The Effect of Platelet-Activating Factor on Histamine Release from Rat Peritoneal Mast Cells

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Abstract—The effect of platelet-activating factor (PAF) on histamine release from peritoneal mast cells of adult and young male rats was investigated. PAF alone tended to release histamine from the mast cells of adult and young rats, although very slightly. On the other hand, PAF significantly inhibited the histamine release induced by the Ca²⁺ ionophore A23187 in mast cells obtained from rats of either age group, but not that by compound 48/80. Such inhibition was not seen with lyso-PAF. CV-3988, a PAF antagonist, antagonized the inhibitory effect of PAF on the A23187-induced histamine release in mast cells from adult and young rats. These results suggest that PAF does not have a strong histamine-liberating action on mast cells, and that PAF inhibits the calcium influx into mast cells through the activation of PAF receptors.

In 1972, Benveniste et al. (1) reported the release of PAF from rabbit platelets. PAF causes a bronchospasm in laboratory animals (2–6) and humans (7, 8). It may be released from various types of inflammatory cells, including macrophages (9, 10), neutrophils, basophils (11), platelets (12), and eosinophils (13) and plays role in the pathogenesis of inflammation and asthma. PAF is also released from rabbit mast cells (14), but not from those of rats and mice (9). On the other hand, the effect of PAF on histamine release from mast cells has not been adequately documented. Therefore, in the present study, the effects of PAF alone and the combination of PAF with well-known histamine liberators on histamine release from rat peritoneal mast cells were investigated.

Materials and Methods

Purification of rat peritoneal mast cells: Adult (250–300 g) and young (60–80 g) male Wistar rats were purchased from Tokyo Laboratory Animals, Inc. The animals were decapitated, and then the peritoneal cavity was washed with ice-cold Tyrode-heparin-gelatin solution (THG solution) (adult: 15 ml, young: 7.5 ml). The lavage fluid was pooled and centrifuged at 200×g, for 15 min, at 4°C. Mast cells were isolated from the pellet using a Percoll gradient method (15, 16). After Percoll gradient centrifugation, the mast cell pellet was resuspended in 7 ml of THG and washed twice by centrifugation (200 x g, 5 min, 4°C) to remove residual Percoll. After staining with 0.017% Toluidine blue the number and purity of mast cells were counted with a hemocytometer under a microscope. The viability was evaluated by staining with 0.2% Trypan blue.

THG buffer composition: The THG solution had the following composition: 137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH₂PO₄, 1.8 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose and 10 mM HEPES. THG buffer was adjusted to pH 7.4 with 5 N NaOH, and gelatin (1 mg/ml) and heparin (5 units/ml) were then added.

Treatment of mast cells with drugs: Purified mast cells were resuspended and each tube contained 4.5×10⁵ cells (adult) or 5.6×10⁴ cells (young) in 0.9 ml of THG. Mast cells were incubated with a given drug for 15 min at 37°C under shaking. To study the effect of PAF on the enhanced histamine release by histamine liberators, PAF was added to the test tube 2 min prior to the 15-min incubation.
with histamine liberators. When CV-3988, a PAF antagonist, was used, CV-3988 was given 2 min prior to the addition of PAF.

**Histamine assay**: The histamine contents of both the pellet and supernatant were assayed by the fluorometric method described by Shore et al. (17). The percentage of histamine release into the supernatant was defined as:

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\frac{\text{Histamine in supernatant}}{\text{Histamine in cells} + \text{Histamine in supernatant}} \times 100(\%)
\]

**Drugs**: Platelet-activating factor (PAF, β-acetyl-γ-O-hexadecyl-L-α-phosphatidylcholine, Sigma) and lyso-PAF (Sigma) were dissolved in PBS/BSA buffer solution. The PBS/BSA buffer solution was prepared as follows: 0.15 M phosphate buffer solution (PBS, Na₂HPO₄ and NaH₂PO₄·2H₂O) was adjusted to pH 7.4 with 1 N NaOH, and then bovine serum albumin (BSA, 2.5 mg/ml, Sigma) was added. Compound 48/80 (Sigma) and CV-3988 (Takeda Chem. Ind.) were dissolved in THG. Calcium ionophore A23187 (Sigma) was dissolved in 1% dimethyl sulfoxide (DMSO) aqueous solution. o-Phthalaldehyde (OPA, Sigma) and Percoll (Sigma) were also used.

**Statistical analysis**: The results were presented as the mean±S.E. Statistical significance of differences between the means was assessed by Student's t-test for unpaired data.

**Results**

PAF (10⁻⁷–2×10⁻⁶ g/ml) alone tended to release histamine from peritoneal mast cells of both adult and young rats, although very slightly (Fig. 1). Significant histamine release was observed at the highest concentration of PAF (3×10⁻⁶ g/ml); that is, 1.5±0.6% in mast cells from adult rats and 58.1±3.9% in mast cells from young rats. Lyso-PAF at 3×10⁻⁶ g/ml also significantly released histamine (Fig. 2).

PAF (10⁻⁷–10⁻⁶ g/ml) did not influence the histamine release induced by compound 48/80 (2×10⁻⁷ g/ml) (Fig. 3). On the other hand, PAF significantly inhibited the histamine release induced by calcium ionophore A23187 (5×10⁻⁷ g/ml) in mast cells from either age group (Fig. 4). A reverse U-shaped inhibition curve was observed in mast cells from adult rats, whereas a concentration-dependent inhibition was seen in mast cells from young rats. The maximal inhibition was 60.8±3.1% in mast cells from adult rats and 35.8±2.7% in mast cells from young rats. Such an inhibition of histamine release induced by A23187 was not seen with lyso-PAF (Figs. 5 and 6). A two-min pretreatment with CV-3988 (6×10⁻⁶ g/ml) efficiently anta-

![Fig. 1](image-url). Effect of PAF on histamine release from peritoneal mast cells of adult and young rats. Spon.: spontaneous release, N.D.: Not determined. N=7. *: P<0.05, **: P<0.01 vs. Spon.
gonized the inhibitory effect of PAF on the A23187-induced histamine release in mast cells from both adult and young rats (Figs. 5 and 6).

**Discussion**

There have been very few reports on the effect of PAF on histamine release from mast cells. Kurosawa (18) reported in a study using only one concentration (10\(^{-7}\) g/ml) of PAF that the agent tended to enhance histamine release from rat peritoneal mast cells induced by calcium ionophore A23187. However, he did not describe the effect of PAF alone on the histamine release from mast cells, so his experiments seem to be preliminary.

In the present study, it was found that PAF (10\(^{-7}\)-2\(\times\)10\(^{-6}\) g/ml) alone tends to release
histamine from mast cells obtained from adult and young rats, but the effect is minimal. Only the highest concentration of PAF (3×10^{-6} g/ml) used caused a significant histamine release. The release by the highest concentration of PAF may be, however, due to a non-
specific action, because lyso-PAF at the same concentration also significantly released histamine.

We subsequently investigated the interaction between PAF and histamine liberators such as compound 48/80 and calcium ionophore A23187 on histamine release from rat peritoneal mast cells. PAF significantly inhibited the histamine release induced by calcium ionophore A23187 in mast cells from rats of either age group, but not that by compound 48/80. CV-3988, a PAF antagonist (19, 20), completely antagonized the inhibitory effect of PAF on the A23187-induced histamine release. There was however no interaction between PAF and compound 48/80. Calcium ionophore A23187 is known to elevate intracellular calcium levels and release histamine by carrying calcium across the cell membrane, that is, by bypassing calcium channels (21). On the other hand, compound 48/80 is characterized by a rapid rate of histamine release, which depends on intracellular, but not extracellular calcium (22). It is therefore suggested that PAF inhibits Ca^{2+} influx from the extracellular space into mast cells, through the stimulation of specific PAF receptors. Lee et al. (23) reported that PAF (5×10^{-3}-5×10^{-7} g/ml) enhanced intracellular calcium level in rabbit platelets. The elevated calcium level may then induce platelet aggregation. However, this is not the case with rat peritoneal mast cells which are not activated by PAF alone as shown above.

Atopic bronchial asthma is known to be related to the heredity of an individual, and children generally suffer from asthma at higher incidence. The onset age for asthma in children is one or two years of age in many cases. The Wistar rats weighing 60-80 g used presently are equivalent in age to one- or two-year-old humans. From our results, a high spontaneous rate for histamine release was observed in young rats (Fig. 1), although the effect of PAF was minimal as was the case with adult rats. A reverse U-shaped curve for the inhibitory effect of PAF on the A23187-induced histamine release was seen in adult rats, while in young rats, PAF showed a concentration-dependent inhibitory effect (Fig. 4). At present, it is difficult to explain the difference. We think the higher concentrations of PAF cause histamine release by itself, partially due to a non-specific effect, which might make the result ambiguous.

In conclusion, the above findings suggest
that PAF by itself does not have any strong histamine liberating action on mast cells, and that PAF does not enhance the effects of other histamine liberators. The effect of PAF on enhanced histamine release from mast cells caused by antigen remains to be studied as a subject of interest.

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