

Pharmacological Study of Phospholipase A₂-Induced Histamine Release from Rat Peritoneal Mast Cells

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The effect of some drugs on phospholipase A₂ (PLA₂)-induced histamine release from rat peritoneal mast cells was investigated.

1. PLA₂ (*Naja naja*) caused the release of histamine from rat peritoneal mast cells in a dose related fashion.
2. The release of histamine by PLA₂ was decreased by the removal of each of calcium and glucose from the reaction medium (Tyrode's solution).
3. *p*-Bromophenacyl bromide and mepacrine, PLA₂ inhibitors, inhibited the release of histamine caused by PLA₂.
4. Cinnarizine and nifedipine, calcium channel blockers, and oxatomide, KC-404, tranilast and disodium cromoglycate, anti-allergic agents, also inhibited the PLA₂-induced histamine release.
5. When prednisolone (0.5 mg/kg) was administered i.p. 2, 3 and 4 h prior to the harvest of the mast cells, the amount of histamine released by PLA₂ *ex vivo* was significantly decreased. Hydrocortisone, prednisolone and dexamethasone clearly inhibited the release of histamine from mast cells when they were administered 4 h prior to harvest of the cells.

Keywords — phospholipase A₂; histamine release; calcium; glucocorticoid

Introduction

There are some reports to indicate the importance of phospholipase A₂ (PLA₂) activation in allergic histamine release from mast cells.¹⁻⁴ However, there is no direct evidence to show the changes of PLA₂ activity in mast cells during histamine release. This is mainly due to the extremely low activity of PLA₂ in the mast cells and the rapid change of the activity after the antigen-antibody reaction. Therefore, some investigators examined the release of histamine from mast cells by extrinsic PLA₂.⁵⁻⁷ Although extrinsic PLA₂-induced histamine release is not an exact model for the intrinsic activated PLA₂ during the histamine releasing process, this method seems to be valuable for studying the pharmacology of anti-allergic agents. In the present study, therefore, we investigated the pharmacological profiles of extrinsic PLA₂-induced histamine release from rat peritoneal mast cells.

Materials and Methods

Animals — Male Wistar rats weighing 120 to 180 g were used. Animals were housed in

wire mesh cages in an air-conditioned room at 24 °C and fed the usual laboratory diet and water *ad libitum* as provided. All animals were purchased from Japan SLC Inc. (Hamamatsu).

Drugs and Chemicals — PLA₂ from *Naja naja* was purchased from Sigma Chemicals (St. Louis, Mo., U.S.A., lot 67F-8085). *p*-Bromophenacyl bromide (PBPB; Wako Junyaku, Osaka), mepacrine (Sigma), nifedipine (Bayer Pharmaceuticals, A.G. Postsach, West Germany), hydrocortisone acetate (Nippon Merk Banyu, Tokyo), prednisolone acetate (Sionogi, Osaka) and dexamethasone acetate (Nippon Merk Banyu) were purchased from these commercial sources. Cinnarizine (Eisai, Tokyo), oxatomide (Kyowa Hakko, Tokyo), KC-404 (Kyorin, Tokyo), disodium cromoglycate (DSCG; Fujisawa, Osaka) and tranilast (Kissei, Matsumoto) were kindly supplied from each company as listed. Drugs without glucocorticoids were dissolved in dimethyl sulfoxide (DMSO). When DMSO was used, the concentration of DMSO against the cells was under 0.05%. Glucocorticoids were suspended in saline.

Histamine Release from Rat Peritoneal

TABLE I. Histamine Release from Rat Peritoneal Mast Cells by PLA₂ from *Naja naja*

Concentration of PLA ₂ (μ/ml)	Histamine release	
	(ng/ml)	(% to total)
0 (Spontaneous)	18.2 ± 2.0	3.0 ± 0.3
0.1	46.7 ± 2.5	7.8 ± 0.4
0.5	97.9 ± 12.9	16.3 ± 2.2
1.0	233.2 ± 19.4	38.8 ± 3.2
5.0	379.5 ± 45.2	63.2 ± 7.5
10.0	508.5 ± 34.6	94.6 ± 6.4
20.0	596.5 ± 35.5	99.3 ± 5.9

Total cellular content of histamine was 600.9 ng/ml. Histamine release was expressed as mean ± S.E.M. of 4 to 5 experiments.

TABLE II. Effect of the Removal of Calcium and Glucose from Tyrode's Solution on PLA₂-Induced Histamine Release from Rat Peritoneal Mast Cells

Condition	Histamine release (ng/ml)	(% to control)
Normal Tyrode's solution	425.5 ± 24.3	100.0
Calcium free Tyrode's solution	39.1 ± 2.2 ^{a)}	9.2
Glucose free Tyrode's solution	296.1 ± 31.3 ^{a)}	69.6

Cells were incubated in calcium free and glucose free Tyrode's solution for 30 min prior to the addition of PLA₂. ^{a)} $p < 0.01$. Each experiment consists of 4 to 5 samples.

Mast Cells — The peritoneal exudate cells containing mast cells were collected after the injection of 10 ml Tyrode's solution and gentle massage of the abdomen. The cells were washed

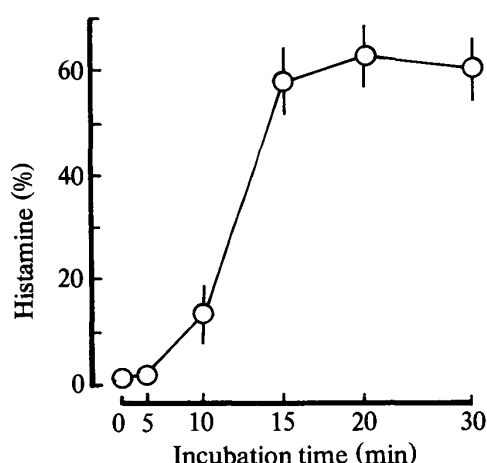


Fig. 1. Histamine Release by PLA₂ from Rat Peritoneal Mast Cells after the Incubation for 0, 5, 10, 15, 20 and 30 min

Total cellular content of histamine was 1001.2 ng/ml. Each point represents the mean S.E.M. of 4 to 8 experiments.

twice with Tyrode's solution and adjusted to a concentration of 1.5×10^5 mast cells/ml. The release of histamine by PLA₂ was caused by the incubation of the peritoneal cell suspension with PLA₂ at 37 °C for 20 min. Released histamine was measured by the fluorescence method according to May *et al.* ⁸⁾ using *o*-phthaldialdehyde (Sigma). The released histamine was expressed as a percentage of the total cellular content of histamine in each experiment.

Statistics — Statistical significance was evaluated by means of Student's *t*-test and $p = 0.05$ was taken as the upper limit of significance.

Results

Release of Histamine by PLA₂

PLA₂ (*Naja naja*) caused the release of histamine from rat peritoneal mast cells in a dose related fashion (Table I). Since PLA₂ at a concentration of 10 μ/ml caused a submaximum release of histamine, the following experiments were done by using 10 μ/ml PLA₂. Figure 1 shows the time course for histamine release by

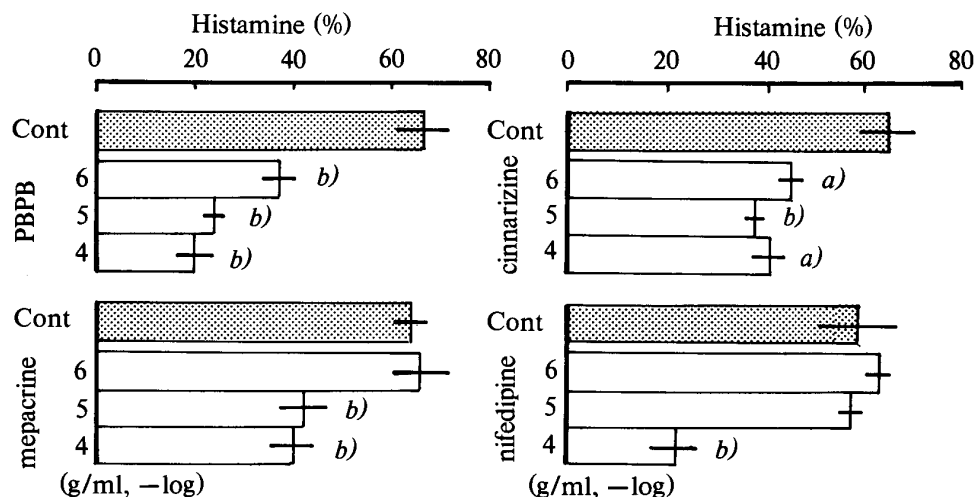


Fig. 2. Effect of PBPB, Mepacrine, Cinnarizine and Nifedipine on PLA₂-Induced Histamine Release from Rat Peritoneal Mast Cells

Each drug was incubated with the cells at 37 °C for 30 min before addition of PLA₂. Total cellular content of histamine was 784.3 ng/ml (PBPB and mepacrine) and 801.1 ng/ml (cinnarizine and nifedipine), respectively. Each column represents the mean S.E.M. of 4 to 9 experiments. a) $p < 0.05$, b) $p < 0.01$.

PLA₂. The release increased gradually until 15 min and then reached the maximum at 20 min. Table II indicates the effect of the removal of calcium ion and glucose from the reaction medium on the PLA₂-induced histamine release. Histamine release was suppressed by the removal of each of calcium and glucose.

The Effect of PBPB, Mepacrine, Cinnarizine, Nifedipine, Oxatomide, KC-404, DSCG and Tranilast

Figure 2 indicates the effect of PBPB, mepacrine, cinnarizine and nifedipine on the release of histamine by PLA₂. All agents clearly inhibited the release of histamine. Oxatomide, KC-404, DSCG and tranilast also clearly inhibited the release of histamine as indicated in Fig. 3. Each drug itself has not caused the release of histamine from mast cells.

Figure 3 indicates the time course for the effect of prednisolone on the PLA₂-induced release of histamine from mast cells. These ex-

Effect of Glucocorticoids

Figure 4 indicates the time course for the effect of prednisolone on the PLA₂-induced release of histamine from mast cells. These ex-

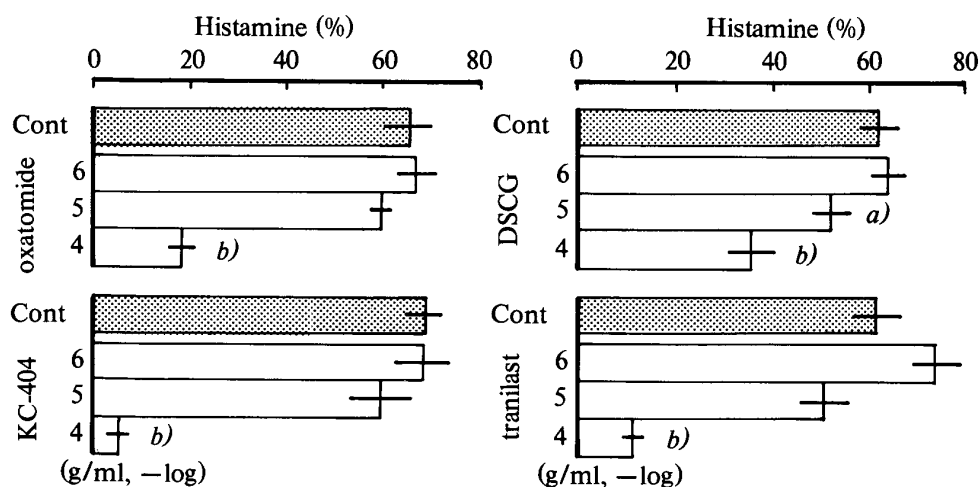


Fig. 3. Effect of Oxatomide, KC-404, DSCG and Tranilast on PLA₂-Induced Histamine Release from Mast Cells

Total cellular content of histamine was 808.0 ng/ml. Each drug was incubated with cells at 37 °C for 20 min before addition of PLA₂. Each column represents the mean S.E.M. of 4 to 8 experiments. a) $p < 0.05$, b) $p < 0.01$.

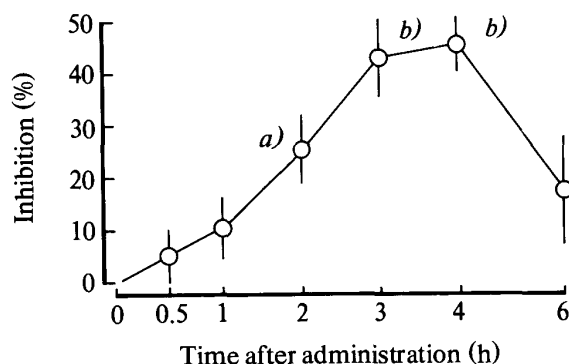


Fig. 4. Time Course for the Inhibition of PLA₂-Induced Histamine Release by Prednisolone

Prednisolone was administered intraperitoneally 30 min and 1, 2, 3, 4 and 6 h prior to harvest the cells. Total cellular content of histamine was 816.3 ng/ml. Each point represents the mean S.E.M. of 4 to 5 animals. a) $p < 0.05$, b) $p < 0.01$.

periments were done *ex vivo*. When prednisolone (0.5 mg/kg) was administered i.p. 2, 3 and 4 h prior to harvest of the cells, the histamine released by PLA₂ was inhibited. But when prednisolone was administered 30 min and 1 and 6 h prior to harvest it did not inhibit the release of histamine significantly. The maximum inhibition was observed at 4 h after administration. Figure 5 indicates the effect of hydrocortisone, prednisolone and dexamethasone on PLA₂-induced histamine release. Hydrocortisone and dexamethasone inhibited the release of histamine in a dose related fashion.

Discussion

The study reported here indicates that PLA₂ (*Naja naja*) caused the released of histamine from mast cells and the release was regulated by some pharmacological agents.

This histamine release was inhibited by BBPB and mepacrine. Each agent is usually used as a PLA₂ inhibitor. Michell *et al.*⁹⁾ and Volwerk *et al.*¹⁰⁾ reported that BBPB inhibits PLA₂ directly. Vane used mepacrine as an inhibitor of PLA₂ for investigating the inflammatory process.¹¹⁾ Markus and Ball¹²⁾ reported that mepacrine inhibited the PLA₂ directly beside the inhibition of triglyceride lipase. The concentration of each drug needed to inhibit PLA₂ activity is similar to

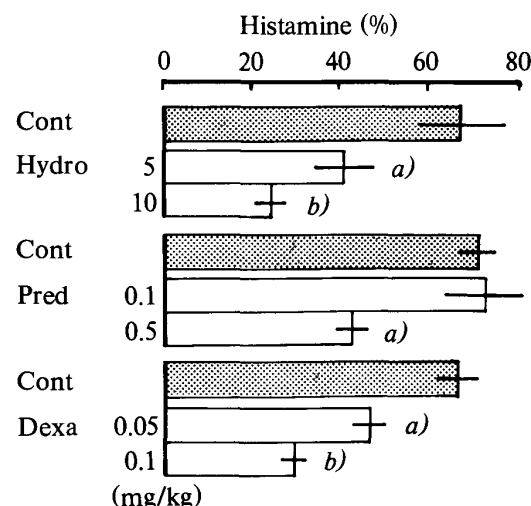


Fig. 5. Effect of Hydrocortisone (Hydro), Prednisolone (Pred) and Dexamethasone (Dexa) on PLA₂-Induced Histamine Release from Mast Cells

Each drug was administered intraperitoneally 4 h prior to harvest the cells. Each column represents the mean \pm S.E.M. of 5 to 7 animals. a) $p < 0.05$, b) $p < 0.01$.

that needed to inhibit the release of histamine in the present study. these results suggest that BBPB and mepacrine may inhibit the release of histamine by interfering with PLA₂ directly.

Since PLA₂ is known as a calcium dependent enzyme, the effect of a calcium free medium and a calcium channel blocker on the PLA₂-induced histamine release was investigated. The results indicated here indicate that PLA₂-induced histamine release is dependent on extracellular calcium. The release of histamine by PLA₂ in extracellular calcium free medium is significantly decreased, and two calcium channel blockers, nifedipine and cinnarizine inhibited the release of histamine by PLA₂. But the inhibitory potency of the two agents are different. Cinnarizine shows more potent inhibitory activity than nifedipine. This also may be due to the heterogeneity of calcium channel on mast cells as indicated in previous reports.^{13,14)} There are some reports relating to the role of extracellular calcium in the release of histamine.^{4,15-20)} Chakravarty and Yu¹⁵⁾ reported that the compound 48/80-induced histamine release is largely independent of extracellular calcium. Foreman and Monger¹⁶⁾ also reported that dextran-induced histamine release does not always require extracellular calcium. These reports indicate that the

mechanism of PLA₂-induced histamine release is different from the compound 48/80- and dextran-induced releases.

In addition to the above data, some anti-allergic drugs including mast cell stabilizer and glucocorticoid, clearly inhibited the release of histamine. The concentrations for given levels of inhibition of the release of histamine by each agent shown in this study are similar to those in immunoglobulin E (IgE)-antibody mediated histamine release indicated in previous reports.^{13,14)} These data suggest that this system provides a suitable method for studying the anti-allergic activity of the agents. Nakazawa *et al.* previously used the method for investigating the mechanism of anti-allergic action of tranilast.²¹⁾ They also indicated direct inhibition of PLA₂ by tranilast. And they concluded an involvement of a PLA₂ inhibitory mechanism for the anti-allergic action of tranilast. The present study confirmed the inhibitory action of tranilast of PLA₂-induced histamine release. We, therefore, support their conclusion. Moreover, the present study indicates the inhibition of histamine release by glucocorticoids. Time lag is necessary for the appearance of inhibition. This seems to be related to the production of new protein such as PLA₂ inhibitory protein by glucocorticoids. Kurihara *et al.*,²²⁾ Nagai *et al.*,²³⁾ Heiman and Crews,²⁴⁾ Wakajtyś-Rode *et al.*,²⁵⁾ Schleimer *et al.*,²⁶⁾ Bergstrand *et al.*²⁷⁾ and Daeron *et al.*²⁸⁾ also reported that the time lag is necessary for the inhibition of antigen-induced histamine release by glucocorticoid to appear. However, nobody demonstrated the participation of PLA₂ inhibitory protein for the inhibition of histamine release has not been demonstrated. If a PLA₂ inhibitory protein is isolated, this method may be a useful technique for investigating the PLA₂ inhibitor.

The histamine releasing mechanism by PLA₂ is not yet clarified from the present data. Postulated mechanisms are as follows; 1) The product which is able to release the histamine is made from the cell (whether mast cell or other cell is not certain) membrane by PLA₂. 2) PLA₂ directly digests the mast cell membrane to cause the release of histamine. 3) PLA₂ preparation contains an unknown substance which is able to

release the histamine. We need more detailed experiments to clarify the mechanism of PLA₂-induced histamine release.

In conclusion, these results indicated that PLA₂-induced histamine release is useful for studying the activity and character of anti-allergic agents.

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