J. Pharm. Pharmacol. 1993, 45: 750–752
Communicated June 22, 1992

Effect of okadaic acid on histamine release from rat peritoneal mast cells activated by anti-IgE

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Abstract—The effect of okadaic acid, a potent inhibitor of protein phosphatase 1 and 2A, on histamine release from mast cells has been investigated. Okadaic acid strongly and dose-dependently inhibited histamine release from mast cells induced by anti-IgE. The IC50 value of okadaic acid on histamine release induced by anti-IgE was 3·2 nM. However, okadaic acid failed to inhibit histamine release induced by A23187 and compound 48/80. Moreover, okadaic acid showed no effect on the initial rise in intracellular Ca^{2+}, Ca^{2+}-mobilization from intracellular Ca^{2+}-stores and the generation of inositol trisphosphate. These results suggest a possible involvement of protein phosphatase 2A in the histamine release from mast cells induced by anti-IgE.

Mast cells and basophils are primary target cells for immunoglobulin E (IgE) and the reaction of cell-bound IgE antibodies with multivalent antigen results in the release of a variety of inflammatory mediators (Ishizaka 1981). Analysis of the biochemical events involved in the antigen-induced triggering of mediator release revealed that the cross-linking of the high affinity receptor for IgE ($FC_\varepsilon R1$) on the cells, results in the activation of various membrane-associated enzymes such as phosphatidylinositol-specific phospholipid phospholipase C (PLC), phospholipase A and adenylate cyclase, and enhances phosphatidylinositol turnover (Ishizaka & Ishizaka 1984). Evidence has been presented that mobilization of intracellular Ca^{2+} ($[Ca^{2+}]_i$) is an initial step for antigen-induced histamine release. Most intracellular signals are transduced by the phosphorylation and dephosphorylation of proteins. Okadaic acid is a polyether monocarboxylic acid toxin (Mr 802) isolated from marine sponges of the genera *Halichondria* (Tachibana et al 1981) and *Pandoros*, and okadaic acid was the first to be regarded as a potent and specific inhibitor of protein phosphatase among in-vitro substances (Bialojan & Takai 1988). It has been reported that okadaic acid induces phosphorylation of myosin light chain in the absence of Ca^{2+} and in the presence of calmodulin inhibitors (Ozaki et al 1987). Okadaic acid treatment has been shown to increase the phosphorylation state of many hepatocyte phosphoproteins (Haystead et al 1989). The role of protein phosphatase on histamine release from activated mast cells is still unknown. In the present study, we examined the effect of okadaic acid on histamine release from rat mast cells induced by anti-IgE.

Materials and methods

Rats were killed by asphyxiation in an atmosphere of CO_2. Rat peritoneal mast cells were obtained from male Wistar rats, 200–300 g, and mast cells were purified using the method of Németh & Röhlich (1980). Viability of the cells was >97% as assessed by trypan blue. Passively sensitized rat mast cells were prepared as described previously (Takei et al 1988).

Histamine release. The assay of histamine release was carried out as described previously (Takei et al 1988). Purified mast cells in Tyrode-HEPES solution (pH 7·4) were incubated in duplicate at 37°C with or without okadaic acid before the various secretagogues. Tyrode-HEPES solution contained (mM): NaCl 124, KCl 4·0, $CaCl_2$ 1, $NaHCO_3$ 10, glucose 5·6, NaH_2PO_4 0·64, $MgSO_4$ 0·5, 4-(2-hydroxyethyl)-1-piperazineethane-sulphonic acid (HEPES) 5, phosphatidylserine 50 mg mL^{-1} and bovine serum albumin (BSA) 50 $\mu g\ L^{-1}$. BSA was omitted from the solution in the experiments on histamine release. The cells were separated from the released histamine in the supernatant by centrifugation (1300 *g*, 10 min, 4°C). Residual histamine in the cells was released by disrupting the cells with 100% trichloroacetic acid (final concentration 10%), and centrifugation (1500 *g*, 15 min, 4°C). Histamine was determined fluorometrically (Shore et al 1959). The amount of histamine released was calculated as a percentage of the total histamine present in a control mast cells suspension.

Measurement of $[Ca^{2+}]_i$. Measurement of $[Ca^{2+}]_i$ was carried out as described previously (Takei et al 1989).

Fluorescence was recorded using a fluorimeter (model 650-40 Fluorescence 100; Hitachi, Japan) with a temperature-controlled cuvette and a magnetically driven stirrer. Purified mast cells (10^6 cells mL^{-1}) were incubated at 37°C for 10 min with 100 μM Quin-2/AM (2-[(2-amino-5-methyl-phenoxy)methyl]-6-methoxy-8-aminoquinoline-*N*,*N*,*N'*,*N'*-tetraacetic acid, tetraacetoxymethyl ester) in Tyrode-HEPES solution. The cell suspension was diluted 1:10 with Tyrode-HEPES solution, kept for 60 min, and then washed twice with the complete solution. A cell suspension (10^5 cells mL^{-1}) was placed in the cuvette described above, and agents were added with a microsyringe, directly into the cuvette, without interrupting the recording. The cell suspension was incubated at 37°C for 5 min with okadaic acid, and challenged with an appropriate amount of secretagogue. Fluorescence excitation and emission wavelengths were 339 and 492 nm, respectively. $[Ca^{2+}]_i$ was calculated by the method described by Tsien et al (1982).

Determination of inositol trisphosphate (IP_3). Measurement of IP_3 was carried out using a commercially available kit (Amersham, UK). Briefly, the passively sensitized mast cells were resuspended in Tyrode-HEPES solution containing 10 mM LiCl. Cell suspension (160 μL, 10^7 cells mL^{-1}) was incubated at 37°C for 5 min with or without okadaic acid, and challenged with 20 μL of an optimal concentration of anti-IgE (200 $\mu g\ mL^{-1}$). The reaction was terminated by the addition of 750 μL of chloroform:methanol (1/2, v/v) after appropriate intervals. Aqueous phase was separated by the addition of chloroform and distilled water (750 μL each), vortex mixing, and centrifugation (400 *g*, 5 min). The chloroform layer obtained from the chloroform/methanol extracts was evaporated under reduced pressure. IP_3 was determined by the D-myoinositol, 1,4,5-triphosphate [^{3}H] assay kit (Amersham, UK).

Okadaic acid, A23187, compound 48/80, and L-α-phosphatidylserine were purchased from Sigma Chemical Co., St Louis, MO, USA, and Quin-2/AM was obtained from Wako Pure Chemical Industries, Osaka, Japan. Anti-IgE was purchased from Miles Laboratories.

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Results

We determined the effect of okadaic acid, a protein phosphorylation phosphatase inhibitor, on the histamine release from rat mast cells induced by various secretagogues. Okadaic acid did not produce histamine release from rat mast cells. Anti-IgE, at its optimal concentration of 200 μg mL^{-1}, induced a time-dependent release of histamine from mast cells. The histamine release was essentially complete within 4–5 min, and 52·1 ± 2·3% of total histamine was released after 10 min. When added to the reaction medium, okadaic acid strongly and dose-dependently inhibited histamine release from mast cells induced by anti-IgE. At 0·1, 1 and 10 nM, the degree of inhibition of okadaic acid on histamine release from mast cells at 10 min after challenge with anti-IgE was 20·5 ± 2·2, 35·1 ± 3·5 and 60·1 ± 4·8%, respectively (Fig. 1a). At 300 nM, 75·8 ± 3·6% inhibition was achieved. The IC50 value of okadaic acid on histamine release induced by anti-IgE was 3·2 nM.

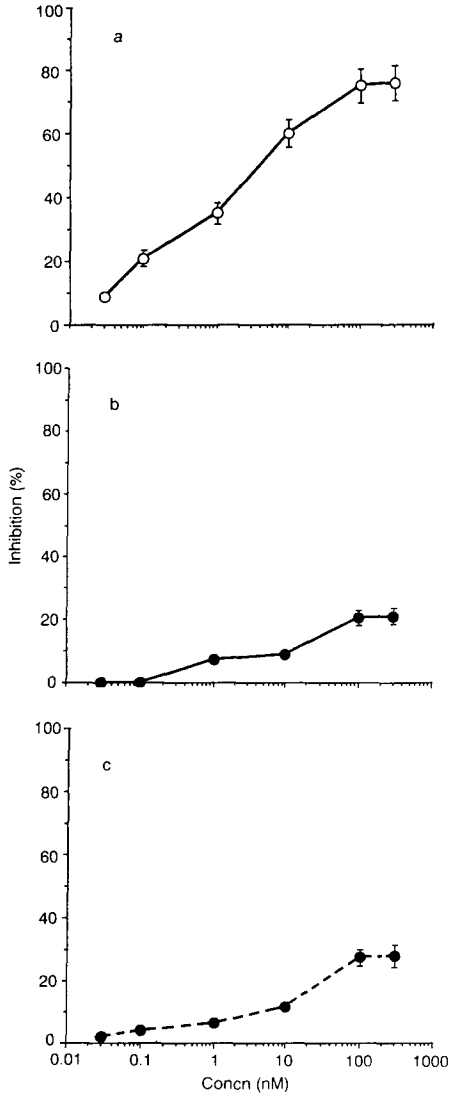

FIG. 1a. Inhibitory effect of okadaic acid on histamine release from passively sensitized mast cells induced by anti-IgE (200 μg mL^{-1}). b and c. Inhibitory effect of okadaic acid on histamine release from mast cells induced by A23187 (0·1 μM) and compound 48/80 (1·0 μg mL^{-1}), respectively. After preincubation with okadaic acid for 5 min, the cell suspension was incubated with anti-IgE, A23187 and compound 48/80 for 10 min at 37°C. Histamine release from rat mast cells induced by anti-IgE (200 μg mL^{-1}), A23187 (0·1 μM) and compound 48/80 (1·0 μg mL^{-1}) in the absence of okadaic acid were 52·5 ± 3·5, 85·2 ± 3·8 and 86·5 ± 3·2%, respectively. Spontaneous histamine release from mast cells was 5·3 ± 1·6%, and this value was subtracted from each experimental value. Each point represents the mean of five experiments and vertical bars indicate s.e.m.

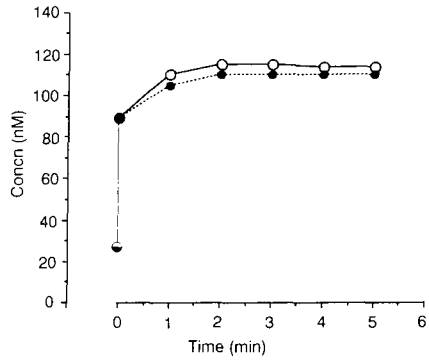

FIG. 2. Effect of okadaic acid on the increase in intracellular calcium $[Ca^{2+}]_i$ in passively-sensitized mast cells induced by anti-IgE. Passively-sensitized mast cells (10^5 cells mL^{-1}) were challenged with 200 μg mL^{-1} of anti-IgE and incubated at 37°C after preincubation for 5 min with okadaic acid (300 nM). Each point represents mean of five experiments and vertical bars indicate s.e.m. O——O Without okadaic acid, ●·····● with okadaic acid (300 nM).

Unlike anti-IgE, incubation with optimal concentrations of A23187 (0·1 μM) and compound 48/80 (1·0 μg mL^{-1}) rapidly caused histamine release, the process of which was complete within 60 s. The maximum rates of histamine release induced by A23187 and compound 48/80 at 60 s were 72·5 ± 1·6 and 86·2 ± 2·3%, respectively. As shown in Fig. 1 b, c, okadaic acid slightly suppressed histamine release induced by A23187 and compound 48/80. At 300 nM, the inhibition by okadaic acid on histamine release from rat mast cells at 5 min after challenge with A23187 and compound 48/80 was 21·2 ± 2·5 and 27·2 ± 4·2%, respectively.

Agonists stimulate a rapid PLC-mediated hydrolysis of PIP2 with generation of two second messengers, 1,2 diacylglycerol (1,2-DA) and IP_3. IP_3 regulates the release of Ca^{2+} from endoplasmic reticulum, an event which initiates the cellular activation process. In the next series of experiments, we examined the effect of okadaic acid on Ca^{2+} mobilization from the intracellular Ca^{2+}-store and the generation of IP_3.

Purified passively sensitized mast cells were challenged with

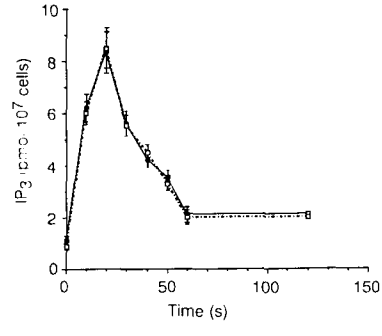

FIG. 3. Effect of okadaic acid on the kinetics of anti-IgE-induced generation of IP_3. Passively-sensitized mast cells were challenged with 200 μg mL^{-1} of anti-IgE and incubated at 37°C after preincubation for 5 min okadaic acid (300 nM). The generation of inositol triphosphate (IP_3) in control mast cells was 0·35 pmol/10^7 cells, and was subtracted from the experimental value. Each point represents the mean of five experiments and vertical bars indicate s.e.m. O——O Without okadaic acid, □·····□ with okadaic acid (300 nM).

200 μg mL^{-1} anti-IgE (Fig. 2). The initial and maximum increases were 62 and 88 nM $[Ca^{2+}]_i$, respectively. The effects of the various concentrations of okadaic acid on the initial rise in $[Ca^{2+}]_i$ in mast cells activated by anti-IgE were examined. As shown in Fig. 2 okadaic acid did not affect the initial rise in $[Ca^{2+}]_i$ or the second rise. Passively sensitized mast cells were challenged with anti-IgE (200 μg mL^{-1}), and the kinetics of IP_3 generation were analysed (Fig. 3). Anti-IgE induced a rapid increase in IP_3 production in mast cells, over 20 s, followed by a decrease for 1 min. The effects of the various concentrations of okadaic acid on IP_3 production in mast cells activated by anti-IgE were examined. Okadaic acid (300 nM) did not inhibit the generation of IP_3.

Discussion

This paper reports that okadaic acid strongly and dose-dependently inhibited histamine release from rat peritoneal mast cells induced by anti-IgE.

Mechanisms involving phosphorylation/dephosphorylation of cell protein play a major role in the regulation of responsibility in many cells. Previously, Takai et al (1987) reported that okadaic acid is a very potent inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), two of the four major protein phosphatases in the cytosol of mammalian cells that dephosphorylate serine and threonine residues (Takai et al 1987). These studies have established that okadaic acid is a specific inhibitor of PP1 and PP2A, which are structurally related enzymes showing 50% amino acid sequence identity in the catalytic domain. Karaki et al (1989) have also reported that okadaic acid inhibits activation of rabbit platelets by thrombin, possibly by potentiation of cAMP-mediated events.

The IC50 value of okadaic acid for histamine release induced by anti-IgE was 3·2 nM. Okadaic acid completely inhibits PP2A at a concentration of 2·0 nM (IC50 1·0 nM), whereas PP1 is affected at 200-fold higher concentrations (IC50 200 nM) (Bialojan & Takai 1988). Therefore, PP2A can be taken as the proportion of activity inhibited by 1 nM okadaic acid, and PP1 can be taken as the activity resistant to 1 nM okadaic acid (but inhibited by 1 μM okadaic acid). The IC50 value of okadaic acid on histamine release in mast cells activated by anti-IgE was in the same range. From these considerations and results it was suggested that okadaic acid might inhibit a protein phosphatase 2A involved in histamine release.

Okadaic acid suppressed histamine release induced by A23187 and compound 48/80, with a much lower potency than the effect on histamine release induced by anti-IgE.

There are several mechanisms involved in the regulation of $[Ca^{2+}]$, some which function to control Ca^{2+} entry into the cell through the plasma membrane voltage-dependent and receptor-operated Ca^{2+} channels and others involved in regulation of Ca^{2+} across intracellular membranes or organelles such as mitochondria and endoplasmic reticulum. Considerable excitement resulted from the finding that Ca^{2+} can be released from an intracellular pool by the second messenger IP_3 (Berridge 1984; Irvine et al 1986). IP_3 is generated along with diacylglycerol from a relatively minor plasma membrane phospholipid, PIP2, by the action of PLC.

Rapid increase in the generation of IP_3 was observed within 15 s after addition of anti-IgE. Okadaic acid showed no effect on the initial rise in $[Ca^{2+}]_i$, Ca^{2+}-mobilization from intracellular Ca^{2+}-stores and generation of IP_3.

It has been shown that higher concentrations of okadaic acid induce contraction in smooth muscle of rabbit aorta and guinea-pig taenia which is attributable to the Ca^{2+}-independent phosphorylation of myosin light chain (Ozaki et al 1987). These results indicate that okadaic acid has a dual effect on smooth muscle contraction: lower concentrations inhibit contractions which is not attributable to a direct effect on the contractile elements, whereas higher concentrations directly activate the contractile elements. In the present study, okadaic acid did not produce histamine release from rat mast cells at higher concentration (1 μM) thus it did not show a dual effect on histamine release from rat mast cells.

From these results it is suggested that protein phosphatase (PP2A) participated in the latter stage of histamine release triggered by IgE-receptor bridging.

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