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11. Concentrations of CH_4 fell rapidly, but some of the deficit was made up by a sharp increase toward the latter part of the cycle. Such an effect may be caused by transport anomalies. The remaining deficit that persisted after the ENSO event was caused by either a greater removal or a lesser production of CH_4 during ENSO. Greater removal is a likely explanation; however, ENSO events also affect temperature and rainfall patterns in the Northern Hemisphere that may change microbial processes in soils and other ecosystems where CH_4 is produced naturally. These effects may have contributed to the observed deficit. Data from Alaska, Hawaii, Samoa, and Tasmania are more than 100 times less frequent than those from Cape Meares. A preliminary analysis shows that the change in concentration was largest at Cape Meares and Alaska, but the effect occurred at all sites.
12. The data are represented by a piecewise linear model. The record is divided into four periods or segments over which the trend of CH_4 is represented by the linear function $C_l = A_1 + B_1t$, where C_l is the concentration of CH_4 in the Ith period and t is time. The first segment (I) consists of 20 months and is followed by second period (II) of another 21 months of slower growth. The third period (III) spans the ENSO event of 1982–83 and is made up of two segments: a phase of rapidly decreasing CH_4 (IIIa) followed by a rapid increase (IIIb). The last period (IV) spans the 22 months after the event. A_1 and B_1 for the first and last periods are estimated by least-squares techniques. For the second period the starting concentration is fixed by the endpoint of the linear function describing the first period. Therefore, only one degree of freedom is used to estimate the slope. Segment IIIa is treated similarly. For segment IIIb the last point of the equation for segment IIIa and the first point of the equation for segment IV are joined. The rates of change in atmospheric CH_4 during the four periods are summarized in Table 1. The average increase in CH_4 is estimated by $b = \Sigma\ w_ib_i$ and $w_i = \delta T_i/\Sigma\delta T_i$, where b_i is the slope in each of the segments I, II, IIIa, IIIb, and IV and T_i is the time over which these rates of change are estimated. The uncertainty of the overall changes in CH_4 that have occurred over the past 6 years may be estimated by propagating the errors so that $S_b^- = [\Sigma\ (w_i)^2\ S_{bi}^2]^{1/2}$. By this method the 90 percent confidence limits of the average increase (16 ppbv per year) are ±3 ppbv per year for the entire 6-year period, so that the total increase was 96 ± 18 ppbv. The rates of increase varied considerably from year to year (Table 1). When the trends are estimated by linear least-squares methods over each 12-month period except for the year of the ENSO event the average of the five yearly rates of increase is 20 ± 10 ppbv. Since observations have been obtained over only one event, an uncertainty of the rate of change during ENSO years cannot be estimated. While the tendency of CH_4 to increase can be explained by growing anthropogenic contributions to the global sources and weakening of the removal processes, these processes are probably changing systematically so that the mean trends and statistical uncertainties in the present record may not be sufficient to allow accurate predictions about CH_4 in the distant future, when perceptible environmental effects are expected.
13. We thank P. Turner and D. Joseph for data management and computer calculations; S. Crawford and D. Stearns for field and laboratory work; E. M. Rasmusson and C. F. Ropelewski for supplying data on the SOI; and R. Gammon for data on CO_2, Fig. 2c, and many discussions. Supported in part by NSF grant ATM-8414020 and by the resources of the Biospherics Research Corporation and the Andarz Company.

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Role of Platelet-Activating Factor–Acether in Mediating Guinea Pig Anaphylaxis

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The pathophysiology of anaphylaxis is very complex, and the sequelae of events are not fully explained in terms of the effects of histamine and peptide leukotrienes alone. Platelet-activating factor (1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, PAF-acether) has been detected in animals undergoing anaphylaxis. Injection of synthetic PAF-acether induces similar effects, including bronchoconstriction, respiratory arrest, systemic hypotension, neutropenia, and thrombocytopenia. The results reported here demonstrate that the histamine- and leukotriene-independent component of guinea pig anaphylaxis in vivo and in isolated lung parenchymal strips in vitro is mediated by PAF-acether. However, PAF-acether is not responsible for the anaphylaxis-induced thrombocytopenia.

A VARIETY OF HUMORAL MEDIATORS, including histamine and the leukotrienes, are involved in the pathogenesis of anaphylaxis in the guinea pig (*1*, *2*). The precise role of these mediators and their interaction with one another have not been well defined. Moreover, the role of platelet-activating factor–acether in mediating some of the components of the anaphylaxis has not been clarified (*3*–*6*). We therefore set out to investigate the temporal sequence and role of histamine, PAF-acether, and leukotrienes in the pulmonary response to immunochallenge in guinea pig anaphylaxis. We found that histamine is responsible for the early component, PAF acether for the middle component, and leukotrienes for the late component of pulmonary anaphylaxis in pulmonary parenchymal strips. All three mediators must be blocked if the bronchoconstrictor response is to be totally eliminated.

Male Hartley guinea pigs (500 to 700 g) were sensitized by the injection of ovalbumin (OA) (100 mg intraperitoneally and intramuscularly) 2 to 3 weeks before immunologic challenge. For in vitro studies of the responsiveness of airway smooth muscles, we prepared lung parenchymal strips (20 mm long and 3 mm in diameter) from the outer segments of the upper lobes of the lung. The strips were transferred to a water-jacketed organ bath (20 ml) preloaded with 1 g of resting force containing oxygenated (95 percent O_2 + 5 percent CO_2) Krebs-Henseleit buffer. We measured changes in smooth muscle tone isometrically as developed force (in milligrams), using Grass FT-03 force-displacement transducers and a Grass model 7 oscillographic recorder. For the in vivo experiments, sensitized guinea pigs were anesthetized with pentobarbital (35 mg/kg). A catheter was introduced into the left carotid artery for measurement of arterial blood pressure, and a special double-lumen cannula was inserted into the distal jugular vein. This cannula allowed continuous withdrawal of peripheral venous blood (85 μl/min) for measurement of the platelet count with a Technicon Autocounter. We prevented blood coagulation by adding sodium citrate (3.8 percent; 1:7 by volume), without allowing citrate to flow into the animal (*7*). Another catheter was inserted into the jugular vein for injection of drugs or their respective vehicles. Airway pressure was measured with a Statham differential gas-pressure transducer connected to a tracheal cannula while the animals breathed spontaneously. All values presented in this study are means ± SEM. All statistical comparisons were made by t test and confirmed by analysis of variance (ANOVA).

Isolated guinea pig lung parenchymal strips challenged by the addition to the bath fluid of OA (2 μg/ml) showed an increase in force of 184 ± 16 mg (n = 38). This increased force persisted for more than 1 hour. Although the amplitude of the OA-induced increases in force showed considerable individual variation, the responses of the four strips taken from each animal were approximately equal. For this reason, the results are given either in absolute values or as a percentage of the responses of control strips taken from the same animal. Addition to the bath of the antihistamine diphenhydramine (30 μ*M*) or of BW-755c (30 μ*M*), the dual inhibitor of arachidonic acid lipoxygenase and cyclooxygenase pathways, prior to the addition of OA did not significantly alter the increase in force (178 ± 19 and 194 ± 28 mg for ten and eight strips, respectively). When both inhibitors were added together,

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the response to OA was significantly reduced to 64 ± 14 mg ($P < 0.02$ versus vehicle). Addition of the PAF-receptor antagonist kadsurenone (400 μ*M*) (*8*) also significantly reduced the increase in force ($P < 0.05$) to 94 ± 15 mg ($n = 7$). However, when all three drugs were added together at these respective concentrations, the contractile response to OA was totally abolished (3 ± 3 mg; $n = 7$) ($P < 0.001$) (Fig. 1). Total antagonism of the OA receptor also occurred when the structurally dissimilar PAF-receptor antagonist alprazolam (*9*) was included instead of kadsurenone in the triple drug combination (13 ± 4 mg; $n = 6$).

These results support the concept that PAF-acether, in addition to histamine and the peptide leukotrienes, is involved in the contraction of guinea pig small airways during anaphylaxis. Since simultaneous antagonism of histamine and PAF receptors and inhibition of eicosanoid formation were required to totally abolish the antigen-induced contraction, it appears that histamine, peptide leukotrienes, and PAF-acether work together in pulmonary anaphylaxis. Lower concentrations of the blocking agents produced smaller degrees of antagonism of the OA contraction. Higher concentrations exerted nonspecific inhibitory effects on the contractile process, as evidenced by impaired KCl-induced contractions.

We tested the effectiveness of mediator inhibition against exogenous contractile agonists such as histamine (30 μ*M*) or PAF-acether (30 μ*M*), which induced increases in force amounting to 170 ± 16 mg ($n = 12$) and 145 ± 23 mg ($n = 5$), respectively. The contractile effects of histamine or PAF-acether were reduced by more than 95 percent each in the presence of diphenhydramine (30 μ*M*) or kadsurenone (400 μ*M*), respectively. Kadsurenone (400 μ*M*) did not significantly alter the contractions induced by histamine (30 μ*M*) or leukotriene D_4 (150 n*M*), indicating a specific antagonism of PAF receptors.

We investigated the role of PAF-acether during systemic anaphylaxis in sensitized guinea pigs in vivo. All anesthetized, nonsensitized guinea pigs survived the observation period of 30 minutes after intravenous injection of OA (0.5 mg/kg) without any alterations in arterial blood pressure, airway pressure, or continuously measured platelet count. Furthermore, when sensitized guinea pigs were injected with 0.9 percent NaCl as the vehicle for OA, no statistically significant alterations in these measurements occurred. In contrast, sensitized animals given an injection of OA (0.5 mg/kg) experienced severe coughing and developed respiratory arrest within 2 minutes. The mean arterial blood pressure of these animals increased transiently from 85 ± 3 to 112 ± 5 mmHg ($P < 0.01$) about 2 to 3 minutes after the injection of OA. Soon after this hyperdynamic phase, mean arterial blood pressure fell abruptly to about 5 mmHg and the peripheral platelet count declined by 70 ± 8 percent of the initial value within approximately 5 minutes (Fig. 2). The animals died within 4 to 6 minutes. The decrease in circulating platelet count was independent of the hypoxemia caused by respiratory arrest. We tested this by injecting another dose of pentobarbital (30 mg/kg) into anesthetized guinea pigs, which induced respiratory depression leading to respiratory arrest. The peripheral platelet count in these animals was unaltered for more than 20 minutes after respiratory arrest, and the arterial blood pressure declined slowly without the occurrence of a hyperdynamic phase.

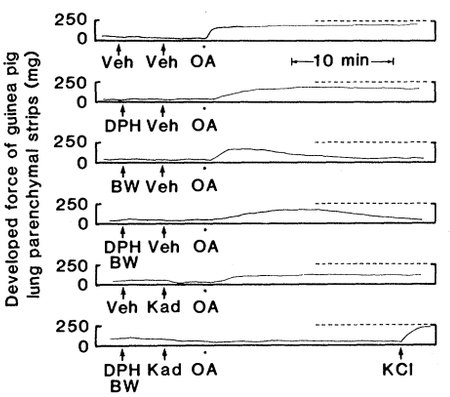
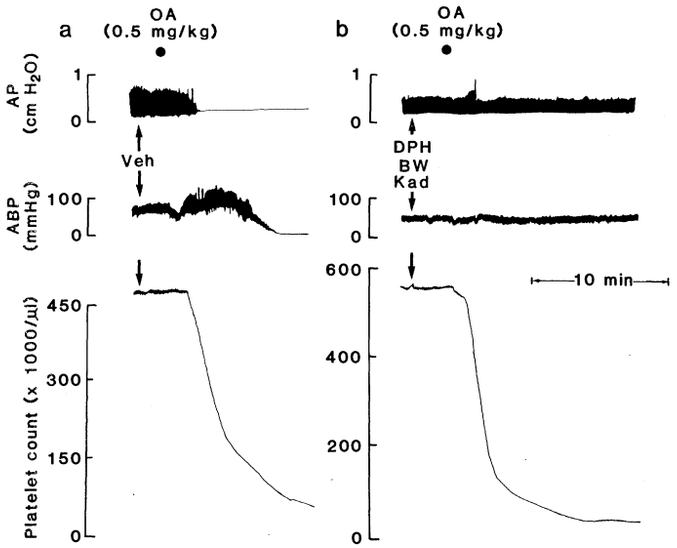


Fig. 1. Development of force by isolated lung parenchymal strips from sensitized guinea pigs in response to the administration of ovalbumin (OA; 2 μg/ml). The rapid increase in developed force induced by OA persisted for more than 1 hour (only 20 minutes of the record is shown) in strips with the vehicle (Veh). The vehicle for all drugs except kadsurenone was 0.9 percent NaCl; for kadsurenone, the vehicle in vitro was dimethyl sulfoxide (0.1 percent) diluted in 0.9 percent NaCl. Pretreatment of the strips with the antihistamine diphenhydramine (DPH; 30 μ*M*) delayed the initial increase but did not alter the resulting force developed. BW-775c (BW; 30 μ*M*), the dual inhibitor of lipoxygenase and cyclooxygenase product formation, did not influence the initial phase of the force development but reduced the duration of contraction significantly. Pretreatment with DPH (30 μ*M*) and BW (30 μ*M*) delayed the initial increase in force and also reduced the duration of the response. Administration of the PAF-receptor antagonist kadsurenone (Kad; 400 μ*M*) led to a slight relaxation of the lung parenchymal strip and reduced the OA-induced increase in force during the initial and the late phase. Pretreatment of the strips with DPH (30 μ*M*), BW (30 μ*M*), and Kad (400 μ*M*) totally abolished the OA-induced increases in force without reducing the effect of KCl (50 m*M*), which gave 90 to 100 percent of the response observed in untreated strips. Use of either of the drugs alone or in combination at these concentrations did not inhibit the contractions of the strips induced by KCl. We tested for this at the end of each experiment to exclude nonspecific inhibitory drug effects interfering with the contractile process. BW-755c (30 μ*M*) inhibited thromboxane B_2 and leukotriene C_4 and D_4 concentrations by >90 percent in response to arachidonic acid or calcium ionophore (A-23187) stimulation.

Pretreatment of sensitized guinea pigs with diphenhydramine (200 μg/kg) or BW-755c (2 mg/kg) or the PAF-antagonist alprazolam (5 mg/kg) did not change the fatal outcome or the sequelae of anaphylaxis. Furthermore, addition of diphenhydramine together with BW-755c in three sensitized guinea pigs did not significantly protect against OA-induced anaphylaxis. In contrast, combined pretreatment with all three drugs at these doses before OA challenge markedly improved survival time. Four out of six guinea pigs survived the entire 30-minute observation period, whereas the other two exhibited a prolonged survival time of 16 and 20 minutes, respectively. Respiratory arrest did not occur in any of the treated animals, nor did the characteristic blood pressure decline followed by circulatory failure occur. Despite these beneficial respiratory and blood pressure effects, platelet count decreased by 77 ± 14 percent ($n = 6$) compared to 70 ± 8 percent in animals treated with 0.9 percent NaCl. When kadsurenone (60 mg/kg) was used in the triple combination instead of alprazolam, two out of three animals survived and the third animal showed a prolonged survival time (26 minutes). Moreover, kadsurenone did not prevent the decrease in platelet count observed upon OA challenge.

These data indicate that histamine, eicosanoids (for example, peptide leukotrienes), and PAF-acether are the primary mediators responsible for bronchoconstriction and circulatory failure in anaphylaxis but do not account for the acute thrombocytopenia. We tested the ability of kadsurenone to antagonize PAF effects in vivo after injection of synthetic PAF-acether (100 ng/kg). Injection of PAF-acether was followed by a decrease in expiratory airway pressure, indicating peripheral bronchoconstriction. We observed a decrease in the arterial blood pressure (by about 25 mmHg) which persisted for approximately 5 to 8 minutes and a marked decrease in platelet count, without complete recovery (Fig. 3). Injection of PAF-acether 20 to 30 minutes later into guinea pigs receiving only the vehicle for kadsurenone (polyethylene glycol 400; 500 μl) resulted in similar circulatory and pulmonary effects and in a further decrease in peripheral platelet count. In contrast, when kadsurenone (60 mg/kg) was given 10 minutes prior to the PAF-acether injection, the deleterious effects on airway pressure and



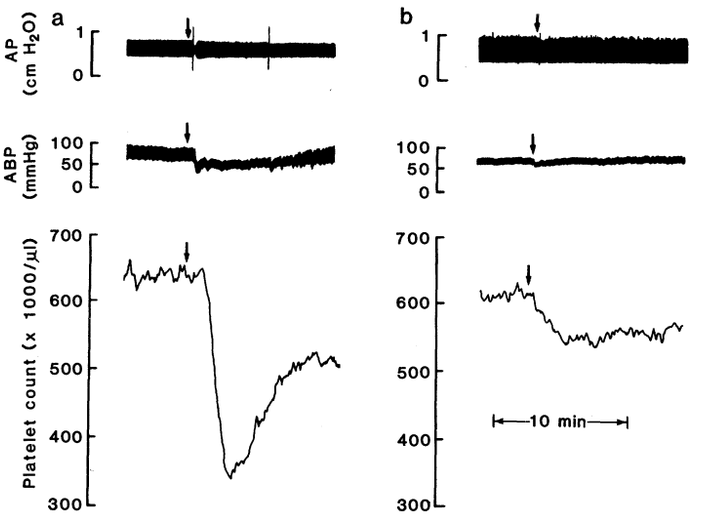


Fig. 2 (left). (a) Airway pressure (AP), arterial blood pressure (ABP), and continuously measured peripheral platelet count of anesthetized, sensitized guinea pigs. Intravenous injection of antigen (OA) (at the filled circle) resulted in coughing and the development of respiratory arrest in vehicle-treated (Veh) animals. The vehicle was a mixture of 0.9 percent NaCl (500 μl) and polyethylene glycol 400 (500 μl). ABP fell to zero after a transient hyperdynamic phase. Peripheral platelet count decreased by 85 percent from the initial value. (b) When sensitized animals were pretreated with diphenhydramine (DPH; 200 μg/kg), BW-755c (BW; 2 mg/kg), and kadsurenone (Kad; 60 mg/kg) before injection of OA, the respiratory arrest and ABP changes did not occur. However, the decrease in platelet count was comparable to that of Veh-treated animals and did not recover within 1 hour after antigen challenge. Fig. 3 (right). Effects of injections of synthetic PAF-acether (100 ng/kg; at the arrow) into guinea pigs pretreated with polyethylene glycol 400 (500 μl) (a) or kadsurenone (60 mg/kg) (b). In vehicle-treated animals PAF-acether injection resulted in a transient decrease in airway pressure (AP) and arterial blood pressure (ABP). Peripheral platelet count showed a marked decrease with only incomplete recovery. Injection of PAF into another guinea pig pretreated with kadsurenone without prior exposure to PAF-acether induced only moderate alterations in AP and ABP and only a modest decrease in platelet count.

arterial blood pressure were largely abolished and the decrease in platelet count was totally prevented (Fig. 3). These results demonstrate that kadsurenone in vivo acts as an effective PAF-acether antagonist, inhibiting the PAF-induced thrombocytopenia in guinea pigs. Therefore, it is highly unlikely that endogenously formed PAF-acether is responsible for the decrease in platelet count observed during anaphylaxis.

These in vivo and in vitro data provide evidence that PAF-acether is an important mediator in anaphylactic shock in guinea pigs, although it is not the only mediator involved, as indicated by the ineffectiveness with which single treatment with kadsurenone or alprazolam inhibited the anaphylactic response. Adams and Lichtenstein (*10*) ascertained the degree of leukotriene involvement in the OA-induced contraction of isolated guinea pig tracheal strips in vitro. The remaining contraction was not accounted for by histamine, leukotrienes, or cyclooxygenase products of arachidonic acid. We were able to confirm their findings in our preparation of guinea pig lung parenchymal strips. The initial phase of the OA-induced increase in force in parenchymal strips is inhibited by diphenhydramine and thus appears to be mediated by histamine. Antagonism of leukotriene effects by the leukotriene receptor antagonist FPL-55,712 or inhibition of eicosanoid formation by BW-755c reduced the amplitude and duration of the late contraction. We preferred to use the dual eicosanoid inhibitor BW-755c instead of FPL-55,712 in most of our experiments because the latter is primarily selective for leukotriene receptors (*11*) and exogenous leukotrienes induced the release of thromboxane A_2 in our in vitro preparations. Because BW-755c inhibited the release of leukotrienes and any auxiliary cyclooxygenase products simultaneously, it essentially isolated all the major eicosanoids from the anaphylactic response.

The availability of two recently characterized PAF-receptor antagonists enabled us to explore the role of PAF-acether in the airway contraction in vitro and in systemic anaphylaxis. Release of endogenously formed PAF-acether appears to be responsible for the increase in force in isolated strips of small guinea pig airways after the effects of histamine and eicosanoid have been eliminated. In guinea pigs, in vivo antagonism of histamine responses and inhibition of eicosanoid formation do not provide sufficient protection from anaphylactic shock leading to respiratory arrest and cardiac failure. However, additional treatment with PAF-receptor antagonists abolished most of the pulmonary and cardiovascular effects of the immediate immune reaction and improved the survival rate from zero to six out of nine animals and prolonged the survival time of the three nonsurvivors. Interestingly, the marked decrease in peripheral platelet count during anaphylaxis was not influenced by PAF-receptor antagonism, although the same dose of kadsurenone inhibited the effects of exogenous PAF-acether on in vivo platelet count. Other mediators such as adenine nucleotides or complement components such as anaphylatoxins may be responsible for the anaphylaxis-induced thrombocytopenia.

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