



The responses of pulmonary and systemic circulation and airway to anaphylactic mediators in anesthetized BALB/c mice[☆]

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ABSTRACT

Aims: Anaphylactic shock sometimes accompanies pulmonary vaso- and broncho-constriction. We previously reported the hemodynamic features of mouse anaphylaxis (Life Sci. 2014; 116: 98–105). However, the effects of anaphylactic chemical mediators on the hemodynamics of *in vivo* mice are not well known. Furthermore, it is uncertain whether the mediators exert the same directional actions. Therefore, we determined their effects systematically on total peripheral resistance (TPR), pulmonary vascular resistance (PVR), or airway pressure (AWP) in anesthetized mice.

Main methods: We measured directly pulmonary arterial pressure, left atrial pressure, systemic arterial pressure, central venous pressure and aortic blood flow to determine PVR and TPR, as well as AWP, following injections of platelet-activating factor (PAF), histamine, serotonin, leukotriene (LT) C₄, and prostaglandin (PG) D₂ in anesthetized open-chest artificially ventilated BALB/c mice.

Key findings: Consecutive administration of any agents increased PVR dose-dependently with the maximal responsiveness being PAF > LTC₄ > serotonin > histamine = PGD₂. Histamine caused a biphasic PVR response, an initial decrease, which was abolished by L-NAME, followed by an increase at high doses. PAF, serotonin, and histamine decreased TPR dose-dependently, while LTC₄ or PGD₂ yielded an increase or no change in TPR, respectively. Serotonin, but not the other agents, increased AWP.

Significance: Anaphylactic mediators exert non-uniform actions on the pulmonary and systemic circulation and airway in anesthetized BALB/c mice: PAF, LTC₄ and serotonin cause substantial pulmonary vasoconstriction, while histamine biphasic responses of the initial nitric oxide dependent vasodilation followed by vasoconstriction; PAF, serotonin, and histamine, but not LTC₄ or PGD₂, evoke systemic vasodilatation; only serotonin induces airway constriction.

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1. Introduction

Mice are currently used as transgenic or knockout mouse models for investigations on anaphylaxis [1,4,26]. However, the studies on physiological characteristics of the mouse pulmonary circulation were limited. The mouse pulmonary vascular responses to various endogenous anaphylactic mediators were previously examined in isolated perfused lungs [7]: platelet-activating factor (PAF) and leukotriene (LT) C₄ caused substantial vasoconstriction, whereas serotonin and histamine

had no or only very small effects. In murine isolated pulmonary arteries [27], serotonin showed strong constriction. However, there is no systematic study in which the effects of anaphylactic mediators on the mouse pulmonary vascular resistance (PVR) and total peripheral resistance (TPR) were determined in *in vivo* mice by measuring cardiac output (CO) and the inflow and outflow pressures of the systemic and pulmonary circulations. Therefore, we measured directly and continuously CO, pulmonary arterial pressure (PAP) and left atrial pressure (LAP), along with systemic arterial pressure (SAP) and central venous pressure (CVP), in order to determine the responses of PVR and TPR to anaphylactic mediators including PAF, histamine, serotonin, LTC₄, and prostaglandin (PG) D₂ in anesthetized BALB/c mice. Airway pressure (AWP) was also measured to determine whether anaphylactic mediators cause bronchoconstriction. We hypothesized that these mediators

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do not exert the same directional actions on pulmonary and systemic circulation and airway.

2. Methods

2.1. Animals

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

2.2. Surgical preparation

Mice were anesthetized with pentobarbital sodium (60 mg/kg, i.p.) and placed supinely on a heating pad (ATC-101B; Unique Medical, Tokyo, Japan) that maintained body temperature at 36–37 °C. The methods for surgical preparation was previously described [24]. Following tracheal cannulation with a stainless tube (18-gauge stainless needle), mice were mechanically ventilated (UGO BASILE 28025 Mouse ventilator, Camerio VA, Italy) with a tidal volume of 8 ml/kg, a respiratory rate of 100/min and a positive end-expiratory pressure of 2.0 cmH₂O [3,20]. AWP was measured through the T-type tube set in the inspiratory line. The left carotid artery was cannulated with a polyethylene catheter (OD: 0.3 mm) for measurement of SAP. The right jugular vein was cannulated with a 22-gauge polyethylene catheter (Terumo, Tokyo, Japan) connected to a Y-type miniature plastic tube, one twig end of which was connected via a water-filled polyethylene tube for measurement of CVP; another twig end of the tube was used to introduce a thin inner polyethylene tube, which was tapered to less than 0.1 mm in diameter over hot air, for an intravenous injection of the drugs [16].

After a midline incision of the chest, a polyethylene catheter (ID: 0.3 mm, OD: 0.5 mm) was advanced into the pulmonary artery via the right ventricle for measurement of PAP. The same-size polyethylene catheter was inserted into the left atrium for measurement of LAP. The pulsed Doppler flow probe (MC1.5PSL, Transonic Systems, Ithaca, NY) was placed on the ascending aorta for continuous measurement of the CO or aortic blood flow (ABF).

2.3. Experimental protocol

All experimental protocols were carried out in anesthetized open-chest mice. SAP, PAP, LAP and CVP, as well as AWP, were monitored using calibrated pressure transducers (TP-400T, Nihon-Kohden, Tokyo, Japan) positioned at the level of the left atrium. HR was measured by triggering the wave of systemic arterial pressure. The vascular and airway pressures, HR and ABF were continuously recorded digitally at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia). Mean SAP (MAP), mean LAP and mean ABF were automatically determined; PVR and TPR were calculated as follows:

$$\text{PVR} = (\text{mean PAP} - \text{mean LAP}) / \text{mean ABF} \quad (1)$$

$$\text{TPR} = (\text{MAP} - \text{CVP}) / \text{mean ABF} \quad (2)$$

At 20 min after surgery, the baseline measurements were performed. Then PAF, histamine, serotonin, LTC₄, or PGD₂ was intravenously administered via the jugular vein as a bolus consecutively from the starting dose of 0.01 nmol/kg, with an injection volume of 50 µl. In addition, the effect of pretreatment with the nitric oxide (NO) synthase inhibitor L-NAME (50 mg/kg, i.v.) at 10 min before histamine injection on the responses to histamine was studied for the post-L-NAME histamine group. When hemodynamic variables returned to the pre-

injection levels within 10 min after preceding administration of a smaller dose, a subsequent higher dose was administered. Each animal received only one anaphylactic mediator. We also performed the control studies in which 50 µl saline alone as the control for the histamine, post-L-NAME histamine, and serotonin groups, or 50 µl saline-diluted ethanol (5%) as the control for the PAF, LTC₄, and PGD₂ groups was intravenously injected at 10 min intervals over 50 min. Seven mice were assigned to each drug and control group.

2.4. Drugs

All drugs were purchased from Sigma Chemical Company, St Louis, MO. Histamine, serotonin and L-NAME were dissolved in saline. PAF, LTC₄, and PGD₂ were dissolved in 95% ethanol for stock solution, which was diluted with saline for the working solution.

2.5. Statistical analysis

Results are expressed as means $\pm$ SEM. Intragroup comparisons were performed using two-way analysis of variance for repeated measures. Intergroup comparisons were performed using one-way analysis of variance. When a significant difference was observed, post-hoc test was performed by using Bonferroni. The statistical analyses were performed by Stat View, version 5.0 (SAS Institute Inc., Cary, NC).

3. Results

3.1. The basal levels of variables in pulmonary and systemic circulation

The basal hemodynamic values for each group were shown in the Table 1. The summarized data of 56 mice were as follows: MAP, 99 ± 3 mmHg; CVP, 3.6 ± 0.2 mmHg; PAP, 16.1 ± 0.4 mmHg; LAP, 5.8 ± 0.2 mmHg; ABF, 9.7 ± 0.3 ml/min; TPR, 9.9 ± 0.5 mmHg·min/ml; PVR, 1.1 ± 0.2 mmHg·min/ml; HR, 495 ± 21 beats/min; peak AWP, 8.6 ± 0.2 cmH₂O.

3.2. Effects of the anaphylactic mediators

3.2.1. PAF

Fig. 1 shows representative recordings of the changes in the variables after injections of PAF at doses ranging from 0.01 nmol/kg (5.42 ng/kg) to 100 nmol/kg (54.2 µg/kg) in an anesthetized mouse. Fig. 2 shows the summarized data of maximal changes in PVR, TPR, and AWP in all groups studied. At 10 nmol/kg, SAP progressively decreased to 31 ± 2 mmHg at 8 min after injection, along with a decrease in ABF. Then, in 3 of 7 mice, SAP did not recover to the baseline; the subsequent dose of 100 nmol/kg was not injected into these mice. Therefore, to obtain 100 nmol/kg data, 3 additional mice were firstly administered of 100 nmol/kg.

TPR tended to decrease, but not significantly, at doses of 0.01–1 nmol/kg, while a significant decrease was observed at 10 nmol/kg. The 100 nmol/kg PAF showed a biphasic TPR response comprising an initial decrease followed by a profound increase in parallel with a marked decrease in ABF. PVR showed dose-dependent increases at doses of 10 and 100 nmol/kg by $141 \pm 10\%$ and $367 \pm 13\%$ of baseline, respectively, along with an increase in PAP (Figs. 1 and 2). AWP did not significantly change at any doses studied.

3.2.2. Histamine

Histamine produced significant decreases in TPR at doses of 1 µmol/kg (111 µg/kg) or higher (Figs. 2 and 3). In contrast to the other agents studied, immediately after an injection of histamine, PVR transiently and significantly decreased by around 10% at 0.01–10 µmol/kg (1.11–1110 µg/kg) and by $16 \pm 1\%$ at 30 µmol/kg (3.33 mg/kg) (Figs. 2 and 3). In Fig. 3, the time when PVR was decreased to the nadir was indicated by black arrow heads. The higher

Table 1
The basal levels of the hemodynamic variables.

Groups	MAP (mmHg)	CVP (mmHg)	PAP (mmHg)	LAP (mmHg)	ABF (ml/min)	AWP (cmH ₂ O)	HR (beats/min)	TPR (mmHg·min/ml)	PVR (mmHg·min/ml)
PAF (n = 7)	102 ± 4	3.5 ± 0.3	16.4 ± 0.4	5.7 ± 0.3	9.5 ± 0.3	8.5 ± 0.2	464 ± 20	10.5 ± 0.4	1.1 ± 0.1
Serotonin (n = 7)	99 ± 2	3.8 ± 0.2	16.5 ± 0.3	5.9 ± 0.2	10.3 ± 0.5	8.4 ± 0.2	520 ± 22	9.3 ± 0.5	1.0 ± 0.1
LTC ₄ (n = 7)	95 ± 3	3.2 ± 0.3	15.5 ± 0.6	5.9 ± 0.2	9.6 ± 0.7	8.4 ± 0.3	471 ± 20	9.8 ± 0.6	1.0 ± 0.1
PGD ₂ (n = 7)	99 ± 4	3.6 ± 0.2	16.1 ± 0.3	5.8 ± 0.3	10.3 ± 0.3	8.5 ± 0.2	525 ± 18	9.2 ± 0.5	1.0 ± 0.1
Histamine (n = 7)	99 ± 3	3.8 ± 0.3	16.0 ± 0.7	5.9 ± 0.2	9.6 ± 0.4	9.1 ± 0.2	476 ± 30	10.0 ± 0.3	1.1 ± 0.1
Post L-NAME									
Histamine (n = 7)	101 ± 5	3.4 ± 0.3	15.8 ± 0.5	5.8 ± 0.3	9.1 ± 0.5	8.3 ± 0.2	515 ± 15	10.7 ± 0.5	1.1 ± 0.1
Saline control (n = 7)	97 ± 3	3.7 ± 0.3	16.3 ± 0.5	5.5 ± 0.3	9.3 ± 0.5	8.5 ± 0.3	486 ± 20	10.0 ± 0.5	1.2 ± 0.1
Ethanol control (n = 7)	98 ± 5	3.7 ± 0.2	16.5 ± 0.5	5.7 ± 0.2	9.7 ± 0.6	9.0 ± 0.3	496 ± 25	9.8 ± 0.5	1.1 ± 0.1

Values are means ± S.E.M. MAP, mean systemic arterial pressure; CVP, central venous pressure; PAP, pulmonary arterial pressure; LAP, left atrial pressure; ABF, aortic blood flow; AWP, airway pressure; HR, heart rate; TPR, total peripheral resistance; PVR, pulmonary vascular resistance.

doses of 10 and 30 $\mu\text{mol/kg}$ produced a biphasic PVR response of the initial decrease followed by a dose-dependent increase, which was reflected by an increase in PAP. The time of peak PVR was indicated by white arrow heads in Fig. 3. However, this subsequent PVR increment was as small as $32 \pm 5\%$ of baseline even at the highest dose of 30 $\mu\text{mol/kg}$ (Fig. 2). Of note, histamine (0.1–30 $\mu\text{mol/kg}$) caused a transient increase in ABF at around 23 s after injection. This increase in ABF was accompanied by increases in SAP, PAP, LAP, and CVP, leading to a decrease in TPR. AWP did not change at any doses.

Next we determined, by L-NAME pretreatment, the roles of NO-induced vasodilation in the responses of the pulmonary and systemic circulation to histamine, because histamine is known to release NO from endothelial cells, and thereby cause vasodilatation [15]. As expected, following pretreatment with L-NAME, the histamine-induced decreases in TPR at early stage, as observed in mice without L-NAME pretreatment (Fig. 3), were completely converted to increases in TPR (Fig. 4), as reflected by the increase in SAP. However, there followed a sustained decrease in TPR at high doses of 10 and 30 $\mu\text{mol/kg}$. Similar to systemic circulation, pretreatment with L-NAME abolished the initial decrease in PVR at 0.01–1 $\mu\text{mol/kg}$, and produced dose-dependent increases in PVR at 1 $\mu\text{mol/kg}$ or higher, as indicated by white arrow heads in Fig. 4. However, at high doses of 10 and 30 $\mu\text{mol/kg}$, the initial decrease in PVR, as seen in mice without L-NAME, was significantly

enhanced in L-NAME pretreated mice, which was accompanied by transient and marked increases in LAP (12.1 ± 0.5 mmHg at 10 $\mu\text{mol/kg}$ and 25.5 ± 3.2 mmHg at 30 $\mu\text{mol/kg}$), as indicated by black arrow heads in Fig. 4.

3.2.3. Serotonin

Serotonin decreased TPR dose-dependently: Serotonin at 100 nmol/kg (21.3 $\mu\text{g/kg}$) caused the maximal decrease by $43 \pm 3\%$ of baseline along with an increase in ABF and a decrease in SAP (Figs. 2 and 5). PVR dose-dependently increased along with an increase in PAP: PVR increased to the peak levels of $130 \pm 4\%$ and $166 \pm 8\%$ of baseline at 100 and 300 nmol/kg (21.3 and 63.9 $\mu\text{g/kg}$), respectively. Notably, similar to histamine, serotonin at 300 nmol/kg caused an increase in ABF, which was accompanied by increases in SAP, PAP, CVP, and LAP (Fig. 5). AWP also significantly increased at doses of 100 and 300 nmol/kg.

3.2.4. LTC₄

In contrast to other mediators, LTC₄ increased SAP dose-dependently, resulting in significant increases in TPR at 10 and 30 nmol/kg (6.3 and 18.9 $\mu\text{g/kg}$) to the peak levels being $123 \pm 6\%$ and $138 \pm 6\%$ of baseline, respectively (Figs. 2 and 6). At 10 nmol/kg, PVR increased to the peak of $170\% \pm 14\%$ of baseline along with a decrease in ABF and no change in PAP. The maximally increased PVR

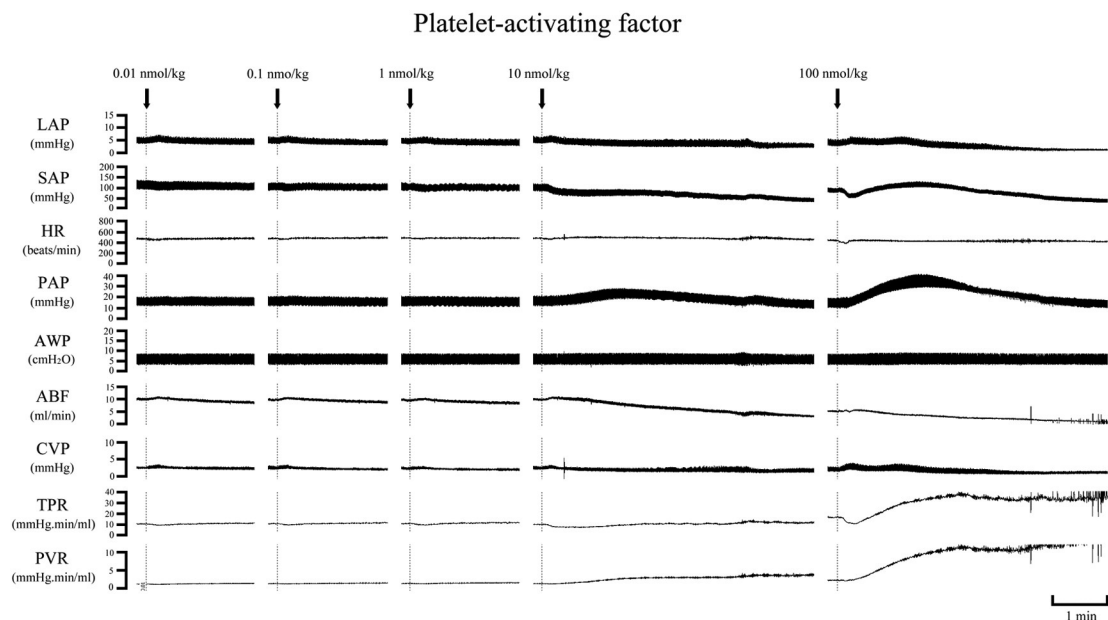


Fig. 1. The representative recordings of the left atrial pressure (LAP), systemic arterial pressure (SAP), heart rate (HR), pulmonary arterial pressure (PAP), airway pressure (AWP), aortic blood flow (ABF), and central venous pressure (CVP) after successive injections of PAF at 0.01, 0.1, 1, 10, and 100 nmol/kg in an anesthetized mouse. Pulmonary vascular resistance (PVR) and total peripheral resistance (TPR) were also shown, which were automatically calculated by Eqs. (1) and (2) with PowerLab.

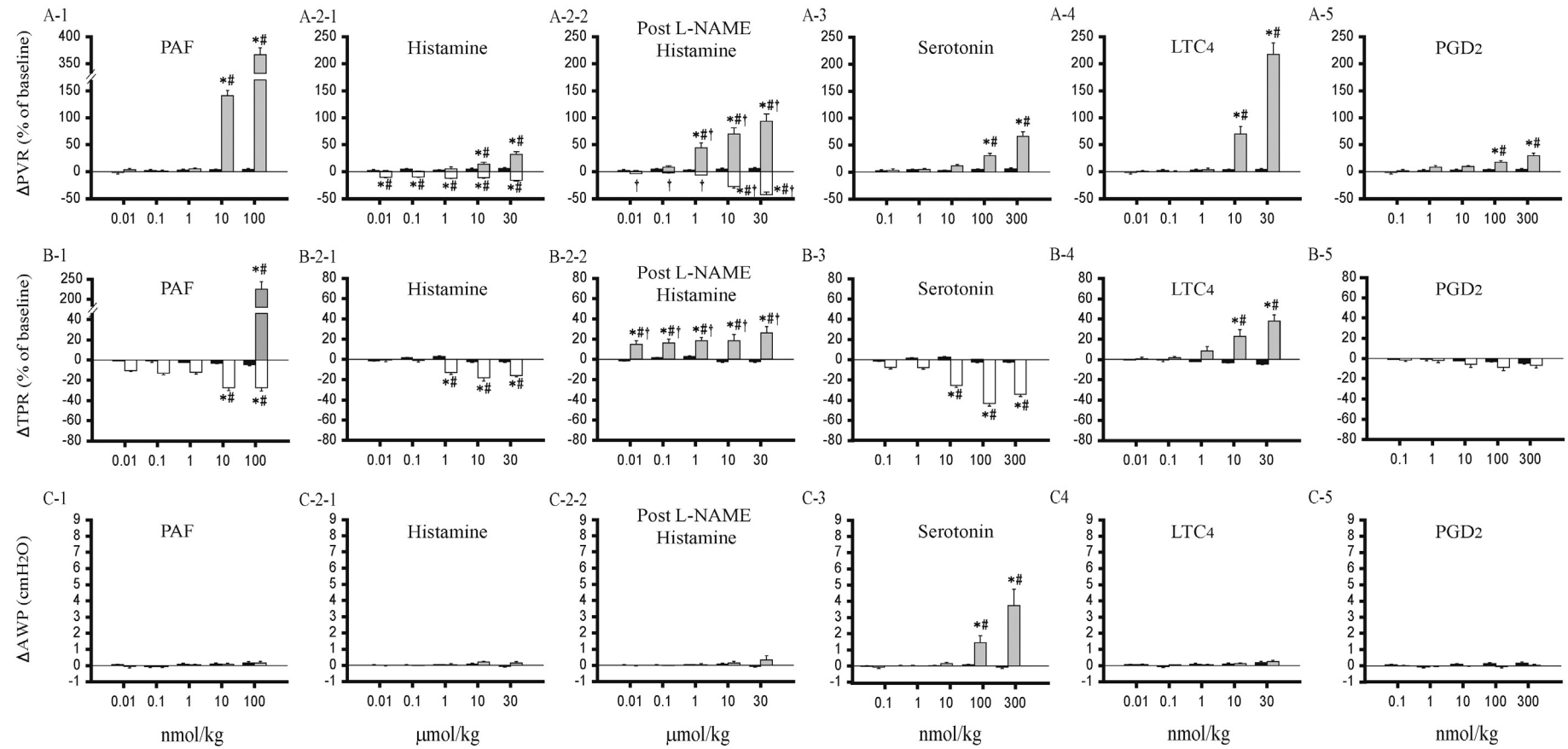


Fig. 2. The maximal changes in pulmonary vascular resistance (PVR), total peripheral resistance (TPR), and airway pressure (AWP) after an injection of PAF ($n = 7$), histamine ($n = 7$), post-L-NAME histamine ($n = 7$), serotonin ($n = 7$), LTC₄ ($n = 7$), PGD₂ ($n = 7$), saline ($n = 7$), and ethanol ($n = 7$). The black bars in each panel indicate the data after injections of saline or ethanol in the control groups. * $P < 0.05$, versus the baseline; # $P < 0.017$, versus the control group (black bars); † $P < 0.017$, versus the histamine group.

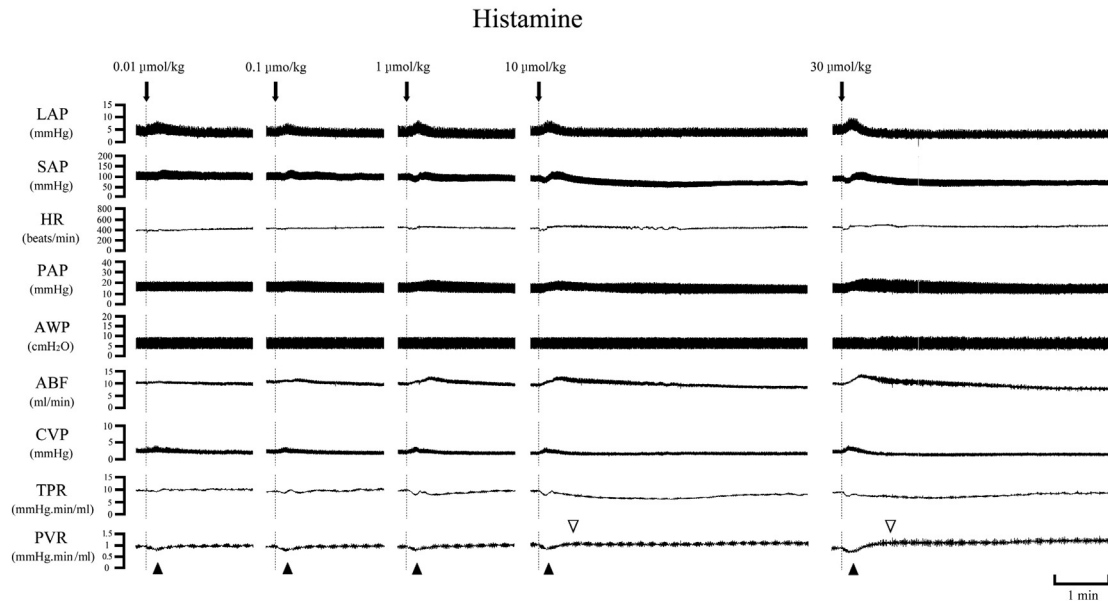


Fig. 3. The representative recordings of the hemodynamic variables and resistances after intravenous injections of histamine at 0.01, 0.1, 1, 10 and 30 $\mu\text{mol/kg}$ in an anesthetized mouse. The black arrow heads indicate the time points for the nadir of the initially decreased PVR, and the white arrow heads indicate the time points for the peak of the secondarily increased PVR.

at 30 nmol/kg was $318 \pm 20\%$ of baseline and was accompanied by an increase in PAP in the presence of a decrease in ABF. AWP did not change significantly.

3.2.5. PGD_2

TPR did not change significantly at 0.1–300 nmol/kg (35.3 ng/kg–105.9 $\mu\text{g/kg}$) (Figs. 2 and 7). PVR increased significantly but slightly at 100 and 300 nmol/kg, with the maximal values being $117 \pm 3\%$ and $129 \pm 5\%$ of baseline, respectively. AWP did not change significantly.

3.2.6. The control study

The hemodynamic variables obtained at 30 s after injections of 50 μl saline alone or 50 μl diluted ethanol were adopted as the data for the time control groups, and their changes were presented in Fig. 2 (black bars). Five consecutive injections for individual doses of saline alone

or diluted ethanol did not affect the hemodynamic variables over 50 min.

4. Discussion

In the current study, we determined the responses of systemic and pulmonary circulation, as well as AWP, to anaphylactic mediators in anesthetized open-chest BALB/c mice. We obtained the following findings: (1) all mediators studied significantly increased PVR although the responsiveness varied among mediators, with the rank of the potency being $\text{PAF} > \text{LTC}_4 > \text{serotonin} > \text{histamine} = \text{PGD}_2$. Histamine also produced an initial decrease in PVR, which was abolished and augmented at low and high doses, respectively, by L-NAME pretreatment. (2) In contrast to pulmonary vessels, systemic circulation responses differed depending on mediators: TPR was decreased by PAF, histamine and serotonin, but not changed by PGD_2 , and rather increased by LTC_4 .

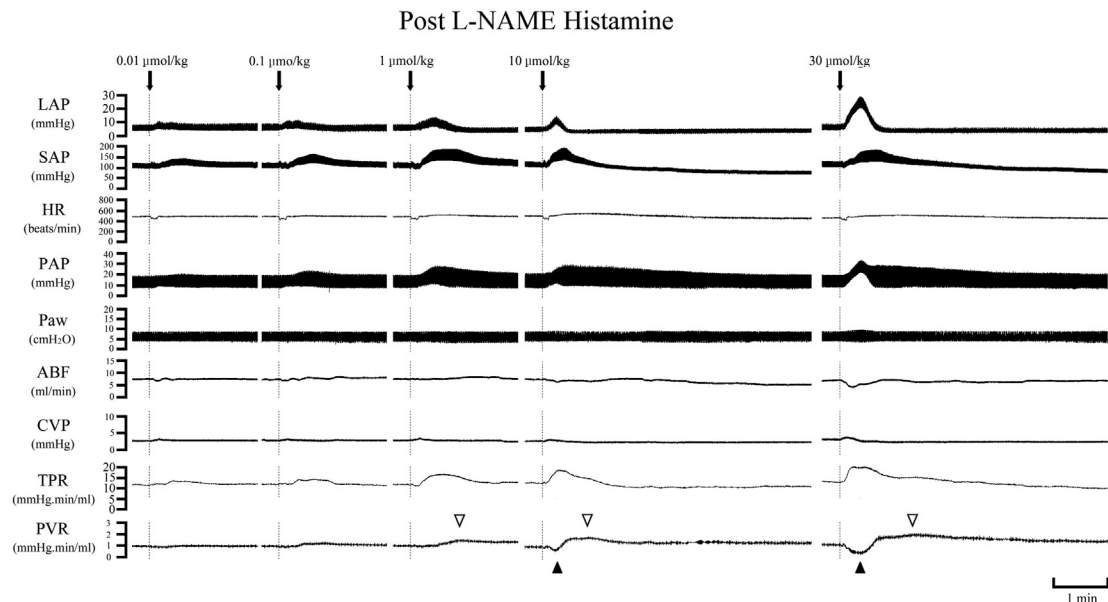


Fig. 4. The representative recordings of the hemodynamic variables and resistances after intravenous injections of histamine at 0.01, 0.1, 1, 10 and 30 $\mu\text{mol/kg}$ following pretreatment with the nitric oxide synthase inhibitor L-NAME (50 mg/kg, i.v.) in an anesthetized mouse.

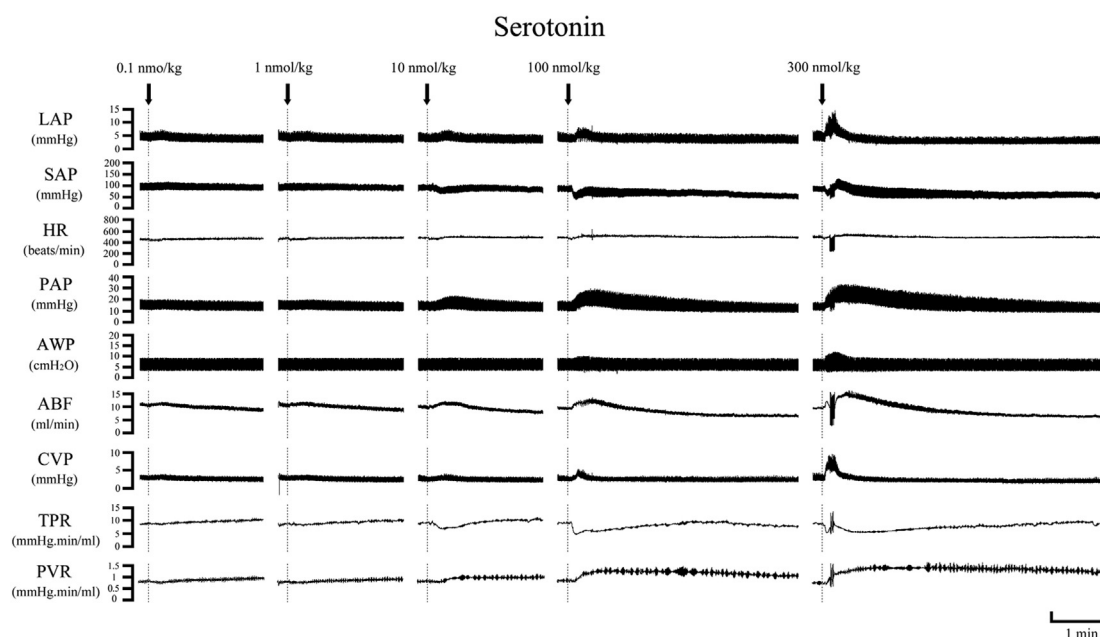


Fig. 5. The representative recordings of the hemodynamic variables and resistances after intravenous injections of serotonin at 0.1, 1, 10, 100 and 300 nmol/kg in an anesthetized mouse.

With L-NAME pretreatment, histamine caused only an increase in TPR. (3) AWP was increased only by serotonin, but not by the other mediators in the present mouse *in vivo* preparations.

In our previous study [24], the antigen-induced increases in PVR in the early phase could not be attenuated by inhibition of PAF combined with either receptor antagonist of LTC₄ or serotonin. We assumed the reason for the inability to inhibit the PVR increases may be related to the redundancy; even if the actions of one or two mediators were blocked, those of other ones still remained. The present study supports this assumption by demonstrating that all of the mediators studied including PAF, histamine, LTC₄, PGD₂, and serotonin cause pulmonary vasoconstriction, although the potency is varied.

We previously reported that pretreatment with histamine H1 antagonist augmented the anaphylactic pulmonary vasoconstriction soon after antigen injection in anesthetized mice [24]. This finding suggests

that histamine released during mouse anaphylaxis may exert a pulmonary vasodilatory action via H1 histamine receptor. Actually, H1 receptor activation can elicit an endothelial-dependent vasodilation [10] via production of the vasodilator NO from endothelium [15]. Consistent with these observations, histamine decreased PVR immediately after injection albeit to a small degree at any doses studied in the present study. Furthermore, pretreatment with L-NAME not only abolished this initial decrease in PVR at low doses, but also augmented a subsequent increase in PVR (Figs. 2 (A-2-2) and 4), a finding demonstrating the involvement of NO-induced pulmonary vasodilation in the PVR response to histamine. However, at high doses, the initial decrease in PVR was augmented dose dependently, which was accompanied by a considerable increase in LAP to induce pulmonary congestion. Thus, this enhanced decrease in PVR seems to be caused by pulmonary congestion. Actually we previously demonstrated that LAP elevation-induced pulmonary

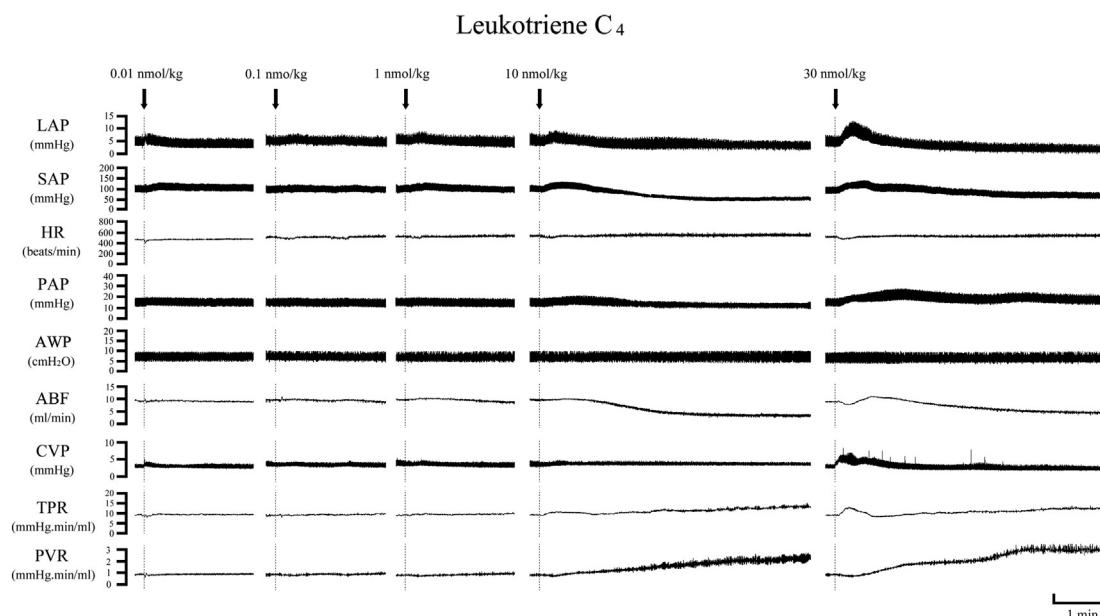


Fig. 6. The representative recordings of the hemodynamic variables and resistances after intravenous injections of LTC₄ at 0.01, 0.1, 1, 10 and 30 nmol/kg in an anesthetized mouse.

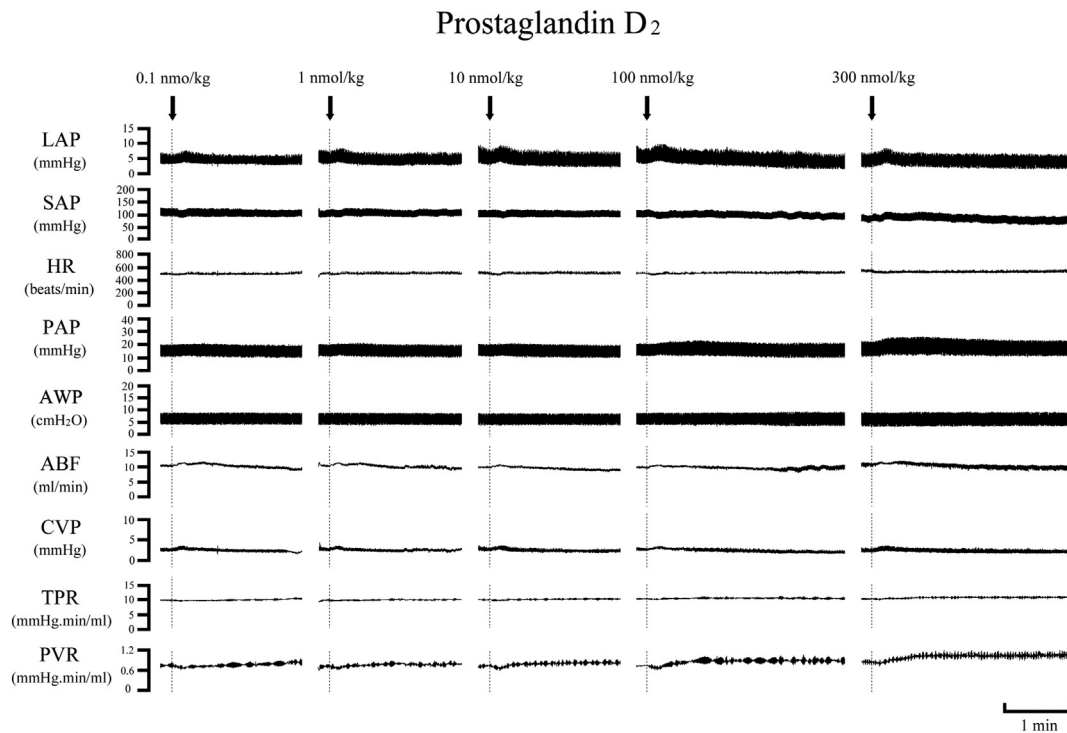


Fig. 7. The representative recordings of the hemodynamic variables and resistances after intravenous injections of PGD₂ at 0.1, 1, 10, 100 and 300 nmol/kg in an anesthetized mouse.

congestion induces a decrease in PVR in anesthetized BALB/c mice [25]. The mechanism for the transient increase in LAP at high doses of histamine under L-NAME pretreatment is not known. It might result from acute left heart failure, which could be caused by histamine-induced coronary vasoconstriction [11,19] that was enhanced by L-NAME [21] and/or left heart overload caused by large increases in SAP due to strong systemic vasoconstriction [25].

In this study, high doses of histamine caused a biphasic PVR response with an initial NO-induced vasodilatation followed by vasoconstriction. A similar biphasic response to histamine was observed in cremaster muscle arterioles of the C57BL/6 mice, with vasodilation manifest at low concentration and vasoconstriction at high concentration [18]. In contrast, in mice with null endothelial NO synthase (eNOS^{−/−}), histamine produced only vasoconstriction. This evidence is consistent with the present results on the effect of L-NAME on the pulmonary vessel response to histamine.

Payne et al. [18] further reported that in mouse cremaster muscle arterioles, both dilation and constriction by histamine are mediated via H1 receptors, with vasodilation dependent on NO production by endothelium and vasoconstriction induced through the activation of L-type calcium channels in smooth muscle. In other species than mice, pulmonary vasodilatation is also induced by activation of vascular smooth muscle H2 receptor, while pulmonary vasoconstriction activation of vascular smooth muscle H1 receptor [2,6,14,23]. Further study is required to determine the histamine receptor subtypes involved in pulmonary vasomotion of mice.

We have previously demonstrated that anaphylactic hypotension is mainly mediated by PAF and, to a lesser extent, by histamine in anesthetized BALB/c mice; pretreatment with PAF receptor antagonist attenuated the antigen-induced decrease in SAP with a greater magnitude than that with histamine H₁ receptor antagonist, and combination of these PAF and H₁ receptor antagonists almost completely inhibited the hypotension [24]. These findings on mouse anaphylactic hypotension were supported by the present results demonstrating the hypotensive actions of PAF and histamine. The present study clearly demonstrates that the initial depressor effect of histamine is caused by NO because L-NAME pretreatment converted the histamine-induced decrease in TPR to an

increase. In contrast to systemic depressors of PAF, histamine and serotonin, PGD₂ did not affect SAP or TPR, while LTC₄ rather increased these two variables in the present anesthetized mice. Hui et al. [9] reported that LTC₄ administered systemically caused an immediate rise in mean SAP that gradually fell to baseline within 3 to 15 min in mice. However, they did not measure CO or TPR; therefore it was not known whether the increased SAP was due to increased CO or vasoconstriction. We here clearly showed that LTC₄ increases TPR and hence causes systemic vasoconstriction.

One of the characteristics of mouse anaphylactic hypotension is the presence of the initial transient increase in SAP, followed by subsequent hypotension [20,24]; this biphasic response could not be observed in other animals such as rats, dogs, or rabbits. This initial SAP elevation was accompanied by an increase in ABF and no apparent change in TPR [24], and may be caused by increased cardiac output, presumably due to increased cardiac contractility. The similar elevation of SAP and ABF was observed in mice injected with histamine and serotonin (Figs. 3 and 5). Indeed, serotonin [12,28] and histamine [5] could exert a positive inotropic action in animals other than mice; however, their information on mouse heart is limited [22]. Nonetheless, histamine and serotonin could at least in part account for the transient systemic hypertension in mouse anaphylaxis.

The AWP was increased only by serotonin, but not by the other mediators studied in the present anesthetized mice. This finding is consistent with the observations that pulmonary conductance and compliance measured in mechanically ventilated mice were decreased by intravenous infusions of serotonin, methacholine, or norepinephrine, and that reproducible responses were not detected after infusions of histamine, PGD₂, LTC₄, or PAF [17]. Held et al. [7] also reported using excised mouse lung slice preparations that serotonin constricted the airway as strongly as methacholine, while either histamine or LTD₄ did not at all.

We here used BALB/c mice to examine the pulmonary and systemic vascular and airway responses to anaphylactic mediators, since BALB/c mouse is the strain frequently used in laboratory animal research [7], and many immunologic examinations have utilized this strain. However, there are interstrain differences in pulmonary vascular and airway

responsiveness to bioactive substances [8,13]. We should be cautious about extrapolating the present information obtained from BALB/c mice to other mouse strains such as C57BL/6 mice, the background strain of many genetically engineered mice.

5. Conclusion

We determined the responses of PVR, TPR and AWP to anaphylactic mediators in anesthetized open-chest BALB/c mice. PVR was increased by all mediators studied with the order of the potency being PAF > LTC₄ > serotonin > histamine = PGD₂. Histamine caused a biphasic PVR response, an initial NO-induced decrease followed by an increase at high doses. In contrast, TPR was decreased by PAF, histamine and serotonin, but not changed by PGD₂, or rather increased by LTC₄. AWP was increased only by serotonin, but not by the other mediators in the present mouse *in vivo* preparations. Thus, anaphylactic mediators exert non-uniform actions on the pulmonary and systemic circulation and airway. These basic characteristics of the anaphylactic mediators in mouse pulmonary and systemic vessels well account for the pathophysiological changes in anaphylaxis and furthermore could be of importance for the experimental studies using mice.

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