Lysophosphatidic Acid (LPA) Induces Plasma Exudation and Histamine Release in Mice via LPA Receptors

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Abstract. Lysophosphatidic acid (LPA), the simplest of the water-soluble phospholipids, can evoke various biological responses. The present study examined the activity of LPA to induce plasma exudation and histamine release in mice. Plasma exudation was assessed by extravasation of Evans blue. Subcutaneous administration of LPA (1–100 µg/site) led to increased plasma exudation in the skin. The LPA-induced plasma exudation was inhibited by ketotifen, a histamine H1-receptor antagonist, and diacylgllycerol pyrophosphate (DGPP), a LPA1/LPA3-receptor antagonist. Moreover, pretreatment with pertussis toxin and DGPP inhibited the histamine release from peritoneal mast cells induced by LPA. These findings indicate that plasma exudation induced by LPA is mediated by histamine release from mast cells via LPA receptor(s), presumably LPA1 and/or LPA3, coupled to Gi/o proteins. Moreover, these findings point to a role of LPA in the pathomechanisms of various allergic disorders.

Keywords: lysophosphatidic acid, plasma exudation, histamine release, diacylglycerol pyrophosphate

Introduction

Lysophosphatidic acid (LPA), the simplest naturally occurring phospholipids, can evoke various biological responses. Administration of LPA in vivo induces airway hyperresponsiveness (1), itch-scratch responses (ISRs) (2), infiltration and activation of eosinophils and neutrophils (3), vascular remodeling (4), and nociceptive flexor responses (5). Previously, we documented that LPA induced histamine release (6). This finding indicates that, at least in part, the biological response induced by LPA may be attributable to histamine release, possibly from mast cells.

Histamine exerts its numerous physiological and pathophysiological functions. In an acute allergic reaction, histamine induces various responses, such as contraction of smooth muscle, plasma exudation, and mucus production. Plasma exudation seems to be important in the airway, because the leakage and subsequent airway-wall edema contribute to the development of airway hyperresponsiveness. Plasma exudation may progress to conjunctival swelling in ocular allergic disorder and nasal blockage in allergic rhinitis, which is a key symptom influencing the quality of life (7). On the basis of these points, we hypothesize that LPA may induce plasma exudation via histamine release, and this effect may contribute to the LPA-induced biological responses, especially development of airway hyperresponsiveness.

Cell responses of LPA are mediated by G protein-coupled receptors such as LPA1, LPA2, and LPA3 (8). Each LPA receptor can be activated differentially by LPA with various acyl chains bound at either the sn-1 or the sn-2 position of the glycerol backbone. For LPA3, the highest reactivity is observed with oleoyl LPA. In contrast, LPA1 and LPA2 showed broad ligand specificity (9). We also reported that differential activation by LPA species was evident in airway responsiveness to acetylcholine; furthermore, LPA-induced airway hyperresponsiveness was attributable to histamine release (10). From these points, we also hypothesize that LPA-induced histamine release may occur via an LPA receptor(s).
Recently, it is reported that several agents have been shown to inhibit LPA-induced responses. Diacylglycerol pyrophosphate (DGPP) has been shown to have a preferential competitive property for LPA\(_2\) over LPA\(_1\) and was ineffective on LPA\(_2\) (11). Idzko et al. (12) reported that DGPP inhibits LPA-induced activation of human eosinophils.

Hence, the present study examined whether LPA induces plasma exudation and effect of ketotifen, a histamine H\(_1\) receptor antagonist, and DGPP in mice. Furthermore, in order to assess the involvement of LPA receptor in histamine release, effects of pertussis toxin (PTX) and DGPP are examined.

Materials and Methods

All experiments were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Animals

Experimental animals included male ICR mice weighing 26 – 40 g (Charles River Japan Inc., Tokyo). Animals were maintained in an air-conditioned animal room at 23 ± 3°C, relative humidity of 50 ± 20%, and a 12-h light-dark cycle (lights on 8:00 to 20:00). Animals received a standard laboratory diet and water was provided ad libitum.

Materials

The following reagents were employed in this study: 1-oleoyl-2-hydroxy-sn-glycero-3-phosphate sodium salt (LPA 18:1; Avanti Polar-Lipids, Inc., Alabaster, AL, USA), diacylglycerol pyrophosphate 8:0 (DGPP; Avanti Polar-Lipids, Inc.), calcium ionophore A23187 (Calbiochem-Behring, La Jolla, CA, USA), bovine serum albumin (BSA; Cohn Fr. V, Sigma Chemical, St. Louis, MO, USA), ketotifen fumarete (Sigma Chemical), pertussis toxin (PTX, Sigma Chemical) histamine dihydrochloride (Wako Pure Chemical, Osaka), n-octahalaldehyde (Wako Pure Chemical), Evans blue (Wako Pure Chemical), 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, Wako Pure Chemical). Other reagents were of the highest grade commercially available.

LPA and DGPP dissolved in deionized water and diluted with physiological saline. A23187 was dissolved in dimethylsulfoxide and diluted with physiological saline. Other reagents were dissolved in physiological saline.

Assessment of plasma exudation

As an indicator of plasma exudation, 1 ml/animal of 0.5% Evans blue was administered intravenously. Subsequently, LPA (1 – 100 µg/site) or histamine (0.1 – 10 µg/site), was administered subcutaneously into the back. Thirty minutes after administration, the mice were sacrificed under anesthesia and the back skin was removed for colorimetric measurement of bluing spots. The amount of dye leakage into the skin was determined as described by Katayama et al. (13).

Drug administration

Ketotifen (1 mg/kg) was administered intravenously 5 minutes prior to the LPA (10 µg/site) or histamine (1 µg/site). Separately, DGPP (0.1 or 1 µg/site), LPA, or their combination was administered subcutaneously into the back and plasma exudation also determined.

Preparation of mast cells

Mice were exsanguinated under anesthesia; subsequently, calcium-free 10 mM HEPES-Tyrode’s solution (2 ml) containing 0.3% BSA and 10 U/ml heparin sodium (pH 7.4) was injected into the abdominal cavity. Following gentle massage of the abdominal wall for 2 – 3 min, peritoneal cells were obtained and washed twice with calcium-free HEPES-Tyrode’s solution (pH 7.4) by centrifugation at 50 × g for 10 min at 4°C. After staining with 0.05% toluidine blue, mast cells were suspended in HEPES-Tyrode’s solution (pH 7.4) and adjusted to a concentration of 1 × 10⁵ cells/ml.

Effects of PTX and DGPP on histamine release from peritoneal mast cells

The cell suspension was pre-warmed for 5 min at 37°C. Following the addition of 100 ng/ml of PTX and 2-h incubation, the cell suspension was challenged with the LPA (final concentration of 10 µg/ml) and further incubated for 10 min. Additionally, effect of DGPP on LPA-induced histamine release from mast cells was investigated. The cell suspension was incubated with 0.1 or 1 µg/ml of DGPP for 5 min at 37°C. The stimulation of LPA and other conditions were the same as those of the PTX.

The reaction was terminated by placement of the test tube in ice-cold water. After centrifugation at 1500 × g for 10 min at 4°C, histamine in the supernatant was determined spectrofluorometrically (14). Total histamine content in mast cells was obtained by boiling. The quantity of histamine released was calculated as percentage of the total histamine content.

Statistical analyses

Values are expressed as means ± S.E.M. Student’s
A *t*-test or Dunnett’s multiple comparison test was employed to calculate statistical significance of differences between the means of the test and control groups. A *P*-value of less than 0.05 was considered significant.

### Results

**LPA-induced plasma exudation**

Figure 1 illustrates the effects of LPA and histamine on dye leakage. Under the present conditions, dye leakage in the saline-treated group was $2.47 \pm 0.65 \mu g/site$. In contrast, LPA (1 – 100 $\mu g/site$) induced a dose-dependent increase in dye leakage; moreover, the amounts of dye at the 1, 10, and 100 $\mu g/site$ were $3.64 \pm 0.78$, $6.82 \pm 1.83$, and $11.9 \pm 2.00 \mu g/site$ respectively. Meaningful differences were observed between the LPA-treated and saline treated group at 10 and 100 $\mu g/site$ (*P* < 0.05 at 10 $\mu g/site$, *P* < 0.01 at 100 $\mu g/site$). Histamine (0.1 – 10 $\mu g/site$) also induced an increase in dye leakage similar to LPA. Statistically significant differences were observed with respect to 1 and 10 $\mu g/site$ (*P* < 0.01).

As a result, 10 $\mu g/site$ of LPA and 1 $\mu g/site$ of histamine were utilized for the following experiments.

**Effect of ketotifen and DGPP on LPA-induced plasma exudation**

The effect of ketotifen, a histamine H$_1$-receptor antagonist, was examined as indicated in Fig. 2. LPA-induced dye leakage was $5.96 \pm 0.41 \mu g/site$. Moreover, the value differed significantly from that of the saline-treated group (*P* < 0.01). Ketotifen significantly lowered the LPA-induced dye leakage to approximately that of the vehicle-treated group. Statistically, significant differences were observed between the ketotifen- and vehicle-treated groups (*P*<0.01). Ketotifen also reduced histamine-induced dye leakage (*P*<0.01).

The potential involvement of LPA receptor in LPA-induced plasma exudation was investigated (Fig. 3). Only the mice treated with 1 $\mu g/site$ of DGPP showed no change in dye leakage. The amount of dye in the vehicle-treated group was $8.40 \pm 2.39 \mu g/site$. There were significant differences between the vehicle- and saline-treated groups (*P*<0.05). LPA-induced dye leakage was dose-dependently inhibited by DGPP at the
doses of 0.1 and 1 μg/site. Statistically significant differences were observed between the vehicle- and DGPP-treated groups at 1 μg/site (P<0.05).

**Effect of PTX and DGPP on LPA-induced histamine release**

Figure 4 presents the effects of PTX on LPA-induced histamine release. Under the present conditions, spontaneous histamine release was 8.19 ± 3.56%. In contrast, histamine release increased in significance (P<0.01) following 10 μg/ml of LPA; moreover, the histamine release value was 30.8 ± 4.96%. Pretreatment with PTX reduced this increase to nearly the level observed in spontaneous group. Statistically significant differences were apparent between both groups at P<0.05. On the other hand, A23187-induced histamine release was not inhibited by PTX.

Effect of DGPP on LPA-induced histamine release is displayed in Fig. 5. One μg/ml of LPA did not produce a significant histamine release. LPA-induced histamine release

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**Fig. 3.** Effects of diacylglycerol pyrophosphate (DGPP) on dermal vascular permeability induced by LPA in mice. LPA (10 μg/site) was administered into the dorsal skin of mice. Dye leakage was assessed 30 min later. Each column represents the mean ± S.E.M. of 5 animals. Significantly different from saline (*P<0.05, Student t-test).

**Fig. 4.** Effect of pertussis toxin (PTX) on histamine release induced by LPA (a) and A23187 (b) from mouse peritoneal mast cells. The cells were pre-warmed for 5 min at 37°C. Following the addition of 100 ng/ml pertussis toxin and 2-h incubation, the cell suspension was challenged with LPA (final concentration of 10 μg/ml) or calcium ionophore A23187 (final concentration of 0.05 μg/ml). Each column represents the mean ± S.E.M. of 4 experiments. Significant difference from spontaneous release (*P<0.01, Student’s t-test).

**Fig. 5.** Effect of diacylglycerol pyrophosphate (DGPP) on histamine release induced by LPA (from mice peritoneal mast cells. The cells were pre-warmed for 5 min at 37°C. Following the addition of 0.1 or 1 μg/ml DGPP and 5-min incubation, the cell suspension was challenged with LPA (final concentration of 10 μg/ml). Each column represents the mean ± S.E.M. of 4 experiments. Significant different from spontaneous release (*P<0.01, Student’s t-test).
release was dose-dependently inhibited by DGPP at concentration of 0.1 and 1 μg/ml. There were significant differences between vehicle- and DGPP-treated groups at 1 μg/ml (P<0.05).

**Discussion**

In the present study, we demonstrated that 1 – 100 μg/site of LPA induced plasma exudation utilizing Evans blue. Evans blue has been shown to combine quantitatively with plasma proteins in vivo (15) and correlates well with extravasation of radiolabeled albumin in the skin (16). Moreover, ketotifen was shown to exert an inhibitory effect. A preliminary study demonstrates that ketotifen produces no change in dye leakage alone. These results indicate that histamine was involved in LPA-induced plasma exudation. Furthermore, histamine release of 30% – 40% occurred in the previously reported in vitro studies (6). It is reported that about 35% of total histamine contents releases in response to 0.1 μg/ml of compound 48/80, a typical agent causing histamine release (17). In addition, a nearly equal dose of compound 48/80 induces dye leakage (18). From these reports, we speculate that histamine release of 30% – 40% can evoke plasma exudation. Hence, LPA-induced plasma exudation was mediated by histamine release from mast cells.

We also showed that LPA-induced plasma exudation was inhibited by DGPP. DGPP inhibited human eosinophils (11) and platelet activation (19) evoked by LPA. However, few reports exist regarding the in vivo effect of DGPP. In pilot experiments, we tested the effect of DGPP on its own to investigate whether LPA receptor(s) are involved in LPA-induced plasma exudation. As a result, DGPP slightly induces the dye leakage on its own at 10 μg/site. Although it is unclear why 10 μg/site of DGPP induces dye leakage, higher dose of DGPP is likely to show an agonistic effect. Consequently, the effect of DGPP on LPA-induced plasma exudation was determined using 0.1 and 1 μg/site of DGPP, because no change in dye leakage was observed at 1 μg/site. The present results indicate that LPA-induced plasma exudation is induced via LPA receptor(s), presumably LPA1 and/or LPA3.

Several experiments have demonstrated that LPA1 and LPA2 can couple to the Gq/11, G12/13, and Gq family, and LPA3 can couple to the Gq/11, and Gq family (8). LPA induces various biological effects through these PTX-sensitive (Gq/11) or -insensitive (G12/13, Gq) G-proteins. Bagga et al. reported that LPA-induced proliferation of mast cells was blocked by PTX; VPC-32179, a competitive antagonist of LPA1; and LPA2 (20). Renbäck et al. reported LPA had a nociception-product-


