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Inhibitory effect of lysophosphatidylcholine on the histamine release from rat peritoneal mast cells

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Abstract

Histamine release from isolated rat peritoneal mast cells induced by compound 48/80 (0.5 μg/ml) or antigen–antibody reaction was inhibited by lysophosphatidylcholine in a dose-dependent fashion at concentrations up to 4 μM. Within the same range of concentration, lysophosphatidylcholine exhibited a membrane-stabilizing action on the model membrane systems decreasing the permeability of lipid bilayer and the fluidity of liposomal membrane in the liquid crystalline state. At concentrations higher than 8 μM, lysophosphatidylcholine damaged the cell membrane and subsequently histamine was released. It was assumed that lysophosphatidylcholine may act as an endogenous membrane stabilizer inhibiting histamine release in normal mast cells.

Introduction

An increase in the turn-over rate of phospholipids in cell membranes has been reported to play an important role in histamine release from mast cells induced by either antigen–antibody reaction or concanavalin A [1, 2]. It has been postulated that phospholipase A_2 is one of the key enzymes in the activation of phospholipid metabolism, since the metabolites produced by this enzyme provide various properties leading to histamine release, not only biochemically but also biophysically [2, 3]. It was shown that lysophosphatidylcholine (lyso-PC) brings about a perturbation of the lipid bilayer in the cell membrane [4, 5]. The content of lyso-PC in the normal mast cell is not high [6] and the increased amount of lyso-PC during cell activation may not be large enough to form micelles in the cell membrane. Disruption of the cell membrane ascribed to micelle formation and the subsequent histamine release have been emphasized [4, 7]. To make clear the role of lyso-PC in histamine release, the present study was carried out.

Materials and methods

Histamine release from isolated rat peritoneal mast cells

Histamine release from rat peritoneal mast cells was measured as described previously [8]. Peritoneal mast cells isolated from the abdominal cavity of male Wistar rats were suspended in physiological-buffered solution (NaCl 154 m*M*, KCl 2.7 m*M*, $CaCl_2$ 0.9 m*M*, glucose 5.6 m*M*, HEPES 5 m*M*, pH 7.4: PBS). Cell suspension was incubated with test compounds at 37°C, and then compound 48/80 (48/80) was added to make the final concentration 0.5 μg/ml. In the case of antigen–antibody reaction, mast cells were sensitized passively with IgE rich anti-egg albumin mice serum [9]. After pretreatment with phosphatidylserine (10 μg/ml) for 15 min, sensitized mast cells were exposed to antigen.

^{45}Ca uptake into rat mast cells

^{45}Ca uptake into rat mast cells was measured according to the method of SPATARO et al. [10]. Rat mast cells purified more than 95% with Percoll [11] were incubated with 1.35 μCi of ^{45}Ca (per 2.5 × 10^5 cells) for 1 min at 37°C. Thereafter, pretreatment with various concentrations of lyso-PC was carried out for 15 min at 37°C. After that, 48/80 (0.5 μg/ml) was added and the cells were incubated for 5 min. The mast cells were washed twice with fresh PBS and solubilized with Triton X-100 (10%). The radioactivity of the solution was measured by a liquid scintillation counter (Aloka, LSC-700). 1-palmitoyl-L-α-lyso-PC was used in all of the experiments.

Uptake of ^{14}C-labelled lyso-PC into mast cells

Purified mast cells incubated with 1-(1^{14}C)-palmitoyl lyso-PC (0.46 μCi/test tube, 4 μM in PBS) at 37°C. The uptake process was stopped by chilling the test tube in an ice-cold bath, and washing the cells twice with fresh PBS. Total lipids of mast cells were extracted by the method of BLIGH and DYER [12], and spotted on Silica gel G plate. After development with $CHCl_3$:MeOH:AcOH:H_2O (30:15:4:1), the plate was divided into 30 fractions and each fraction was extracted with $CHCl_3$:MeOH (2:1), and the radioactivity was measured by a liquid scintillation counter.

Carboxy fluorescein (CF) release from liposomes

CF containing liposomes were made with egg yolk PC and cholesterol (5:1) according to a modification of the

method after KLAUSNER et al. [13]. Sonicated liposomes, prepared in HEPES-buffered saline (NaCl 154 m*M*, HEPES 5 m*M*; pH 7.4) containing 100 m*M* CF, were applied to Sepharose 4B column, and eluted with HEPES-buffered saline to remove free CF and to collect the CF-trapped unilamellar liposome selectively. The liposome suspension was preincubated with lyso-PC at 37°C for 5 min, and then 48/80 (5 μg/ml) was added into the medium [8]. The fluorescence of CF-containing liposome was not detectable, since self-quenching takes place at a high concentration of CF sequestered in the liposome. However, when CF was released from the liposomes by 48/80, its fluorescence became apparent. Thus, the sequential process of CF release was recorded by monitoring the fluorescence intensity measured at 515 nm with an exciting beam of 470 nm [13].

Procedure for the measurement of steady-state fluorescence polarization

The fluidity of liposomal membrane was determined by fluorescence polarization of 2- or 16-(9-anthroyloxy)palmitic acid (2-AP, 16-AP) [14, 15]. Liposomes prepared with dipalmitoylphosphatidylcholine (DPPC) containing various amounts of lyso-PC were labelled with either of the two fluorophores. Steady-state fluorescence polarization (*P*) was calculated according to the following equation by measuring the fluorescence intensities (470 nm) polarized parallel ($I//$) and perpendicular ($I\perp$) to the direction of the polarized excitation beam (375 nm) [15].

$$P = (I// - I\perp)/(I// + I\perp)$$

Hypotonic haemolysis of rat erythrocytes

The effect of lyso-PC on the hypotonic haemolysis of rat erythrocytes was determined by a modification of the method after SEEMAN [16]. Hypotonic buffer was composed of NaCl 68 m*M* and HEPES 5 m*M* (pH 7.4). Erythrocytes were incubated in hypotonic buffer containing various concentrations of lyso-PC at 37°C for 5 min. After centrifugation, the optical density of the supernatant was measured at 543 nm

Determination of critical micelle concentration (CMC) of lyso-PC

CMC of lyso-PC was determined by measuring the fluorescence of 1-anilino-8-naphthalene sulphonate (ANS) under hydrophobic circumstances [8].

Viability of mast cells

The viability of the mast cells was determined by dye exclusion test using 0.2% Trypan Blue.

Results

When isolated rat peritoneal mast cells were incubated with lyso-PC, histamine release was not provoked at concentrations lower than 4 μ*M*. However, histamine release induced by either 48/80 or antigen–antibody reaction was inhibited dose-dependently by pretreatment with lyso-PC up to concentrations of 4 μ*M* (Fig. 1). Palmitic acid and phosphorylcholine, the decomposed compounds of lyso-PC, affected neither the spontaneous histamine release nor histamine release due to 48/80 even at 100 μ*M*. The uptake of ^{45}Ca into mast cells was increased about 2.5 times after treatment with 48/80. But, lyso-PC inhibited the uptake of ^{45}Ca dose-dependently up to 4 μ*M*. At this concentration, Ca uptake was suppressed close to the control level. When ^{14}C labelled lyso-PC was incubated with mast cells, most of the radioactivity was detected in lyso-PC fraction, but the rest was allocated separately in palmitic acid (front) and PC fractions.

To investigate the inhibitory mechanism of lyso-PC on histamine release, the effect of

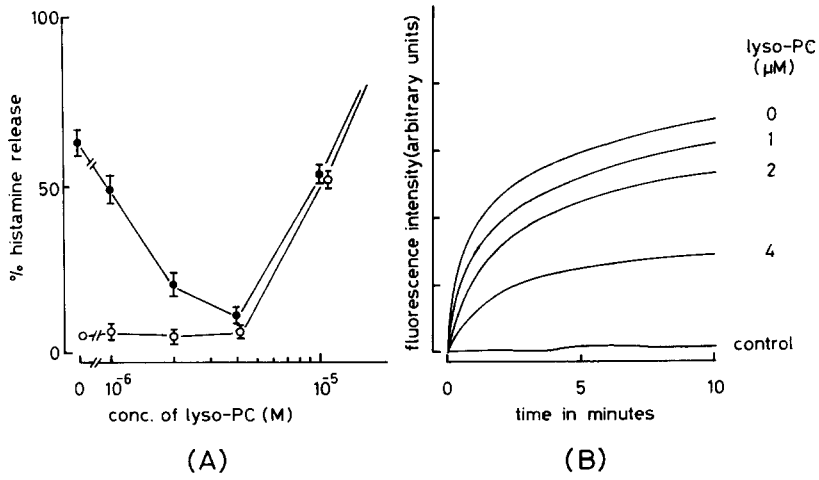

Figure 1
Effects of lysophosphatidylcholine on (A) histamine release from isolated rat peritoneal mast cells due to compound 48/80 (0.5 μg/ml), ●——●: +48/80, ○——○: −48/80 and (B) carboxy fluorescein release induced by compound 48/80 (5 μg/ml) from liposomes made of egg-PC and cholesterol.

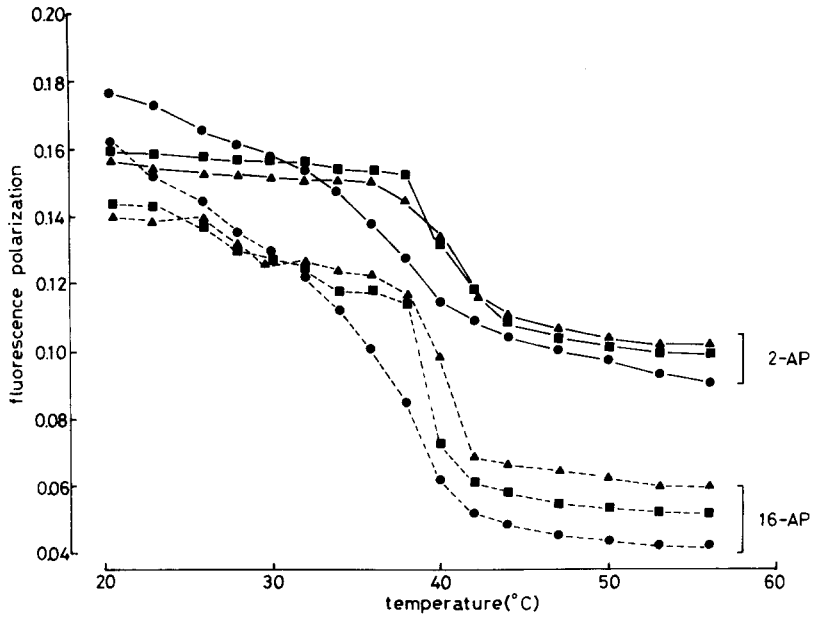

Figure 2
Temperature dependence of the fluorescence polarization of 2- or 16-(9-anthroyloxy)palmitic acid incorporated into the liposomes made of dipalmitoylphosphatidylcholine and 1-palmitoyl lysophosphatidylcholine. ●——● (DPPC; lyso-PC = 100:0), ■——■ (DPPC:lyso-PC = 85:15), ▲——▲ (DPPC:lyso-PC = 70:30).

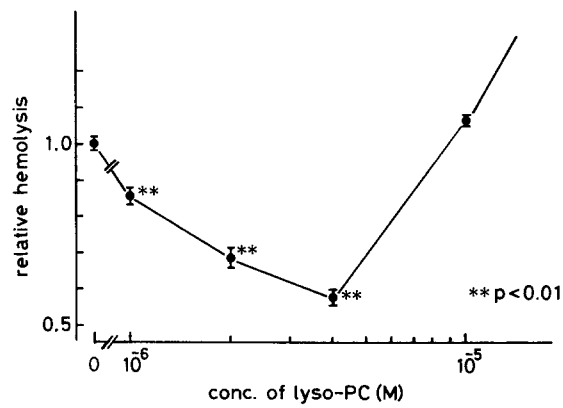

Figure 3
Influence of lyso-PC on hypotonic haemolysis. The data represent mean ± SEM, $n = 6$.

lyso-PC on lipid bilayers was studied using model membrane systems. When the CF-containing liposomes were exposed to 48/80, CF release was brought about rapidly. However, CF release was inhibited by preincubation with lyso-PC dose-dependently (Fig. 1). Figure 2 shows the effect of lyso-PC on the fluidity of the liposomal membrane. *P* value is conversely related to the extent of disarrangement of fluorescent probes in the lipid bilayer [14, 15]. In the case of the liposome made of DPPC without lyso-PC, *P* values of 2-AP and 16-AP showed sudden and large decrease around the phase transition temperature of DPPC. Changes in *P* values determined with 2-AP may reflect the alteration of the fluidity around the hydrophilic head and those measured with 16-AP may reflect the fluidity change near the hydrophobic terminal [14]. The incorporation of lyso-PC into DPPC liposomes fluidized the gel state under 30°C, but rigidified the liquid crystalline state in a dose-dependent fashion. When rat erythrocytes were exposed to the hypotonic buffer, lyso-PC inhibited the haemolysis at concentrations lower than 4 μM, but higher than this concentration, lyso-PC enhanced haemolysis (Fig. 3). The CMC of lyso-PC determined by ANS method, was 8 μM. More than 95% of the mast cells were viable after treatment with lyso-PC at concentrations lower than 4 μM, but almost all of mast cells were swollen markedly at concentrations higher than 10 μM of lyso-PC.

Discussion

There are many reports suggesting that lyso-PC acts as surface active agents causing

histamine release from mast cells [1–3]. This can be deduced from the fact that the content of lyso-PC increases at the early stage of histamine release [17], and phospholipase A_2 can be activated in accordance with histamine release [3]. Therefore, phospholipase A_2 was postulated as a key enzyme in the process of histamine release [2, 3]. However, so far no direct evidence has been shown that the amount of lyso-PC yielded in the mast cell membrane is large enough to destroy the cell membrane.

In the present study, we observed the direct action of lyso-PC on mast cells and lipid bilayer. It was shown that lyso-PC inhibited the histamine release and uptake of ^{45}Ca induced by 48/80 or antigen–antibody reaction at lower concentrations. The results indicate that lyso-PC may act in some way to protect mast cells against the stimuli. It was shown that when lyso-PC interact with mast cells, both deacylation and acylation take place. However, most of the lyso-PC incubated with the mast cells remained as it was. The fact that both palmitic acid and phosphorylcholine are not able to inhibit histamine release, and that lyso-PC inhibited the CF release from liposomes induced by 48/80, suggests that lyso-PC acted directly on the lipid bilayer of the cell membrane to restrain the increase of the membrane permeability caused by histamine releasers. It was shown that lyso-PC acted to stabilize the lipid bilayer membrane so as to decrease the fluidity of the liquid crystalline state. A similar effect on liposomes has been displayed by anti-allergic drugs which remarkably inhibit the histamine release from mast cells [8, 18]. One of the important mechanisms of lyso-PC on histamine release inhibition can be ascribed to the membrane stabilization acting on the lipid bilayer of the plasma membrane. The inhibitory effect of lyso-PC on hypotonic haemolysis indicates that the membrane stabilizing action of lyso-PC is not necessarily limited to mast cells.

On the contrary, lyso-PC at concentrations higher than CMC, caused histamine release from mast cells and enhanced haemolysis. In such cases, micelles of lyso-PC may be formed in the cell membrane, leading to cell disruption. In accordance with this, the turning point from inhibition to enhancement of histamine release or haemolysis is in good agreement with CMC. An increase of lyso-PC in the early stages of antigen–antibody reaction of the mast cell may not be enough to exceed CMC, so it is rather reasonable to assume that the endogenous lyso-PC in the mast cell membrane may inhibit histamine release by its stabilizing action on the cell membrane.

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