

PAF, rather than Histamine, Participates in Mouse Anaphylactic Hypotension

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Key Words

Anaphylactic shock · Platelet-activating factor · Histamine · Hepatic circulation, antigen, portal venous pressure, mouse

Abstract

We determined the roles of platelet-activating factor (PAF) and histamine in anaphylactic hypotension in ovalbumin-sensitized anesthetized BALB/c mice. The effects of PAF and histamine on hemodynamic variables were studied by measuring the systemic arterial (Psa), portal venous (Ppv) and central venous (Pcv) pressures. Intravenous PAF evoked a biphasic Psa response, an initial rapid and transient drop followed by marked hypotension, accompanied by a decrease in Pcv. Histamine caused only mild systemic hypotension. Both agents similarly increased Ppv by approximately 4 cm H₂O at high doses. After an injection of antigen, Psa initially increased slightly and then decreased from the baseline of 94 ± 1 mm Hg to 46 ± 1 mm Hg at 10 min after antigen administration, with Pcv decreasing by 2.5 cm H₂O. Ppv increased by 3.5 cm H₂O at 5 min after antigen injection. Pretreatment with either CV-6209 (PAF receptor antagonist, 1 mg/kg) or diphenhydramine (histamine H₁ receptor antagonist, 20 mg/kg) significantly attenuated an antigen-induced decrease in Psa. The inhibitory action of CV-6209 was greater than that of diphenhydramine, and the combination of these 2 antagonists almost completely inhibited the anaphylactic hypotension. In contrast, the antigen-induced

increase in Ppv was attenuated by CV-6209 alone but augmented by diphenhydramine. It is concluded that anaphylactic hypotension is mainly mediated by PAF and, to a lesser extent, by histamine in anesthetized BALB/c mice.

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Introduction

Anaphylactic hypotension is sometimes a life-threatening allergic disorder, but the hemodynamic mechanism of hypotension is not fully understood [1]. Anaphylactic models in mice could be beneficial because of the development of genetic engineering. Anaphylaxis in humans is the classic well-established immunoglobulin-E-dependent reaction, in which mast cells and histamine are involved [2]. On the other hand, it was recently suggested that an alternative pathway involving IgG, Fc- γ RIII, macrophages and platelet-activating factor (PAF) is more important in the anaphylactic response to antigen challenge in mice [3, 4]. However, the role of histamine or PAF in the mouse anaphylactic hypotension has not been systematically evaluated, despite a study using the genetically PAF-receptor-deficient mice [5].

We have proposed that the liver and splanchnic vascular beds are involved in anaphylactic hypotension [6–8] and recently confirmed their importance in the pathogenesis of mouse anaphylactic hypotension [8]. Thus, us-

ing a PAF receptor antagonist, CV-6209, and a nonspecific histamine H₁ receptor antagonist, diphenhydramine (DPH), we determined the roles of PAF and histamine in both systemic and hepatic circulation during anaphylactic hypotension in anesthetized BALB/c mice. Initially, the effect of PAF or histamine itself on hemodynamic variables was evaluated.

Materials and Methods

Animals

The experiments of the present study were approved by the Animal Research Committee of Kanazawa Medical University. Eighty-two male BALB/c mice (SLC, Shizuoka, Japan) weighing 25 ± 1 g were used. The mice were maintained at 23°C and under pathogen-free conditions on a 12:12-hour dark/light cycle, with food and water ad libitum.

Sensitization

The mice were actively sensitized by the subcutaneous injection of an emulsion made by mixing aluminum potassium sulfate adjuvant (2 mg) with 0.01 mg ovalbumin (grade V, Sigma) dissolved in physiological saline (0.2 ml) [8]. The antigen emulsion was injected again 1 week after the first antigen injection. One week after the second injection, the mice were used for the following experiments.

Protocol

The mice were anesthetized with pentobarbital sodium (90 mg/kg, i.p.) and placed on a thermostatically controlled heating pad (ATC-101B; Unique Medical, Japan) to maintain the body temperature at $37 \pm 0.2^\circ\text{C}$ throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during tail pinch. Supplemental doses of anesthetic (10% of the initial dose) were given intraperitoneally if necessary. To ensure airway patency, tracheotomy followed by insertion of a tracheal tube (18G stainless steel needle) was performed. The right femoral artery was catheterized to measure the systemic arterial pressure (Psa) with a transducer (TP-400T, Nihon Kohden, Japan). The right external jugular vein was catheterized with a 0.67-mm OD, 0.47-mm ID polyethylene catheter. The catheter tip was positioned at the confluence of the superior vena cava and the right atrium. This catheter was connected to a Y-type miniature plastic tube, one twig end of which was connected via a water-filled polyethylene tube to the pressure transducer for measurement of the central venous pressure (Pcv), and another twig end was used to introduce a thin inner polyethylene tube, which was tapered to ~ 0.3 mm in diameter over hot air, for an intravenous injection of drugs or antigen [8]. Following a laparotomy, a catheter (ID = 0.47 mm, OD = 0.67 mm) was inserted into the main portal vein for continuous measurement of the portal venous pressure (Ppv). The heart rate (HR) was determined by triggering the R wave of the electrocardiogram. The Psa, Pcv, Ppv and HR were continuously measured and displayed on a thermal physiograph (RMP-6008, Nihon Kohden, Japan). Outputs were also digitally recorded at 20 Hz with PowerLab (AD Instruments, Sydney, Australia).

The following experimental protocols were employed:

(1) The effects of PAF or histamine itself on hemodynamic variables: PAF (1.7–340 pmol; 25 μl ; $n = 24$) or histamine (0.2–1.4 μmol ; 25 μl ; $n = 18$) was intravenously administered.

(2) The effect of inhibitors of PAF and/or histamine on anaphylactic hypotension: either CV-6209 (PAF receptor antagonist, 1 mg/kg; 100 μl ; $n = 10$) or DPH (histamine H₁ receptor antagonist, 20 mg/kg; 100 μl ; $n = 10$) or combined CV-6209 and DPH was intravenously administered to sensitized mice. After 10 min, 0.01 mg of ovalbumin antigen (in 100 μl saline) was intravenously administered. The dose of CV-6209 or DPH employed in this study was high enough to completely block a change in Psa induced by 340 pmol of PAF and 1.4 μmol of histamine, respectively, in the anesthetized mice in our preliminary studies. All drugs were purchased from Sigma Chemical Company (St. Louis, Mo., USA).

Statistics

All results are expressed as the means $\pm$ SE. Statistical analysis was performed by repeated measures analysis of variance. Comparison of individual points within and among the groups was made by analysis of variance followed by the Bonferroni post-test correction method. Differences were considered statistically significant at p values less than 0.05.

Results

The Action of PAF or Histamine on Hemodynamic Variables

An intravenous injection of PAF evoked systemic hypotension in a dose-dependent manner, as shown in figure 1. The low doses of 1.7–17 pmol of PAF caused only a rapid and transient decrease in Psa. On the other hand, the doses higher than 170 pmol produced a biphasic hypotensive response, characterized by an initial similar drop followed by a secondary drastic and prolonged systemic hypotension: Psa decreased transiently by 30–40 mm Hg within 1.5 min after injection, which was followed by a rise to normal and then sustained a decrease to 37 ± 4 and 28 ± 2 mm Hg at 170 and 340 pmol of PAF, respectively. Ppv significantly increased from the baseline of 6.2 cm H₂O to approximately 8.8 and 10 cm H₂O after injections of PAF at doses of 1.7–17 and 170–340 pmol, respectively. Pcv increased immediately after PAF injection and then returned to the baseline. Thereafter, Pcv significantly decreased in parallel with systemic hypotension at 170–340 pmol of PAF. HR increased in a dose-dependent manner immediately after an injection of PAF.

An intravenous injection of histamine caused an initial dose-dependent elevation of Psa, as shown in figure 2. Then Psa at 0.2 μmol of histamine returned to the baseline, while it further decreased below the baseline

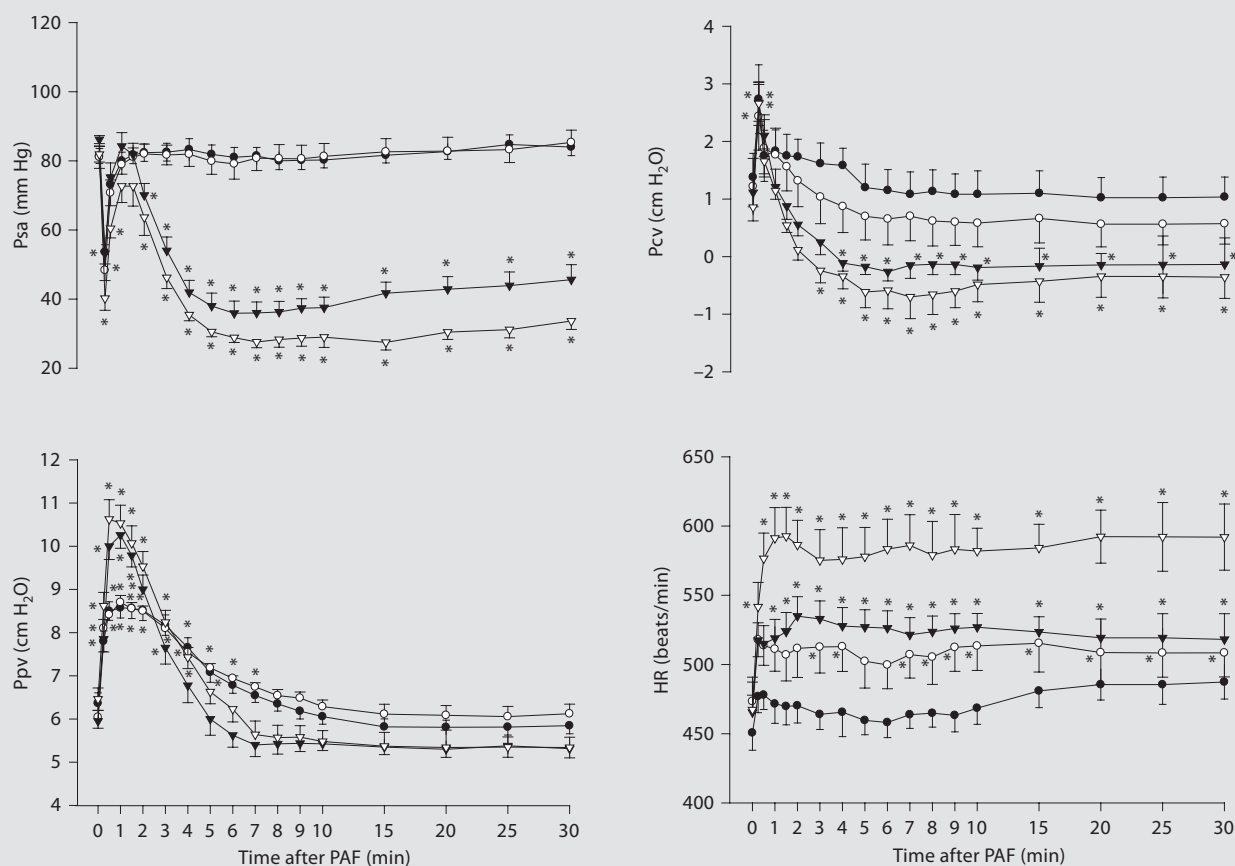


Fig. 1. Changes in Psa, Ppv, Pcv and HR after an intravenous injection of PAF. Closed circle = PAF 1.7×10^{-12} mol (n = 6); open circle = PAF 1.7×10^{-11} mol (n = 6); closed triangle = PAF 1.7×10^{-10} mol (n = 6); open triangle = PAF 3.4×10^{-10} mol (n = 6). Values are means $\pm$ SE. * p < 0.05 vs. the baseline values.

levels to the bottom of 72 ± 4 and 57 ± 4 mm Hg at 0.6 and 1.4 μ mol of histamine, respectively. After the injections of 0.2 and 0.6 μ mol of histamine, Ppv significantly increased from the baseline of 6.2 to 8.6 ± 0.2 and 9.7 ± 0.3 cm H₂O, respectively. However, the Ppv peak level (10.2 ± 0.2 cm H₂O) at the highest dose of 1.4 μ mol was similar to that at 0.6 μ mol. Pcv did not decrease but transiently increased in a dose-dependent manner immediately after injection of histamine.

The Effects of CV-6209 and/or DPH on Systemic Anaphylaxis

Figure 3a shows representative examples of the response to intravenous injection of ovalbumin antigen in a sensitized mouse. Figure 4 presents the summary data for the time course of changes in Psa, Ppv, Pcv and HR.

After an antigen injection, Psa and Ppv increased in the anaphylaxis control group. Psa initially increased from the baseline of 94 ± 1 to the peak of 107 ± 2 mm Hg and then progressively decreased to the bottom of 46 ± 1 mm Hg at 10 min after antigen administration. Ppv increased from the baseline of 5.7 ± 0.1 cm H₂O to the peak of 9.2 ± 0.3 cm H₂O at 5 min after antigen injection. Immediately after antigen administration, Pcv significantly increased and then decreased from the baseline of 0.3 ± 0.1 to -2.2 ± 0.2 cm H₂O at 10 min in parallel with a decrease in Psa. HR significantly increased from the baseline of 438 ± 5 to 506 ± 9 beats/min at 5 min after antigen administration.

To examine the roles of PAF and histamine in mouse anaphylactic hypotension, the effects of pretreatment with CV-6209 (PAF receptor antagonist) and/or DPH

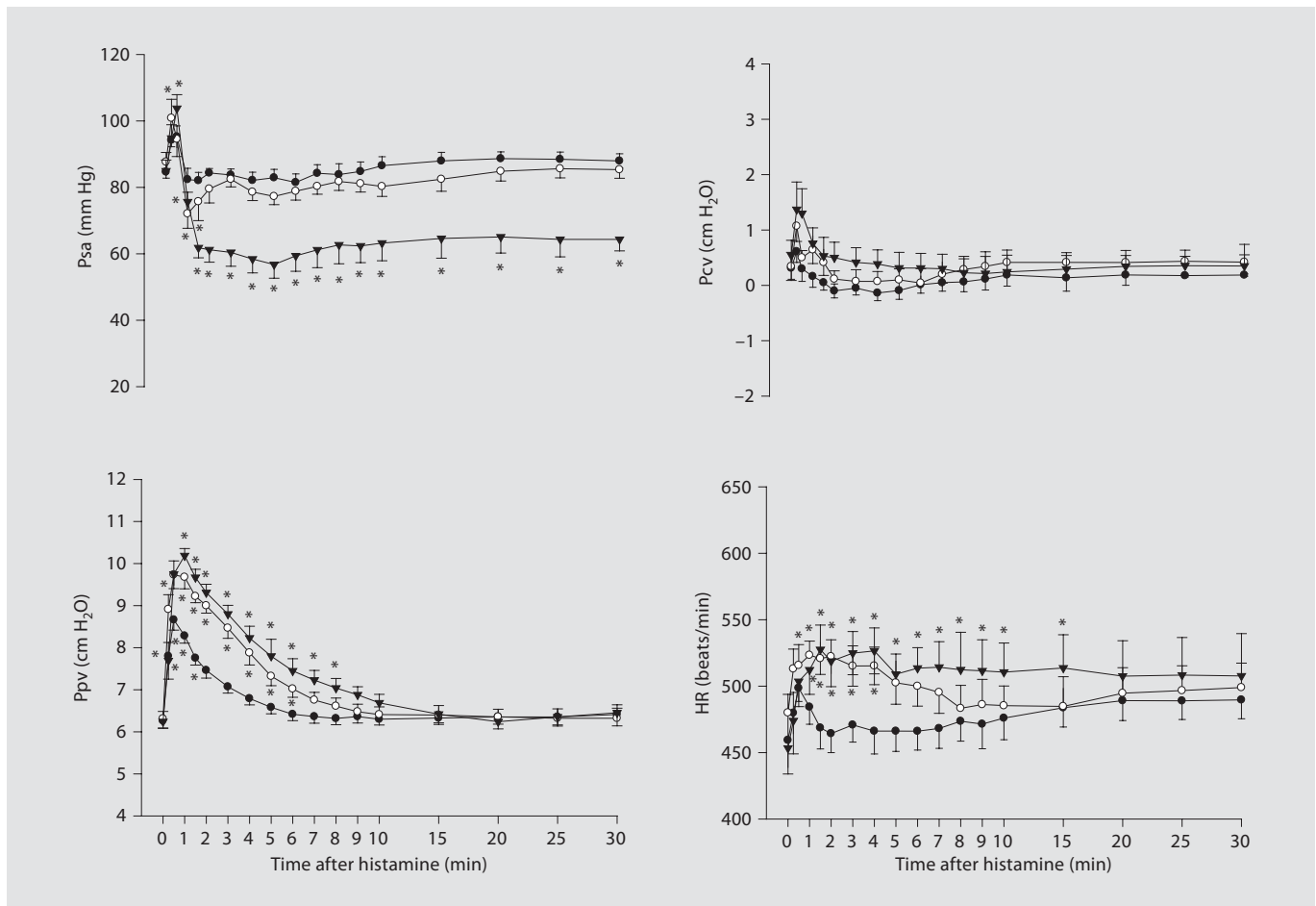


Fig. 2. Changes in Psa, Ppv, Pcv and HR after an intravenous injection of histamine. Closed circle = histamine 2×10^{-7} mol ($n = 6$); open circle = histamine 6×10^{-7} mol ($n = 6$); closed triangle = histamine 1.4×10^{-6} mol ($n = 6$). Values are means $\pm$ SE. * $p < 0.05$ vs. the baseline values.

(histamine H_1 receptor antagonist) were studied in the ovalbumin-sensitized anesthetized mice. As shown in figure 4, either CV-6209 or DPH significantly attenuated an antigen-induced decrease in Psa. The inhibitory action of CV-6209 was greater than that of DPH, and the combination of these 2 drugs almost completely prevented the anaphylactic hypotension. The antigen-induced decrease in Pcv was significantly attenuated by CV-6209 but not by DPH.

Although the antigen-induced decrease in Psa was extensively attenuated in the CV-6209 + DPH group, CV-6209 alone most effectively reduced the antigen-induced increase in Ppv, as shown in figures 3b and 4. Contrary to the Ppv increment in response to histamine (fig. 1), DPH did not attenuate but augment the increase in Ppv after antigen administration. Combined CV-209 and

DPH significantly reduced the antigen-induced increase in Ppv only at the early stage. The basal HR was significantly smaller in the mice pretreated with DPH than that in the control mice. After antigen injection HR significantly increased in all the receptor antagonist groups, but the increase was less than that in the control group, as shown in figure 4.

Discussion

The present study used pharmacological interventions to investigate the roles of PAF and histamine in anaphylactic hypotension and portal hypertension of ovalbumin-sensitized anesthetized mice. In addition, we studied the effects of PAF or histamine alone on the systemic

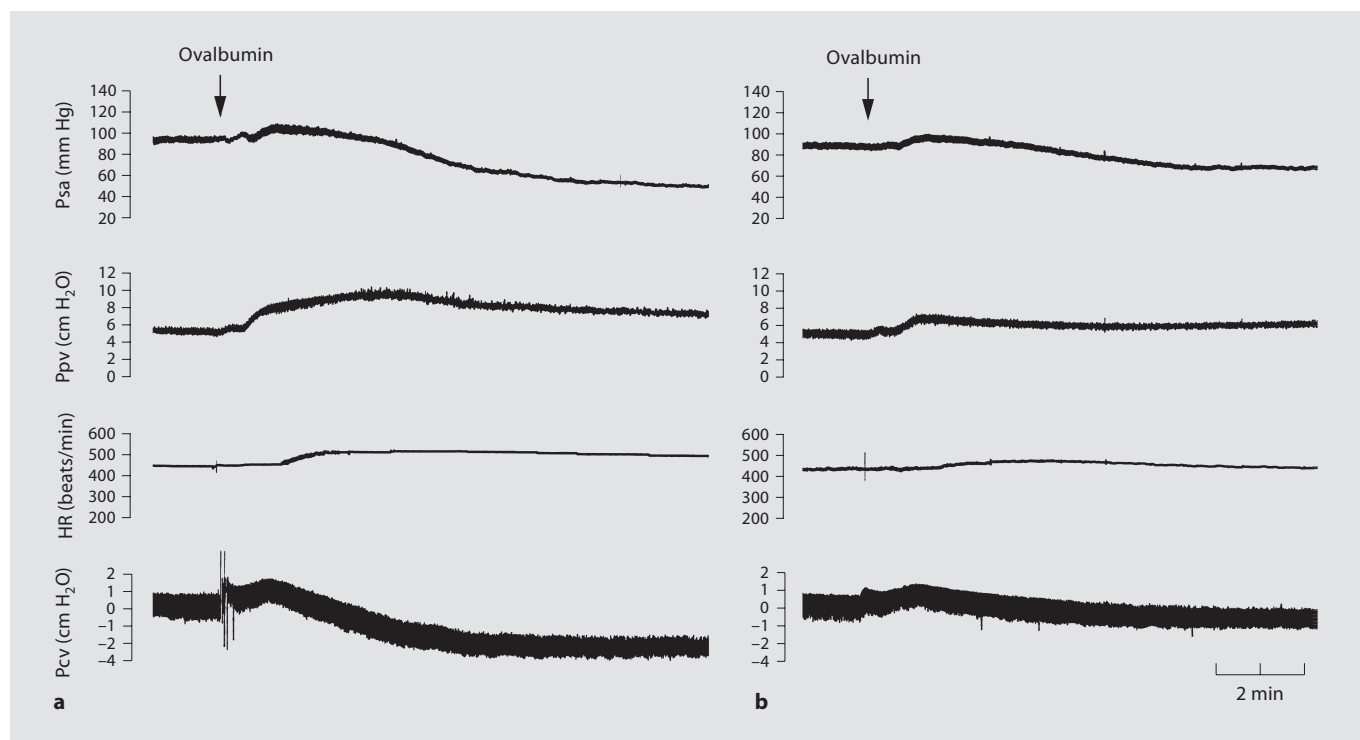


Fig. 3. Representative recordings of the response to ovalbumin antigen of an anesthetized mouse in the anaphylaxis control group (**a**) and the CV-6209 group (**b**). The arrow on each trace indicates the antigen injection.

and hepatic circulations. Our findings show that anaphylactic hypotension is mainly mediated by PAF and, to a lesser extent, by histamine in anesthetized BALB/c mice and that PAF, but not histamine, is the primary mediator for anaphylactic portal hypertension.

In an ovalbumin-sensitized anesthetized anaphylactic shock mouse model [8], the hemodynamic characteristics are the initial short-lasting systemic pressor response followed by profound systemic hypotension, which is accompanied by mild portal hypertension and a decrease in Pcv, as shown in the present study. These features of systemic anaphylaxis are quite similar to the responses to high doses of PAF (fig. 1 and 4), although an initial rise in Psa was not observed in mice challenged with PAF. In contrast, the response to histamine was different from the anaphylactic reaction in that the systemic hypotension was mild and did not accompany a decrease in Pcv. The systemic anaphylaxis in mice was substantially inhibited by the PAF receptor antagonist but not by the histamine receptor antagonist. These findings support the assumption that PAF rather than histamine is the main mediator of anaphylactic hypotension in anesthetized mice. Actually, in mice lacking a PAF receptor, anaphylactic hypotension was substantially attenuated but not abolished: Psa in the mice without a PAF receptor decreased only by 40% of the baseline level at 30 min after antigen administration, while the Psa decrease in the wild control mice was lethal [5]. In addition, during mouse systemic anaphylaxis, PAF could be released enough not only from mast cells but also from macrophages, as Finkelman's group proposed [3, 4], whereas histamine is released substantially only from mast cells. This may also support the responsibility of PAF rather than histamine for mouse anaphylaxis.

We have recently demonstrated that PAF [8] and histamine [9] could constrict the hepatic vasculature in isolated perfused mouse livers. We for the first time reported that in anesthetized mice, PAF and histamine increased Ppv, which reached the maximal levels of 10–11 cm H₂O. This PAF-induced increase in Ppv is consistent with the finding that the PAF receptor antagonist alone extensively attenuated the anaphylactic portal hypertension. However, the hepatic venoconstrictive ability of histamine is apparently contradictory to the finding that the histamine H₁ receptor antagonist did not attenuate, but rather augment, the antigen-induced increase in Ppv

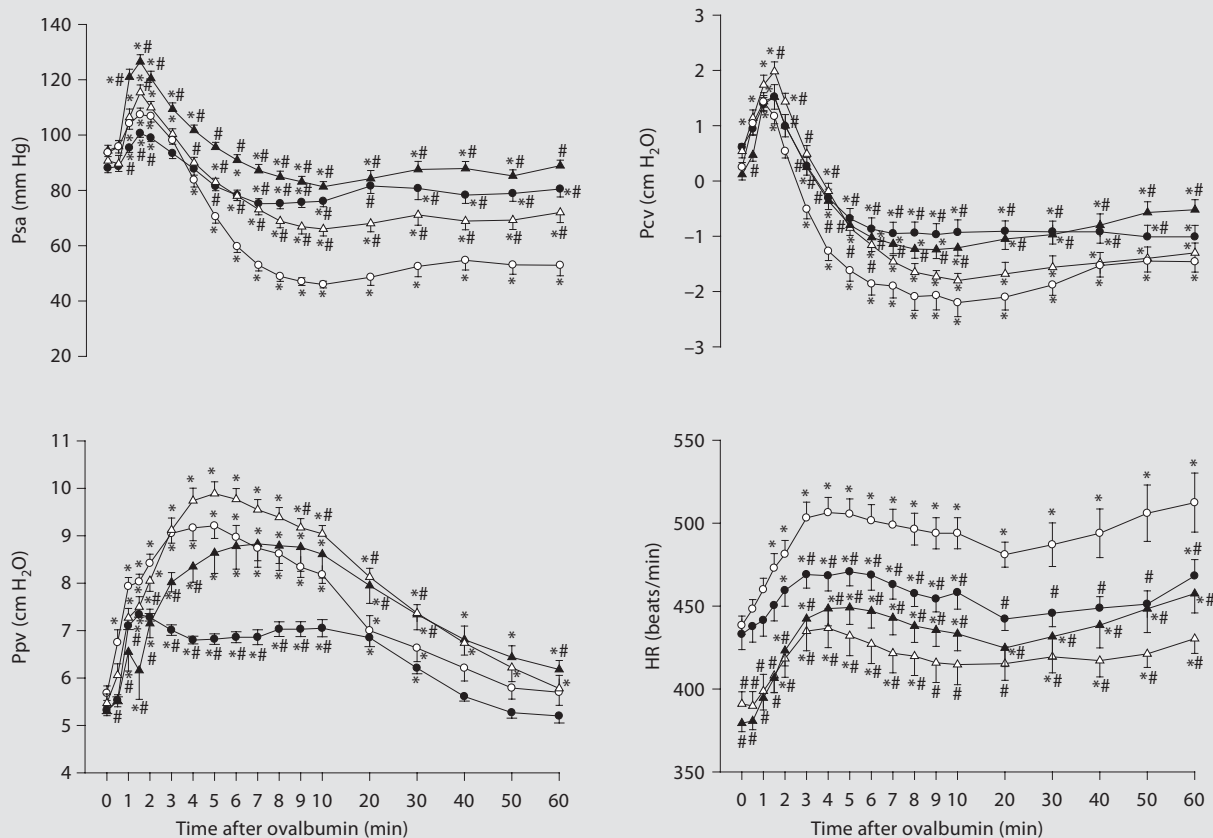


Fig. 4. Changes in the Psa, Ppv, Pcv, HR after antigen injection in the anaphylaxis control group (open circles; $n = 10$), the CV-6209 group (closed circles; $n = 10$), the DPH group (open triangles; $n = 10$) and the CV-6209 + DPH group (closed triangles; $n = 10$). Values are means $\pm$ SE. * $p < 0.05$ vs. the baseline values; # $p < 0.05$ vs. the anaphylaxis control group.

(fig. 4). The reason for this inconsistency is not known. We assume that histamine exerted vasodilator actions when the hepatic vessel was constricted by other vasoactive substances such as PAF. Actually, histamine could produce the vasodilator nitric oxide from the vascular endothelium. Moreover, the vascular smooth muscle relaxation in response to histamine is mediated through H_1 receptors on endothelial cells [10, 11]. On the other hand, we previously reported that a NOS inhibitor of L-NAME did not enhance the PAF-induced hepatic venoconstriction [12], suggesting the absence of modulation by NO. This is consistent with the present finding that PAF yielded a straightforward venoconstriction of the hepatic vessels in mouse anaphylactic hypotension.

The PAF receptor-antagonist-induced reduction of the Ppv increase by the antigen could be ascribed to the

decreases in either hepatic vascular resistance or hepatic blood flow. However, we could not clarify which is responsible for this attenuation of anaphylactic portal hypertension because we did not measure the blood flow to the liver. Nevertheless, we speculate that PAF receptor block prevented PAF-induced hepatic venoconstriction, resulting in the reduction of portal hypertension. As an interesting finding of the present study, the inhibition of PAF alone, rather than the combined inhibition of PAF and histamine, most extensively attenuated the portal hypertension, although the combined inhibition attenuated anaphylactic hypotension more extensively than PAF alone.

In summary, we investigated the roles of PAF and histamine in anaphylactic hypotension of ovalbumin-sensitized anesthetized mice. Pretreatment with the PAF re-

ceptor antagonist attenuated a fall in Psa and an increase in Ppv after antigen injection. In contrast, pretreatment with the histamine H₁ receptor antagonist augmented anaphylactic portal hypertension and attenuated anaphylactic hypotension to a lesser degree than PAF antagonist. The results suggest that PAF rather than histamine mainly contributes to anaphylactic hypotension in anesthetized mice.

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