

Materials and Methods

Chemicals. All chemicals were from the Sigma Chemical Co. (St Louis, MO, U.S.A.), except orthophthalaldehyde (Merck, Darmstadt, F.R.G.), GRF (Serono, Spain), Adriamycin (Farmitalia Carlo Erba, Italy), vinorelbine (navelbine) from Pierre Fabre, France, mitoxantrone (Cyanamid Ibérica, Spain), OKA (LC Services, MA, U.S.A.) and *Bordetella pertussis* (Wako, Germany). Okadaic acid was dissolved in 10% DMSO, and the final

* Abbreviations: OKA, okadaic acid; PP, protein phosphatase; GRF, growth hormone releasing factor; IgE, immunoglublin E; PS, phosphatidylserine.

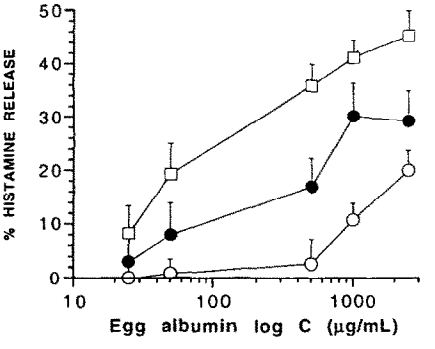

Fig. 1. Effect of a 10 min preincubation with OKA on histamine release from rat peritoneal and pleural mast cells elicited by egg albumin. Filled circles represent histamine release after pretreatment with 1 μM OKA. Open circles represent percentage release after pretreatment with 10 μM OKA. Open squares indicate control values. Mean of three experiments ± SEM.

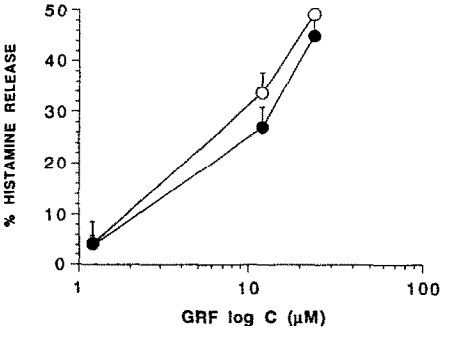

Fig. 2. Effect of preincubation for 10 min with 1 μM OKA on histamine release from rat peritoneal and pleural mast cells elicited by GRF. Open circles represent control. Filled circles represent histamine release after pretreatment with 1 μM OKA. Mean of three experiments ± SEM. A similar plot was obtained with 10 μM OKA.

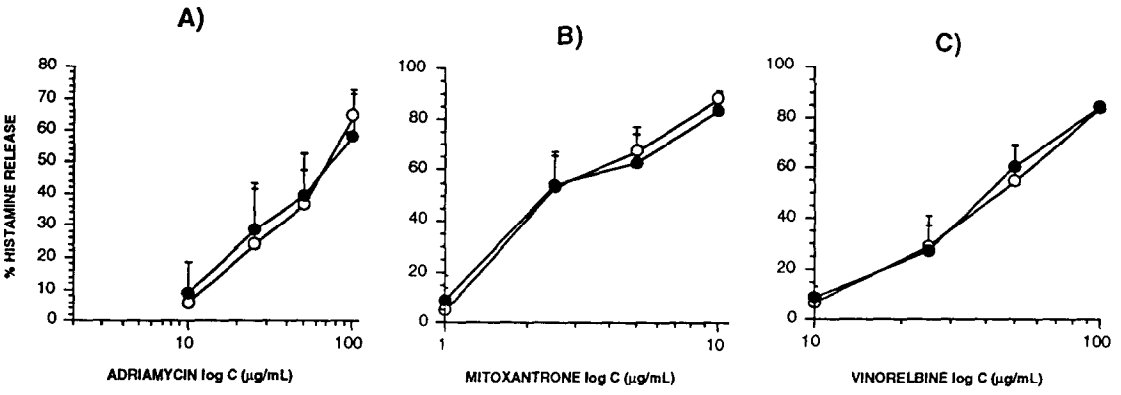

Fig. 3. Effect of preincubation for 10 min with 1 μM OKA on histamine release from rat peritoneal and pleural mast cells elicited by the antineoplastic drugs Adriamycin (A), mitoxantrone (B) and vinorelbine (C). Open circles represent control. Filled circles represent histamine release after pretreatment with 1 μM OKA. Mean of three experiments ± SEM. Similar plots were obtained with 10 μM OKA.

concentration of DMSO (lower than 0.1%) did not affect mediator release.

Sensitization of rat mast cells. Sprague–Dawley rats weighing 200–300 g were sensitized by i.m. injection in the back extremities of egg albumin (15 mg each rat) and adjuvant (9×10^9 killed *Bordetella pertussis* each rat) in saline solution. Two weeks later, the rats were killed and the mast cells isolated.

Mast cell preparation. Mast cells were obtained by lavage of pleural and peritoneal cavities of Sprague–Dawley rats (200–400 g) as described previously [9]. Physiological saline composition was (mM): Na^+, 142.3; K^+, 5.94; Ca^{2+}, 1; Mg^{2+}, 1.2; Cl^-, 126.1; CO_3^-, 22.85; $PO_4H_2^-$, 1.2; SO_4^{2-}, 1.2, giving a final osmotic pressure of 300 ± 5 mOsm/kg H_2O. The final pH was adjusted to 7.0, and in all experiments the incubation medium was equilibrated previously with CO_2. The unpurified cellular suspension contained 4–8% mast cells, with an average of $1.5–2 \times 10^6$ mast cells per rat.

Cell incubation. Twenty-five microliters of a freshly prepared concentrated solution of each drug was added to sufficient incubation medium to attain a final volume of 0.925 mL, and preincubated. When the medium reached 37°, 25 μL of cell suspension, containing $1–1.5 \times 10^5$ mast cells, was added to each tube. Incubations were carried out in a bath at 37° for 10 min, and for a further 10 min after addition of the stimulus. Incubations were stopped by immersing the tubes in a cold bath. After centrifugation at 1000 g_{max} for 5 min, the supernatants were collected and decanted into other tubes for histamine determination. Appropriate controls to determine spontaneous histamine release in the absence of stimuli were executed in every experiment. Immunological stimulation was carried out in the presence of 30 μg/mL PS. This concentration of PS did not activate histamine secretion by itself.

Histamine release assay. Histamine was assayed spectrofluorometrically both in the pellet (residual histamine) and supernatants (released histamine) by Shore's method [10] in a spectrofluorometer Kontron SFM 25. However, 0.1% orthophthalaldehyde was employed. Trichloroacetic acid was added (7%, final concentration) to prevent reaction because protein interferes with histamine assay. To ensure total histamine, pellets were sonicated for 60 sec in 0.8 mL of 0.1 N HCl. Results are expressed as a percentage of histamine released with respect to the total histamine content.

Statistical analysis. Results were analysed using the Student's *t*-test for unpaired data. A probability level of 0.05 or smaller was used for statistical significance. Results are expressed as the mean ± SEM.

Results

We examined the activity of OKA, after a 10 min preincubation, on histamine release elicited by egg albumin (50–2500 μg/mL) in peritoneal and pleural mast cells. The 10 min preincubation time was chosen based on previous results that show, for the highest concentration of stimuli used, maximal inhibition after 3–5 min. Figure 1 shows that the secretion of histamine is clearly decreased after pretreatment of mast cells with 1 μM OKA, the inhibition being undetectable with 0.1 μM. When the concentration of OKA is increased to 10 μM, the inhibition is higher, histamine release being detectable only with 1000 and 2500 μg/mL of egg albumin.

We have recently reported that GRF elicits the release of histamine from rat mast cells by a non-immunological mechanism [11]. When we study the effect of 1 μM OKA on the rat mast cell response to GRF, as shown in Fig. 2, there is a slight, not significant inhibition of the response to GRF. We have also reported the histamine releasing activity of several antineoplastic drugs and antineoplastic drug combinations [12, 13], which effects we tested in the presence of OKA. Figure 3 shows the effect of the antineoplastic drugs Adriamycin (10–100 μg/mL), mitoxantrone (1–10 μg/mL) and vinorelbine (10–100 μg/mL) both in the presence and the absence of OKA. OKA (10 μM) shows the same profile as 1 μM OKA, both with GRF and antineoplastic drugs (results not shown). There are small differences between the responses elicited by the drug and the drug plus OKA, which are not statistically significant.

Discussion

Our results reflect that OKA has no effect on the shape of the histamine release dose–response curve obtained with non-immunological secretagogues, suggesting that the proteins phosphorylated after non-immunological activation, at least with GRF and antineoplastic drugs, are not substrates of PP1 and PP2A. This same absence of effect was previously reported with compound 48/80 [14].

In contrast, the effect of OKA on immunologically stimulated cells is markedly inhibitory. There are two

possibilities to explain the inhibitory effect of OKA in antigen-mediated histamine release. The first is that the substrates for both PP1 and PP2A inhibit the secretory process. This possibility is based on the fact that after immunologic stimulation, several membrane proteins are phosphorylated with a time course similar to that of histamine release, and a 78 kDa protein, the phosphorylation of which peaks when secretion finishes, suggesting an auto-inhibitory role of this protein on mediator secretion [15]. Hence the action of OKA might enhance or accelerate this effect. The second explanation is that the effector protein is a phosphatase which can be inhibited by OKA. This possibility is based on the observation that the G-protein that controls the exocytosis, namely Ge-protein, is a phosphatase and protein dephosphorylation would be an enabling reaction of the secretory process [16].

Similar results were obtained with human basophils; that is, complete inhibition of the response with IgE-dependent stimulation and no effect when using the chemotactic peptide f-Met-Leu-Phe [17]. This suggests that the effect of OKA is related to the cross-linking of receptors, since the stimulation of f-Met-Leu-Phe is mediated through univalent receptors that do not require any aggregation. Although this mechanism differs from the stimuli studied in this paper, which do not use receptors but directly activate G-proteins [4], they all share a lack of cross-links to activate the cell. Therefore, OKA appears to inhibit some mechanism necessary for the IgE receptor to aggregate. However, this mechanism, which does not involve membrane receptors, but direct activation of G-proteins [4], differs from that induced by the stimuli studied in this paper since all share a lack of cross-linking processes to activate the cells.

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REFERENCES

1. Kurosawa M, Protein phosphorylation and inositol phospholipid metabolism in activated mast cells. *Clin Exp Allergy* **20**: 7–12, 1990.
2. Gomperts BD, GE: a GTP-binding protein mediating exocytosis. *Annu Rev Physiol* **52**: 591–606, 1990.
3. Diamant B, New insights into the mechanisms of histamine release from rat peritoneal mast cells. *Chem Immunol* **49**: 141–172, 1990.
4. Aridor M, Traub LM and Sagi-Eisenberg R, Exocytosis in mast cells by basic secretagogues: evidence for direct activation of GTP-binding proteins. *J Cell Biol* **111**: 909–917, 1990.
5. Usagawa T, Nishimura M, Itoh Y, Uda T and Yasumoto T, Preparation of monoclonal antibodies against okadaic acid prepared from the sponge *Halichondria okadai*. *Toxicon* **27**: 1323–1330, 1989.
6. Ingebritsen TS, Foulkes JG and Cohen P, The protein phosphosphates involved in cellular regulation 2. Glycogen metabolism. *Eur J Biochem* **132**: 263–274, 1983.
7. Cohen P, The structure and regulation of protein phosphatases. *Adv Second Messenger Phosphoprotein Res* **24**: 230–235, 1990.
8. Haystead TA, Sim AT, Carling D, Honnor RC, Tsukitani Y, Cohen P and Hardie DG, Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism. *Nature* **337**: 78–81, 1989.
9. Eleno N, Botana LM and Espinosa J, Veratridine elicits histamine release and promotes calcium uptake in mast cells. *Biochem Pharmacol* **37**: 3523–3525, 1988.
10. Shore PA, Fluorometric assay of histamine. *Methods Enzymol* **17**: 842–845, 1971.
11. Estévez MD, Botana MA, Arnaez E, Vieytes MR and Botana LM, Histamine release on rat pleural and peritoneal mast cells elicited by human GRF(1–29)NH2. *Int Arch Allergy Immunol* **100**: 86–88, 1993.
12. Botana LM, Arnaez E, Vieytes MR, Alfonso A, Bujan MJ, Louzao MC and Cabado A, Non-immunological release of histamine from rat mast cells elicited by antineoplastic agents. *Cancer Chemother Pharmacol* **29**: 495–498, 1992.
13. Botana LM, Arnaez E, Vieytes MR, Alfonso A and Louzao MC, Non-immunological release of histamine from rat mast cells elicited by antineoplastic agents: Effect of drug combinations. *Cancer Chemother Pharmacol* **30**: 487–490, 1992.
14. Botana LM, Alfonso A, Botana MA, Vieytes MR, Louzao MC and Cabado AG, Influence of protein kinase C, cAMP and phosphatase activity on histamine release produced by compound 48/80 and sodium fluoride on rat mast cells. *Agents Actions* **37**: 1–7, 1992.
15. Quarto R and Metzger H, The receptor for immunoglobulin E: examination for kinase activity and as a substrate for kinases. *Mol Immunol* **23**: 1215–1223, 1986.
16. Churcher Y, Kramer LM and Gomperts BD, Evidence for protein dephosphorylation as a permissive step in GTP-gamma-S-induced exocytosis from permeabilized mast cells. *Cell Regulation* **1**: 523–530, 1990.
17. Botana LM and MacGlashan DJ, Effect of okadaic acid on human basophil secretion. *Biochem Pharmacol* **45**: 2311–2315, 1993.

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