Effects of Platelet-Activating Factor on Rat Airways

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Abstract—Effects of platelet-activating factor (PAF) on the rat airways were investigated. Male Wistar rats were anesthetized, and PAF was inhaled into the lungs through a tracheal cannula for 5 min using an ultrasonic nebulizer. The bronchomotor response was measured with a modified Konzett-Rössler method in rats immobilized with decamethonium bromide. The inhalation of PAF caused a marked bronchoconstriction, dose-dependently, in a concentration range of 0.0001 to 0.01%. The bronchoconstrictor potency of PAF was about ten times higher than that of ACh. On the other hand, histamine inhalation gave only a slight bronchoconstriction even at the high concentration of 0.1%. The bronchomotor response to PAF was accompanied by a marked, sustained decrease in systemic blood pressure, in a dose-dependent manner. Repeated inhalations of PAF (0.001%) at an interval of 60 min resulted in a pronounced tachyphylaxis in the bronchoconstrictor response, but not in the hypotensive response. Combined inhalations of PAF with ACh or histamine did not produce a potentiation by PAF of the bronchoconstrictor responses to ACh and histamine. These findings show that PAF is a strong bronchoconstrictor agent in rats and that there is no interaction between PAF and other mediators in the acute bronchoconstrictor response.

Chemical mediators involved in human bronchial asthma are still ambiguous. In 1972, Benveniste et al. (1) reported the existence of platelet-activating factor (PAF) and showed that PAF is released in vitro in allergic reactions.

Since then, the pathophysiological role of PAF, for example, that in bronchial asthma, has attracted much attention. PAF is released from antigen-stimulated alveolar macrophages of asthmatic patients (2). It is also known that PAF constricts the airways in guinea pigs (3–6), monkeys (7) and humans (8, 9). However, the effects of PAF on the airways of rats which are often used in experimental asthma models have been poorly documented.

In the present study, bronchoconstrictor effects of inhaled PAF and the interactions between PAF and acetylcholine or histamine in the bronchomotor response were investigated in rats.

Materials and Methods

Procedures: Male Wistar rats (310–390 g) purchased from Nippon Biosupp. Center were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and immobilized with decamethonium bromide (0.8 mg/kg, i.v., in 2 min and 0.4 mg/kg, i.v., in 1 min when a supplement was necessary). Animals were ventilated artificially through a tracheal cannula at a frequency of 70 beats/min. The preparation used in the present study was according to the previous paper (10). In anesthetized rats, the bronchomotor tone was measured by a modification of the Konzett-Rössler method (11). The lung was inflated at a fixed volume of air at room temperature and humidity under a constant pressure (8 cmH2O), and ventilation overflow was continuously recorded with a pneumotachograph (MFP-1T, Nihon Kohden) as an index of change in airway resistance. Systemic arterial blood pressure was monitored with a pressure transducer.
(MPU-0.5, Nihon Kohden) from a cannula inserted into the left carotid artery, and heart rate was measured with a tachometer (RT-5, Nihon Kohden) using systolic blood pressure as the trigger. All the above parameters were recorded on a polygraph (Nihon Kohden, RM-85).

PAF, ACh and histamine solutions were aerosolized by an ultrasonic nebulizer (TUR-3000, Nihon Kohden) with a specially devised plastic cylindrical chamber (10) and inhaled into the animals.

To obtain the dose-response curves for PAF, ACh or histamine, these drugs were inhaled by each animal from low to high concentrations of aerosol, at an interval of 30 to 60 min (PAF) or 15 to 20 min (ACh and histamine).

To test drug interactions, the bronchoconstrictor responses to inhalation of ACh (0.01%) or histamine (0.1%) alone was compared with those to combined inhalation of ACh (0.01%) or histamine (0.1%) with PAF (0.0001%). The concentration of 0.0001% PAF was the threshold concentration for bronchoconstrictor activity of PAF alone. Before the combined inhalation was made, the control responses to ACh, histamine and PAF in the given concentrations were observed in each animal. When PAF and ACh or histamine were inhaled concomitantly, two times higher concentrations of PAF and ACh or histamine were mixed at a 1:1 ratio to obtain the final concentrations of 0.0001% (PAF), 0.01% (ACh) and 0.1% (histamine), respectively.

**Drugs:** Platelet-activating factor (PAF, \(\beta\)-acetyl-\(\gamma\)-O-hexadecyl-L-\(\alpha\)-phosphatidylcholine, Sigma) was dissolved in 70% ethanol-30% water at a concentration of 1 mg/ml and stored at \(-20^\circ\text{C}\); at the time of use, the stock solution was diluted with saline. Acetylcholine chloride (ACh, Ovisot, Daiichi) and histamine dihydrochloride (Wako Pure Chemicals) were dissolved in saline. The doses of ACh and histamine were expressed in terms of the respective base.

**Statistical analysis:** All values were expressed as the mean with S.E. Statistical significance of difference was determined by Student's t-test.

**Results**

The dose-response curve for PAF-induced bronchoconstriction: Typical recordings of the bronchoconstrictor responses to ACh and histamine are shown in Fig. 1. Five-min inhalation of ACh (0.01 and 0.03%) markedly increased ventilation overflow, the maximal response being obtained immediately after the end of inhalation. The increased ventilation overflow was returned to the pre-level within about 9 min. On the other hand, inhalation of histamine (0.01%, 0.03%) only slightly constricted the airways. Inhalations of PAF (0.001 and 0.003%) caused a gradual increase in

![Figure 1](image.png)

Fig. 1. Responses of the rat airway muscles to inhalations of ACh and histamine (Hist). B.P.: systemic blood pressure, H.R.: heart rate and V.O.: ventilation overflow.
ventilation overflow, and the bronchoconstriction reached the maximum immediately after the end of inhalation, and ventilation overflow returned to the pre-level within about 13 min (Fig. 2).

The dose-response curves for the bronchoconstriction by the above three drugs are shown in Fig. 3. The bronchoconstrictor activity of PAF was about ten times stronger than that of ACh, and it was very much stronger than that of histamine.

The PAF-induced bronchoconstriction was usually accompanied by a decrease in systemic blood pressure (Figs. 2 and 4). Even at the concentration of 0.000001% (10 ng/ml), a significant hypotension was produced. At 0.001%, the maximal decrease in blood pressure was 72±6 mmHg, and blood pressure returned to the pre-level within about 5 min after the end of 5-min inhalation. However, inhalations of ACh and histamine caused only a slight hypotension as compared with PAF.

**Tachyphylaxis to PAF-induced bronchoconstriction:** The results of inhalations of PAF (0.001%) repeated three times at an interval of 60 min are shown in Figs. 5 and 6. A pronounced increase in ventilation overflow
(2.9±0.4 ml) was observed at the first inhalation of PAF, but the responses to PAF markedly and significantly decreased at the 2nd (0.8±0.3 ml) and 3rd (0.8±0.3 ml) inhalations of PAF.

On the other hand, the hypotensive response to PAF (0.001%) was not significantly reduced by repeated inhalations, the decreases in blood pressure being 79±8 mmHg (first response), 69±8 mmHg (second response) and 63±7 mmHg (third response).

Interactions of PAF with ACh or histamine: ACh (0.01%) and histamine (0.1%) were inhaled in combination with PAF (0.0001%). The bronchoconstrictions induced by ACh and histamine were not significantly changed by combination with PAF (Figs. 7 and 8). The hypotensive response to PAF was not changed by combination with ACh or histamine either (Fig. 7).

Discussion

PAF is known to produce a variety of effects such as activations of platelets, neutrophils and macrophages, hypotension, increase in capillary permeability and contraction of the ileum (12). PAF is released from macrophages, platelets, alveolar lining cells (12), mast cells (13), etc. during allergy and
anaphylaxis. Therefore, PAF is considered to be one of the important chemical mediators involved in allergic bronchial asthma. In fact, PAF has been found to be released in asthmatic patients (2), and i.v. administration of PAF in guinea pigs induces a strong bronchoconstriction (3–6). Challenges with aerosol of PAF in dogs (14), rhesus monkeys (7) and normal subjects (8) result in a dose-dependent bronchoconstriction.

On the other hand, there has been no paper on the pulmonary effects of PAF in rats, except for the paper (2) reporting that a bronchoconstriction was not induced in rats by i.v. injection of PAF. In that paper, the authors did not provide detailed descriptions about their experiments. Rats are the small animals that have been recently used for screening antiasthmatic drugs, because IgE antibody is mainly responsible for the allergic bronchial responses in most cases. The detailed investigation of PAF effects on airway resistance in rats is therefore required. In the present study, the inhalation technique for small animals previously described (10) was used. Inhalation can deliver agents to the airways efficiently with minimal systemic distribution. It was found that PAF has a potent bronchoconstrictor effect in rats. The threshold concentration that showed a significant airway constriction was 0.0001% (1 μg/ml, about 2×10⁻⁶ M). The bronchoconstrictor potency of PAF was about 10 times higher than that of ACh and much higher than that of histamine. In rhesus monkeys, challenges with aerosol of 1–100 μg/ml PAF (15) resulted in an increase in pulmonary resistance. In normal subjects, the effective dose of PAF for causing an increase in pulmonary resistance was 0.5 μg/ml (8, 9). Therefore, the sensitivity of rat airway tissues to PAF seems to be roughly equivalent to those of monkeys and humans.

![Graph](image-url)
Tachyphylaxis develops to the bronchoconstrictor responses to PAF in guinea pigs (3) and humans (8, 9). In the present study, a marked tachyphylaxis to the PAF-induced bronchoconstriction was observed in rats. The mechanism of bronchoconstriction by PAF is still unclear. From some in vitro studies using isolated airway tissues, PAF has no or very weak direct contractile effect (5, 16, 17). Thus, the in vivo bronchomotor effects probably seems to result from the release of certain substances such as thromboxane A2 by platelets and other cells (18). Tachyphylaxis to the PAF-induced bronchoconstriction may be explained by a possible mechanism secondary to the release of other mediator(s).

A marked hypotension was observed even if PAF was inhaled into the airways. The potency of the hypotensive activity of PAF was about one thousand times higher than those of ACh and histamine. It is well-known that PAF causes a strong and sustained hypotension when injected i.v. in guinea pigs (3, 6), rats (19, 20), dogs (12) and rabbits (21). PAF may be easily absorbed through the airway mucosa into the circulation. In rat DNP-Ascaris antigen-induced bronchial asthma, inhalation of the antigen provokes a negligible effect on systemic blood pressure (10), although similar bronchoconstriction is observed. Thus, the systemic effect of inhaled PAF is different from that of inhaled antigen.

PAF-induced bronchial hyperreactivity has been recently reported in guinea pigs (22, 23) and humans (8, 24). In these reports, the airways were exposed to PAF more than one day prior to methacholine inhalation, resulting in sustained hyperreactivity which lasts for 1 to 4 weeks. As shown in the present study, concomitant administrations of PAF and other bronchoconstrictor agents caused no hyperreactivity. Therefore, the reported PAF-induced bronchial hyperreactivity might be due to a chronic effect of PAF such as airway inflammation.

The results of the present study show the following properties of PAF: 1) PAF is a strong bronchoconstrictor in rats. 2) Tachyphylaxis develops to its bronchoconstrictor effect. 3) It causes a marked decrease in systemic blood pressure. 4) It has no potentiating activity on other bronchoconstrictors when it is administered acutely. Thus, it is suggested that PAF is not a chief chemical mediator of bronchial asthma and a cause of airway hyperreactivity in asthma.

References

1 Benveniste, J., Henson, P.M. and Cochrane, C.G.: Leukocyte dependent histamine release from rabbit platelets: The role of IgE, basophilic and platelet activating factor. J. Exp. Med. 136, 1356–1377 (1972)
2 Vargaftig, B.B. and Benveniste, J.: Platelet-activating factor today. Trends Pharmacol. Sci. 4, 341–343 (1983)
3 Vargaftig, B.B., Lefort, J., Chignard, M. and Benveniste, J.: Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. Eur. J. Pharmacol. 65, 185–192 (1980)
4 Chignard, M., Wal, F., Lefort, J. and Vargaftig, B.B.: Inhibition by sulphinpyrazone of the platelet-dependent bronchoconstriction due to platelet-activating factor (PAF-acether) in the guinea pig. Eur. J. Pharmacol. 78, 71–79 (1982)
5 Bonnet, J., Thibaudeau, D. and Bessin, P.: Dependency of the PAF-acether induced bronchospasm on the lipoxygenase pathway in the guinea-pig. Prostaglandins 26, 457–466 (1983)
6 Berti, F., Ferri, V., Pallavicini, M., Pretolani, M., Tremoli, E., Valoti, E. and Villa, L.: Cardiovascular and pulmonary activity of platelet-activating factor (PAF-acether) and its derivatives. Pharmacol. Res. Commun. 18, 557–562 (1986)
7 Patterson, R. and Harris, K.E.: The activity of aerosolized and intracutaneous synthetic platelet activating factor (AGEPC) in rhesus monkeys with IgE-mediated airway responses and normal monkeys. J. Lab. Clin. Med. 102, 933–938 (1983)
8 Cuss, F.M., Dixon, C.M. and Barnes, P.J.: Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. Lancet II, 189–192 (1986)
9 Rubin, A.E., Smith, L.J. and Patterson, R.: Effect of platelet activating factor (PAF) on normal human airways. Am. Rev. Respir. Dis. 133, A91 (1986)
10 Misawa, M., Takenouchi, K., Abiru, T., Yoshino, Y. and Yanaura, S.: Strain difference in an allergic asthma model in rats. Japan. J. Pharmacol. 45, 63–68 (1987)
11 Konzett, H. and Rössler, R.: Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. Arch. Exp. Pathol. Pharmacol. 195, 71–74 (1940)
12 Braquet, P., Touqui, L., Shen, T.Y. and Vargaftig,
B.B.: Perspectives in platelet-activating factor research. Pharmacol. Rev. 39, 97–110 (1987)

13 Mencia-Huerta, J.M., Lewis, R.A., Razin, E. and Austen, K.F.: Antigen-initiated release of platelet-activating factor (PAF-acether) from mouse bone marrow-derived mast cells sensitized with monoclonal IgE. J. Immunol. 131, 2958–2964 (1983)

14 Chung, K.F., Aizawa, H., Leikauf, G.D., Ueki, I.F., Evans, T.W. and Nadel, J.A.: Airway hyper-responsiveness induced by platelet-activating factor: Role of thromboxane generation. J. Pharmacol. Exp. Ther. 236, 580–584 (1986)

15 Patterson, R., Harris, K.E., Lee, M.L. and Houlihan, W.J.: Inhibition of rhesus monkey airway and cutaneous responses to platelet-activating factor (PAF) (AGEPC) with the anti-PAF agent SRI 63-072. Int. Arch. Allergy Appl. Immunol. 81, 265–268 (1986)

16 Prancan, A., Lefort, J., Barton, M. and Vargaftig, B.B.: Relaxation of the guinea pig trachea induced by platelet activating factor and by serotonin. Eur. J. Pharmacol. 80, 29–35 (1982)

17 Smith, P.F., Palmer, J.D., Holmes, T., Cutcher, A., Dunn, A.M. and Halonen, M.: The responsiveness of rabbit bronchial rings to antigen, AGEPC and histamine. Immunopharmacology 12, 89–96 (1986)

18 Hamasaki, Y., Tai, H.H. and Said, S.I.: Synthesized platelet activating factor (AGEPC) stimulates the production of TXA2, which partially mediates airway and pulmonary vascular constriction on guinea pigs. Kokyu To Junkan 31, 755–761 (1983) (Abs. in English)

19 Sybertz, E.J., Sabin, C., Baum, T., Eynon, E., Nelson, S. and Moran, R.: Studies on the interactions of acetyl glycerol ether phosphorylcholine with the sympathetic nervous system in rats. J. Pharmacol. Exp. Ther. 223, 594–598 (1982)

20 Baranes, J., Hellegouarch, A., Le Hegarat, H., Viossat, I., Auguet, M., Chabrier, P.E. and Braquet, P.: The effects of PAF-acether on the cardiovascular system and their inhibition by a new highly specific PAF-acether receptor antagonist BN 52021. Pharmacol. Res. Commun. 18, 717–737 (1986)

21 McManus, L.M., Hanahan, D.J., Demopoulos, C.A. and Pinckard, R.N.: Pathobiology of the intravenous infusion of acetyl glyceryl ether phosphorylcholine (AGEPC) a synthetic platelet-activating factor (PAF), in the rabbit. J. Immunol. 124, 2919–2924 (1980)

22 Page, C.P., Archer, C.B., Paul, W. and Morley, J.: PAF-acether: a mediator of inflammation and asthma. Trends Pharmacol. Sci. 5, 239–241 (1984)

23 Page, C.P., Paul, W., Dewar, A., Wood, L., Basran, G.S. and Morley, J.: PAF-acether: a putative mediator of asthma and inflammation. Agents Actions 13, 177–183 (1983)

24 Barnes, P.J. and Chung, K.F.: PAF closely mimics pathology of asthma. Trends Pharmacol. Sci. 8, 285–287 (1987)