

## The Effect of Platelet-Activating Factor on Histamine Release from Guinea Pig Peritoneal Mast Cells

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The effect of platelet-activating factor (PAF) on histamine release from the peritoneal mast cells of male guinea pigs at 4 weeks of age and one week of age (weaning) was investigated. PAF as well as compound 48/80 and concanavalin A were not found to release histamine from the mast cells of either age of guinea pigs. On the other hand,  $\text{Ca}^{2+}$  ionophore A23187 showed a significant, concentration-dependent histamine release from the mast cells obtained from guinea pigs of either age group. PAF ( $3 \times 10^{-7}$ – $3 \times 10^{-6}$  g/ml) significantly inhibited the histamine release induced by  $\text{Ca}^{2+}$  ionophore A23187 from the mast cells of guinea pigs at one week of age, but not from those of the older ones. Such an inhibition was not seen with lyso-PAF in either age group. CV-3988, a PAF antagonist, neutralized the inhibitory effect of PAF on the A23187-induced histamine release from the mast cells of guinea pigs at one week of age. These results indicate that PAF does not have a histamine-liberating action on guinea pig peritoneal mast cells, and that PAF inhibits the effect of A23187 on histamine release from mast cells through activation of PAF receptor in guinea pigs at one week of age.

**Keywords** — platelet-activating factor (PAF); mast cell; histamine release; age difference

### Introduction

In 1972, Benveniste *et al.*<sup>1)</sup> reported the release of platelet-activating factor (PAF) from rabbit basophils. PAF causes a bronchospasm in laboratory animals and humans.<sup>2)</sup> It is now recognized that PAF is a product of a variety of inflammatory cell types including alveolar macrophages, neutrophils, platelets, eosinophils and vascular endothelial cells, and may play a role in the pathogenesis of inflammation and bronchial asthma.<sup>2)</sup> PAF is also reportedly released from rabbit<sup>3)</sup> and human pulmonary mast cells,<sup>4)</sup> but not from those of rats and mice.<sup>5)</sup>

On the other hand, the effect of PAF on histamine release from guinea pig mast cells has not yet been documented. Pearce *et al.*<sup>6)</sup> investigated histamine release from guinea pig lung mast cells by some histamine liberators, but did not examine the effect of PAF.

Previously, we reported the effect of PAF on histamine release from rat peritoneal mast cells.<sup>7)</sup> PAF was found to have a slight histamine liberating action on rat mast cells.

In the present study, the effect of PAF alone and interaction of PAF with well-known histamine liberators on histamine release from the

peritoneal mast cells of guinea pigs were investigated.

### Materials and Methods

**Purification of Guinea Pig Peritoneal Mast Cells** — Male Hartley guinea pigs at 4 weeks of age (270—350 g) and at one week of age (weaning) (100—150 g) were purchased from Tokyo Laboratory Animals, Inc. The animals were bled for about 1 min after a blow on the head. The peritoneal cavity was washed with ice-cold Tyrode–heparin–gelatin solution (THG solution) (4 weeks of age: 15 ml, one week of age: 7.5 ml). The peritoneal lavage fluid was pooled and centrifuged at  $200 \times g$ , for 15 min, at 4 °C. Mast cells were isolated from the pellet using a Percoll gradient method.<sup>8,9)</sup> After Percoll gradient centrifugation, the mast cell pellet was resuspended in THG and washed twice by centrifugation ( $200 \times g$ , 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 cell viability was evaluated by

staining with 0.2% Trypan blue.

**THG Buffer Composition** — The THG solution (pH 7.4) had the following composition: 137 mM NaCl, 2.7 mM KCl, 0.4 mM  $\text{NaH}_2\text{PO}_4$ , 1.8 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , 5.6 mM glucose and 10 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethane-sulphonic acid (HEPES). Gelatin (1 mg/ml) and heparin (5 units/ml) were also added.

**Treatment of Mast Cells with Drugs** — Each test tube contained  $1.5 \times 10^5$  cells (4 weeks of age) or  $3.6 \times 10^4$  cells (one week of age) 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 the histamine liberator. When CV-3988, a PAF antagonist, was used, CV-3988 was given further 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.*<sup>10)</sup> The percentage of histamine release into the supernatant was defined as:

$$\frac{\text{histamine in supernatant}}{\text{histamine in cells} + \text{histamine in supernatant}} \times 100 (\%)$$

**Drugs** — PAF ( $\beta$ -acetyl- $\gamma$ -*O*-hexadecyl-L- $\alpha$ -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;  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ) 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), concanavalin A (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 (polyvinylpyrrolidone-coated silica, Sigma) were also used.

**Statistical Analysis** — The results were presented as the mean  $\pm$  S.E. Statistical significance of differences between the means was

assessed by Student's *t*-test for unpaired data.

## Results

The number of peritoneal mast cells yielded from one guinea pig was  $8.4 \times 10^5$  cells in guinea pigs at 4 weeks of age and  $8.1 \times 10^4$  cells in those at one week of age, on an average. Mast cell preparations were about 93% pure, and more than 94% were viable in either age. The average histamine contents contained in mast cells were  $1.33 \pm 0.14 \mu\text{g}/10^5$  cells ( $N=13$ ) in the older and  $6.87 \pm 0.36 \mu\text{g}/10^5$  cells ( $N=12$ ) in the younger guinea pigs. Spontaneous histamine release for a 15-min incubation at 37 °C was  $29.1 \pm 5.2\%$  ( $N=12$ ) and  $33.4 \pm 2.9\%$  ( $N=6$ ) in guinea pigs at one week of age and 4 weeks of age, respectively.

No significant histamine release was induced from the peritoneal mast cells of guinea pigs at either age by compound 48/80 ( $3 \times 10^{-8}$ ,  $10^{-7}$ ,  $3 \times 10^{-7}$  g/ml) and concanavalin A ( $10^{-5}$ ,  $3 \times 10^{-5}$ ,  $10^{-4}$  g/ml) (data not shown). On the other hand,  $\text{Ca}^{2+}$  ionophore A23187 ( $10^{-6}$ ,  $3 \times 10^{-6}$ ,  $10^{-5}$  g/ml) caused a significant concentration-dependent histamine release in either age (Fig. 1). PAF in concentration range of  $10^{-7}$  to  $3 \times 10^{-6}$  g/ml had no histamine liberating action on mast cells of either age group (Table I).

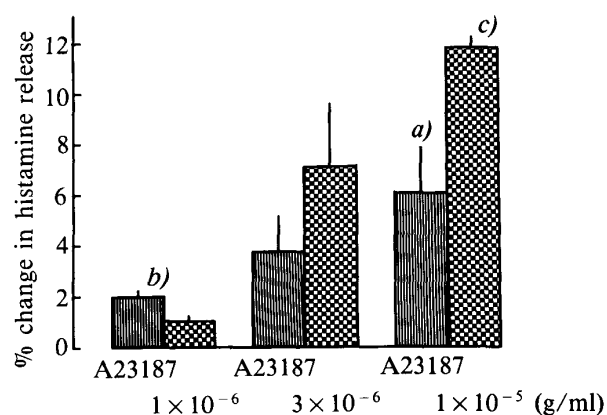


Fig. 1. Effect of  $\text{Ca}^{2+}$  Ionophore A23187 (A23187) on the Histamine Release from the Peritoneal Mast Cells of Guinea Pigs at 4 Weeks of Age and One Week of Age

% change in histamine release represents the change from % spontaneous histamine release. ■, 4 weeks guinea pig; ▨, 1 week guinea pig.  $N=5$ , a)  $p<0.05$ , b)  $p<0.01$ , c)  $p<0.001$  vs. zero (spontaneous release).

TABLE I. Effect of PAF on Histamine Release from Peritoneal Mast Cells of Guinea Pigs at 4 Weeks of Age and One Week of Age

(g/ml)	N	4 weeks (%)	1 week (%)
PAF $1 \times 10^{-7}$	4	$0.9 \pm 0.4$	$-0.1 \pm 0.2$
$3 \times 10^{-7}$	4	$0.4 \pm 0.1^{b)}$	$0.4 \pm 0.2$
$1 \times 10^{-6}$	4	$0.6 \pm 0.2$	$0.7 \pm 0.5$
$3 \times 10^{-6}$	4	$0.4 \pm 0.1^{a)}$	$0.5 \pm 0.4$

The values represent the change from % spontaneous histamine release. a)  $p < 0.05$ , b)  $p < 0.01$  vs. zero (spontaneous release).

PAF ( $3 \times 10^{-7}$  g/ml) tended to inhibit and PAF ( $1 \times 10^{-6}$ ,  $3 \times 10^{-6}$  g/ml) significantly inhibited the histamine release induced by  $\text{Ca}^{2+}$  ionophore A23187 ( $10^{-5}$  g/ml) from the mast cells of one week age guinea pigs (Fig. 2), but not from mast cells of animals at 4 weeks of age. Such an inhibition of histamine release induced by A23187 was not seen with lyso-PAF ( $10^{-6}$  g/ml) (Fig. 3). A two-min pretreatment with CV-3988 ( $6 \times 10^{-6}$  g/ml) efficiently antagonized the inhibitory effect of PAF on the A23187-induced histamine release from mast cells of the younger guinea pigs (Fig. 3). On the other hand, additions of THG as the solvent for

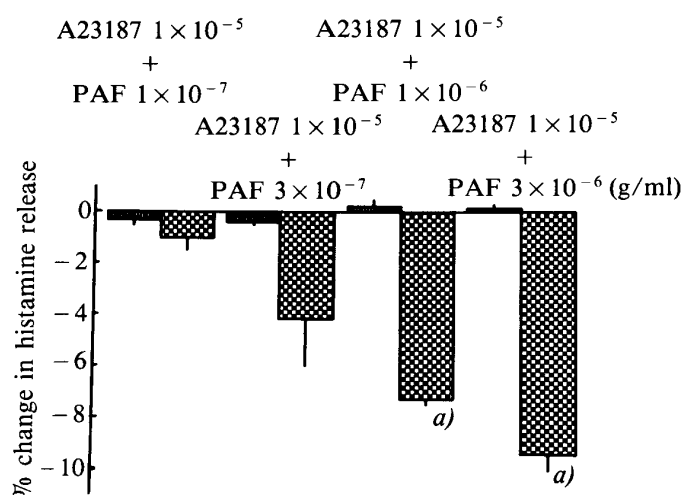


Fig. 2. Influence of PAF on the Histamine Release by  $\text{Ca}^{2+}$  Ionophore A23187 from the Peritoneal Mast Cells of Guinea Pigs at 4 Weeks of Age and One Week of Age  
% change in histamine release represents the change from the % histamine release induced by A23187 alone. ■, 4 weeks guinea pig; ▨, 1 week guinea pig.  $N=5$ , a)  $p < 0.001$  vs. zero (A23187 alone).

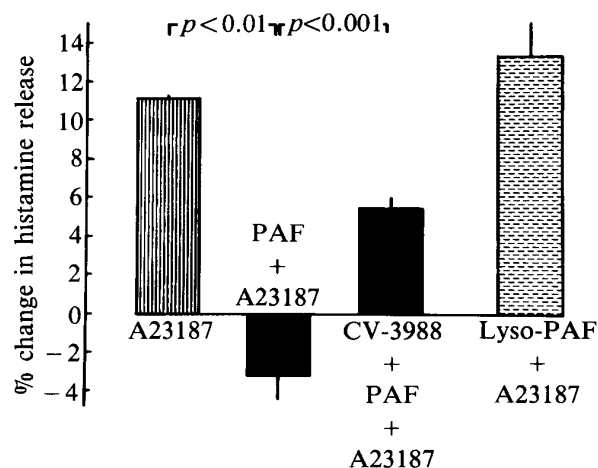


Fig. 3. Antagonistic Effect of CV-3988 ( $6 \times 10^{-6}$  g/ml) on the PAF-Induced Inhibition of Histamine Release by A23187 ( $10^{-5}$  g/ml) from the Peritoneal Mast Cells of Guinea Pigs at One Week of Age

The doses of PAF and lyso-PAF used were  $10^{-6}$  g/ml.  $N=5$ .

CV-3988 4 min prior to, and PBS/BSA as the solvent for PAF 2 min prior to the administration of A23187 did not influence the histamine release level induced by A23187.

## Discussion

The effects of PAF on histamine release from isolated rat peritoneal mast cells have been reported previously.<sup>7)</sup> In that paper, PAF ( $10^{-7}$ — $2 \times 10^{-6}$  g/ml) alone tended to release histamine, although very slightly, from the peritoneal mast cells of both adult and young rats. However, the present study indicates that the peritoneal mast cells of guinea pigs at 4 weeks of age and one week of age are substantially insensitive to PAF as well as to compound 48/80 and concanavalin A, whereas a significant and concentration-dependent histamine release was induced by  $\text{Ca}^{2+}$  ionophore A23187. As regards histamine liberators, our data are consistent with those of Pearce *et al.*<sup>11-13)</sup> that compound 48/80 and concanavalin A caused no histamine release from the lung mast cells of guinea pigs. The spontaneous rate for histamine release was much the same in guinea pig peritoneal mast cells ( $29.1 \pm 5.2\%$ ) at one week of age as that in peritoneal mast cells of weaning rats ( $36.2 \pm 0.9\%$ ).<sup>7)</sup> The spontaneous rate for hista-

mine release from mast cells of the older guinea pigs ( $33.4 \pm 2.9\%$ ) was slightly higher than that from mast cells of older rats ( $12.9 \pm 0.6\%$ ). Barrett *et al.*<sup>11)</sup> and Pearce<sup>12)</sup> have emphasized the existence of differences in histamine release from mast cells from species to species and from tissue to tissue. The differences in spontaneous release rate may be due to species and age differences. We subsequently investigated the interaction between PAF and  $\text{Ca}^{2+}$  ionophore A23187 on histamine release from guinea pig peritoneal mast cells. PAF significantly inhibited the histamine release induced by  $\text{Ca}^{2+}$  ionophore A23187 in guinea pigs at one week of age, but not in guinea pigs at 4 weeks of age. CV-3988, a PAF antagonist,<sup>14,15)</sup> neutralized the inhibitory effect of PAF on the A23187-induced histamine release in younger guinea pigs.  $\text{Ca}^{2+}$  ionophore A23187 is known to directly elevate the intracellular calcium levels across the cell membrane to release histamine.<sup>16)</sup> It is therefore suggested that PAF inhibits the calcium influx from the extracellular space into mast cells through specific PAF receptors in guinea pigs at one week of age. Lee *et al.*<sup>17)</sup> reported that PAF ( $5 \times 10^{-9}$ – $5 \times 10^{-7}$  g/ml) enhanced the intracellular calcium level in rabbit platelets. The enhanced calcium level may then induce platelet aggregation. This is not, however, the case with guinea pig peritoneal mast cells which were not activated by PAF alone as described above. The results obtained in the present study are similar to those obtained in the peritoneal mast cells of young rats in which PAF ( $3 \times 10^{-7}$  to  $3 \times 10^{-6}$  g/ml) inhibited the A23187-induced effect, in a concentration-dependent manner.<sup>7)</sup> PAF significantly inhibits the A23187-induced increase in calcium influx into rat peritoneal mast cells (unpublished data). The concentrations of PAF required to activate human platelets, eosinophils and neutrophils are reportedly  $4 \times 10^{-8}$ – $4 \times 10^{-7}$  g/ml,<sup>18)</sup>  $1 \times 10^{-9}$ – $4 \times 10^{-8}$  g/ml<sup>19)</sup> and  $3 \times 10^{-7}$ – $3 \times 10^{-6}$  g/ml,<sup>20)</sup> respectively. In the present study, the PAF concentrations required to inhibit the A23187-induced histamine release were  $3 \times 10^{-7}$ – $3 \times 10^{-6}$  g/ml, indicating that higher concentrations of PAF may be necessary in this response than those in the responses of cells referred to above.

The reason for the difference in the PAF effect on the A23187-induced histamine release observed between guinea pigs at 4 weeks of age and one week of age is not known, but may reflect variations in the membrane composition of the mast cells of animals at either age. 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 to three years of age in many cases.<sup>21,22)</sup> The Hartley guinea pigs at one week of age used presently are equivalent in age of one- to two-year-old humans (personal communication from Tokyo Laboratory Animals, Inc.). From the present results, the histamine release response of mast cells to  $\text{Ca}^{2+}$  ionophore A23187 in guinea pigs at one week of age was more marked than that in ones at 4 weeks of age, and the histamine content contained in mast cells of animals at one week of age was about five times more than that at 4 weeks of age. We consider a possibility that these differences in nature of mast cells between the animals at one week of age and 4 weeks of age might, in a part, be relevant to the higher incidence rate of asthma in children.

In conclusion, the above findings indicate that PAF by itself does not have any histamine liberating action on guinea pig mast cells, and that PAF inhibits the effect of a histamine liberator,  $\text{Ca}^{2+}$  ionophore A23187.

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