

Nitric oxide and β_2 -adrenoceptor activation attenuate pulmonary vasoconstriction during anaphylactic hypotension in anesthetized BALB/c mice

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ABSTRACT

Systemic anaphylaxis accompanies pulmonary vasoconstriction and bronchoconstriction, which may contribute to increased right heart afterload, and finally anaphylactic hypotension. However, the pulmonary response to anaphylaxis is not known in mice. We determined the pulmonary vascular and bronchial response to systemic anaphylaxis in anesthetized BALB/c mice. We also clarified the roles of β -adrenoceptors, nitric oxide, and cyclooxygenase metabolites in these responses. Anaphylaxis was induced by an intravenous injection of the ovalbumin antigen into open-chest artificially ventilated sensitized mice. Mean arterial pressure, systolic pulmonary arterial pressure, central venous pressure, airway pressure, and aortic blood flow were continuously measured. In sensitized control mice, mean arterial pressure, and aortic blood flow substantially decreased soon after the antigen injection, while systolic pulmonary arterial pressure and airway pressure did not increase. In contrast, in mice pretreated with either the β_2 -adrenoceptor antagonist ICI 118,551 (0.2 mg/kg; $n = 6$), or L-NAME (50 mg/kg; $n = 6$), but not with the β_1 -adrenoceptor antagonist atenolol (2 mg/kg; $n = 6$) or indomethacin (5 mg/kg; $n = 6$), systolic pulmonary arterial pressure increased by 7 mmHg at 1.5 min after antigen. In L-NAME pretreated mice, pulmonary hypertension was sustained over 30 min of the experimental period. Airway pressure did not significantly change after antigen in any mice studied. In conclusion, pulmonary response to systemic anaphylaxis does not increase the right heart afterload and, therefore, may not contribute to the initial decrease in venous return and anaphylactic hypotension in anesthetized mice. β_2 -adrenoceptor activation and nitric oxide, but not β_1 -adrenoceptor activation or cyclooxygenase metabolites, attenuate the antigen-induced pulmonary vasoconstriction.

KEYWORDS anaphylaxis, ICI 118,551, L-NAME, pulmonary arterial pressure

INTRODUCTION

Anaphylactic hypotension is a life-threatening allergic disorder, but the hemodynamic mechanism of hypotension is not fully understood [1]. Anaphylactic hypotension appears to be caused primarily by a decrease in blood flow to the heart, because left ventricular function is relatively well preserved during

anaphylactic shock [1, 2]. The decreased venous return is at least in part caused by a decrease in effective circulating blood volume due to vasodilation with the peripheral pooling and increased vascular permeability with a shift of intravascular fluid to the extravascular space [1, 2]. Increased right heart load, which is caused by pulmonary vasoconstriction and bronchospasm [3–8], also could contribute to the anaphylaxis-induced decrease in venous return. In fact, there are some reports that pulmonary arterial pressure (PAP) and pulmonary vascular resistance (PVR) were increased during anaphylactic shock in dogs [6, 7], monkeys [5], rabbit [8], and sheep [4]. In contrast, we previously reported that PAP does not

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increase during anaphylactic shock in anesthetized rats [9]. However, there is no study in which PAP was measured during anaphylactic hypotension in mice, which have been most frequently used for a variety of physiological and immunological studies because of development of genetic engineering. Thus, we here evaluated PAP and PVR during anaphylactic hypotension in mice.

Constriction of the pulmonary artery and bronchi can be modulated by endogenously released vasoactive mediators such as endothelium-derived nitric oxide (NO), prostaglandin I₂, and catecholamines. Activation of β_2 -adrenoceptors attenuates contraction of smooth muscle cell of the pulmonary arteries [10, 11] and trachea-bronchial trees [12]. Indeed, β_2 -adrenoceptor agonists are administered in favor of inhibiting pulmonary vasoconstriction and bronchoconstriction in canine systemic anaphylaxis [13, 14]. NO has also been shown to play a primary and harmful role in anaphylactic shock, causing progressive refractory hypotension which ultimately leads to multiple organ dysfunction and death [15–18]. Contractile response of guinea pig pulmonary artery to the antigen is attenuated by NO [19]. Finally, prostanoids could affect pulmonary artery [20]. However, it is not known whether or how NO, prostanoid, or β -adrenoceptor activation affects anaphylactic pulmonary venoconstriction and bronchoconstriction in mice. We, therefore, determined the pulmonary vascular and bronchial response to systemic anaphylaxis in anesthetized BALB/c mice. We also clarified the roles of β -adrenoceptors, nitric oxide, and cyclooxygenase metabolites in these responses.

METHODS

Animals

Forty-two male BALB/c mice (Japan SLC, Shizuoka, Japan) weighing 29 ± 1 g were used in this study. Mice were maintained at 23°C and under pathogen-free conditions on a 12:12 h 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.

Sensitization

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 Chemical Co., St. Louis,

MO, USA) dissolved in physiological saline (0.2 mL) [21]. The antigen emulsion was injected again one week after the first antigen injection. Non-sensitized mice were injected with aluminum potassium sulfate adjuvant and ovalbumin-free saline. One week after the second injection, the mice were used for the following experiments.

Surgical Preparation

Mice were anesthetized with pentobarbital sodium (60 mg/kg, i.m.) and placed supinely on a heating pad (ATC-101B; Unique Medical, Tokyo, Japan) that maintained body temperature at 36°C–37°C. The trachea was cannulated with a stainless tube (18-gauge stainless needle), and mice were mechanically ventilated (UGO BASILE 28025 Mouse ventilator, Camerio VA, Italy) with a tidal volume of 8 mL/kg, a respiratory rate of 120/min and a positive end-expiratory pressure of 2.0 cmH₂O [22]. Based on the preliminary study, under this artificial ventilation, PaCO₂ and PaO₂ were kept at 35–40 mmHg and 100–110 mmHg, respectively, throughout the experimental period of 30 min. The airway pressure was measured through the T-type tube set in the inspiratory line. A polyethylene catheter (OD: 0.3 mm) was inserted into the left carotid artery for measurement of the mean arterial pressure (MAP). The right external jugular vein was catheterized with a 22 Fr polyethylene catheter (Terumo, Tokyo, Japan) (ID: 0.6 mm, OD: 0.9 mm). This catheter was connected to a Y-type miniature plastic tube, one twig end of which was connected via a water-filled polyethylene tube 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 ~0.1 mm in diameter over hot air, for an intravenous injection of antigen or drugs [21].

After a midline incision of the chest, polyethylene catheter (ID: 0.3 mm, OD: 0.5 mm) was advanced into the right ventricle for measurement of the systolic right ventricular pressure, which was used as systolic PAP. The pulsed Doppler flow probe (MC1.5PSL, Transonic Systems, Ithaca, NY) was placed on the ascending aorta for continuous measurement of the cardiac output or aortic blood flow (ABF).

Experimental Protocol

All experimental protocols were carried out in anesthetized open-chest mice. MAP and Pcv, as well as airway pressure, were monitored using calibrated pressure transducers (TP-400T, Nihon-Kohden, Tokyo, Japan) positioned at the level of the left atrium. Heart rate was measured by triggering the

wave of systemic arterial pressure. The vascular and airway pressures, heart rate, and ABF were continuously recorded digitally at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia). For determination of PVR, we could not obtain correct PVR because we did not measure the left atrial pressure or pulmonary arterial wedge pressure. Thus, in this study, we determined quasi-PVR using the following equation:

$$\text{quasi-PVR} = \text{systolic PAP} / \text{ABF}. \quad [1]$$

One of the following inhibitors was intravenously administered at 10 min before an intravenous injection of the antigen (0.01 mg) into the sensitized mice: 1) the selective β_1 -adrenoceptor antagonist atenolol (2 mg/kg; 50 μ L; $n = 6$), 2) the selective β_2 -adrenoceptor antagonist ICI 118,551 (0.2 mg/kg; 50 μ L; $n = 6$), 3) a NO synthase inhibitor, N^G -nitro-L-arginine methyl ester (L-NAME; 50 mg/kg; 50 μ L; $n = 6$) [23, 24], 4) N^G -nitro-D-arginine methyl ester (D-NAME) (50 mg/kg; 50 μ L; $n = 6$), 5) indomethacin (5 mg/kg; 50 μ L; $n = 6$) [25]. For control studies, drug-free saline (50 μ L) was intravenously injected into the sensitized control mice ($n = 6$) and non-sensitized control mice ($n = 6$) before an intravenous injection of the antigen (0.01 mg).

Drugs

All drugs were purchased from Sigma Chemical Company, St Louis, MO. ICI 118,551, atenolol, L-NAME and D-NAME were dissolved in saline. Indomethacin was dissolved in 5% sodium bicarbonate.

Statistical Analysis

Results are expressed as means $\pm$ S.E.M. The Student t -test was used to compare the basal variables before and after the pretreatment with β -adrenoceptor antagonists, NO synthase inhibitors or indomethacin. For the analysis of the variables after the antigen injection, intragroup, and between-group comparisons were performed using one-way and two-way analysis of variance for repeated measures. When a significant difference was observed with the two-way analysis of variance, paired comparisons were made within a group and also between groups by using a Fisher post-test. Significance was assumed when P -value was less than 0.05 (two-tailed). All statistical analyses were performed by Stat View, version 5.0 (SAS Institute Inc., Cary, NC).

RESULTS

Table 1 shows the effects of β -adrenoceptor antagonists, L-NAME and indomethacin on the basal levels of the variables. ABF was significantly decreased by injections of atenolol, ICI118,551, and L-NAME, while quasi-PVR was increased by injections of atenolol and L-NAME. MAP was significantly increased by L-NAME. Heart rate decreased significantly after administration of atenolol.

Figure 1 shows representative recordings of the changes in the variables measured during the first 10 min after an antigen challenge in the sensitized control, ICI 118,551 pre-treated and L-NAME pre-treated groups. Figure 2 shows the averaged changes in the variables for the sensitized control and non-sensitized control groups for 30 min after antigen. In the sensitized control group, MAP, and systolic PAP initially increased slightly, but not significantly, from the baseline of 86 ± 6 and 22.7 ± 0.5 mmHg to peak of 96 ± 6 and 25.0 ± 0.7 mmHg, respectively, at 2 min after antigen. Thereafter, MAP significantly decreased to 57 ± 6 mmHg (Figure 2A), while systolic PAP decreased slightly, but not significantly (Figure 2B). ABF decreased progressively from the baseline of 7.6 ± 0.7 to 4.4 ± 0.5 mL/min (Figure 2C), quasi-PVR did not change significantly until 7 min after antigen; thereafter it increased approximately two times in the presence of 50% decrease of ABF (Figure 2D).

Anaphylactic responses of the variables in ICI 118,551- or atenolol-pretreated mice are shown in Figure 3. In the β_2 -adrenoceptor antagonist ICI 118,551 pre-treated group, the antigen-induced initial increases in MAP and systolic PAP were significantly greater than those of the sensitized control group, by 250% and 230%, respectively: MAP and systolic PAP increased from the baseline of 81 ± 4 mmHg and 21.9 ± 0.7 mmHg to the peak of 116 ± 8 mmHg and 28.5 ± 0.8 mmHg, respectively, at 1.5 min after antigen (Figures 3A, 3B). Along with the decrease in ABF, quasi-PVR significantly increased as early as 4.5 min after antigen (Figures 3C, 3D). After that, MAP and ABF decreased progressively. The quasi-PVR significantly increased from the baseline of 3.1 ± 0.1 to 8.7 ± 0.9 mmHg·min/mL at 15 min after antigen. The decrease in ABF of the ICI 118,551 pre-treated group was significantly greater than that of the sensitized control group at 8–15 min after antigen. The increase in quasi PVR was significantly greater in the ICI 118,551 pre-treated group than the sensitized control group at 10, 15, 25, and 30 min after antigen. In contrast, the responses of the atenolol group to the antigen were similar to that of the sensitized control group, and

TABLE 1. The Basal Level of the Variables Before and After Administration of β -adrenoceptor Antagonists, L-NAME and Indomethacin in Sensitized Mice.

Group	Sensitized control ($n = 6$)		Atenolol ($n = 6$)		ICI 118,551 ($n = 6$)		L-NAME ($n = 6$)		Indomethacin ($n = 6$)	
	before	after	before	after	before	after	before	after	before	after
Mean arterial pressure (mmHg)	86 $\pm$ 5	86 $\pm$ 6	88 $\pm$ 6	76 $\pm$ 6	89 $\pm$ 5	82 $\pm$ 4	94 $\pm$ 5	136 $\pm$ 4**	83 $\pm$ 5	83 $\pm$ 3
Systolic pulmonary arterial pressure (mmHg)	24.3 $\pm$ 0.8	22.7 $\pm$ 0.5	21.8 $\pm$ 0.4	20.5 $\pm$ 0.5#	23.9 $\pm$ 1.1	22.0 $\pm$ 0.7	24.8 $\pm$ 1.8	21.4 $\pm$ 0.8	22.3 $\pm$ 0.9	22.2 $\pm$ 0.6
Aortic blood flow (mL/min)	8.7 $\pm$ 0.4	7.6 $\pm$ 0.5	9.1 $\pm$ 0.4	6.4 $\pm$ 0.3**	9.1 $\pm$ 0.5	7.2 $\pm$ 0.4*	8.5 $\pm$ 0.7	5.1 $\pm$ 0.5#	9.0 $\pm$ 0.7	7.4 $\pm$ 0.8
quasi pulmonary vascular resistance (minHg/mL/min)	2.8 $\pm$ 0.1	3.0 $\pm$ 0.2	2.4 $\pm$ 0.2	3.2 $\pm$ 0.2*	2.6 $\pm$ 0.2	3.1 $\pm$ 0.3	3.0 $\pm$ 0.5	4.4 $\pm$ 0.6#	2.5 $\pm$ 0.2	3.0 $\pm$ 0.3
Heart rate (beats/min)	440 $\pm$ 12	480 $\pm$ 17	440 $\pm$ 13	371 $\pm$ 8**	476 $\pm$ 30	477 $\pm$ 34	461 $\pm$ 24	440 $\pm$ 24	423 $\pm$ 16	432 $\pm$ 28

Note: Values are means $\pm$ S.E.M. In the sensitized control group, drug-free saline was injected. * $P < 0.05$ vs. before administration; #, $P < 0.05$ vs. sensitized control group

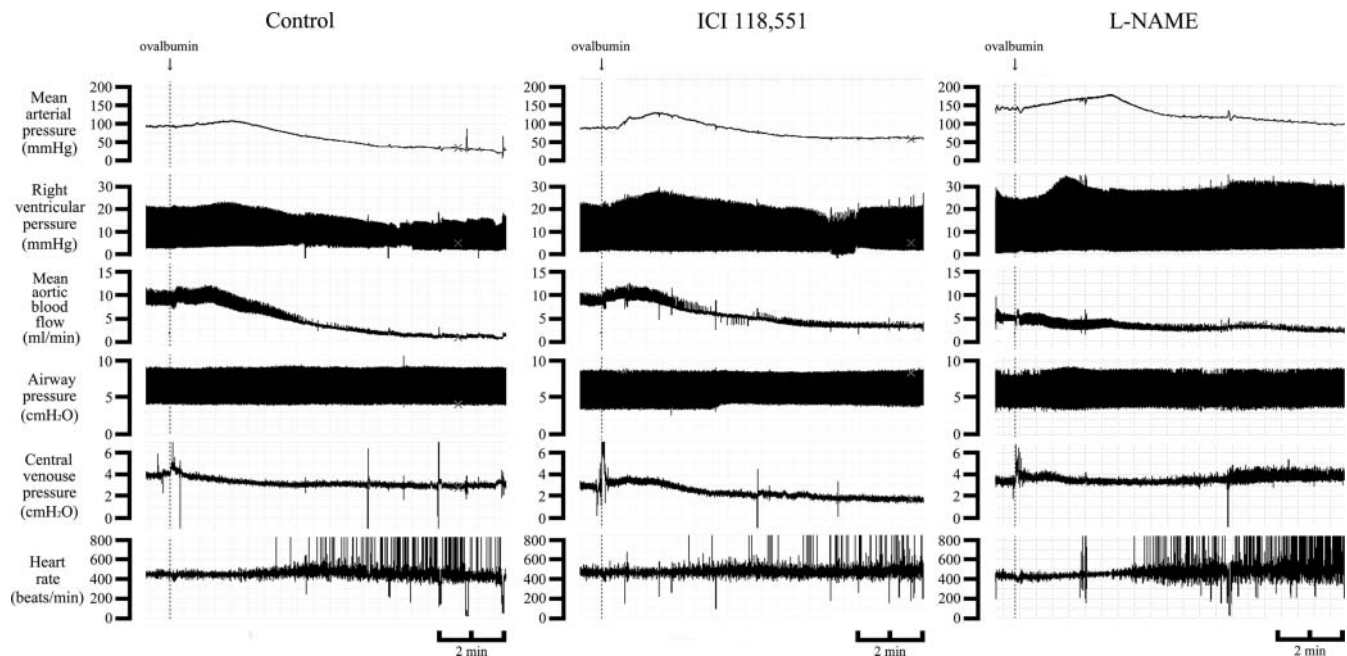


FIGURE 1. The representative recordings of the mean arterial pressure, right ventricular pressure, aortic blood flow, central venous pressure, heart rate, and airway pressure after an intravenous injection of the antigen of ovalbumin (0.01 mg) in a sensitized control, ICI 118,551- and L-NAME-pretreated mouse.

there were no significant differences in the variables between these two groups (Figures 3C, 3D).

In the L-NAME pre-treated group, prior to the injection of the antigen, L-NAME caused systemic vasoconstriction with resultant marked increase in MAP and decrease in ABF, as shown in Table 1. The quasi-PVR, but not systolic PAP, was also increased significantly by pretreatment with L-NAME. The initial responses of systolic PAP and MAP to the antigen in the L-NAME group were greater than those in the sensitized control group. MAP increased from the baseline of 135 ± 4 mmHg to the peak of 162 ± 4 mmHg at 2 min after antigen (Figure 4A). The systolic PAP increased from the baseline of 21.4 ± 0.1 mmHg to peak of 28.9 ± 2.0 mmHg at 2 min, and then this high level of systolic PAP was maintained to the end of the experimental period (Figure 4B). After antigen, ABF was progressively decreased from the baseline of 5.0 ± 0.5 mL/min to 3.0 ± 0.2 mL/min (Figure 4C). Quasi-PVR significantly increased from baseline of 4.4 ± 0.5 to 10.3 ± 1.3 mmHg·min/mL at 10 min after antigen and remained at this high level to the end of the experiment (Figure 4D). In the D-NAME group, there were no significant differences in the measured variables as compared with the sensitized control group (Figure 4).

In the cyclooxygenase inhibitor indomethacin-pretreated group, anaphylactic hypotension occurred in a manner similar to the sensitized control group with no significant differences in any variables mea-

sured between these two groups (Figure 5). The airway pressure did not significantly change throughout the experimental period in any groups studied.

DISCUSSION

In the current study, we examined the pulmonary vascular and bronchial responses to anaphylaxis in anesthetized open-chest BALB/c mice. We also determined the role of β -adrenoceptors, NO, and cyclooxygenase metabolites in modulation of the anaphylactic responses. There are two major findings. First, after the antigen administration systolic PAP and airway pressure did not significantly increase in the sensitized mice. Second, systolic PAP, but not airway pressure, significantly increased after antigen in the sensitized mice pre-treated with ICI 118,551 and L-NAME, but not atenolol or indomethacin. This finding clearly indicates that activation of β_2 -adrenoceptors and NO, but not β_1 -adrenoceptor activation or cyclooxygenase products, attenuates the antigen-induced pulmonary vasoconstriction in anesthetized mice.

Anaphylactic hypotension is primary caused by a decrease in blood flow to the heart [1]. An increased resistance to venous return is important in the pathogenesis of circulatory collapse in anaphylactic shock [2]. We assume that anaphylaxis causes pulmonary vasoconstriction, resulting in increased right heart

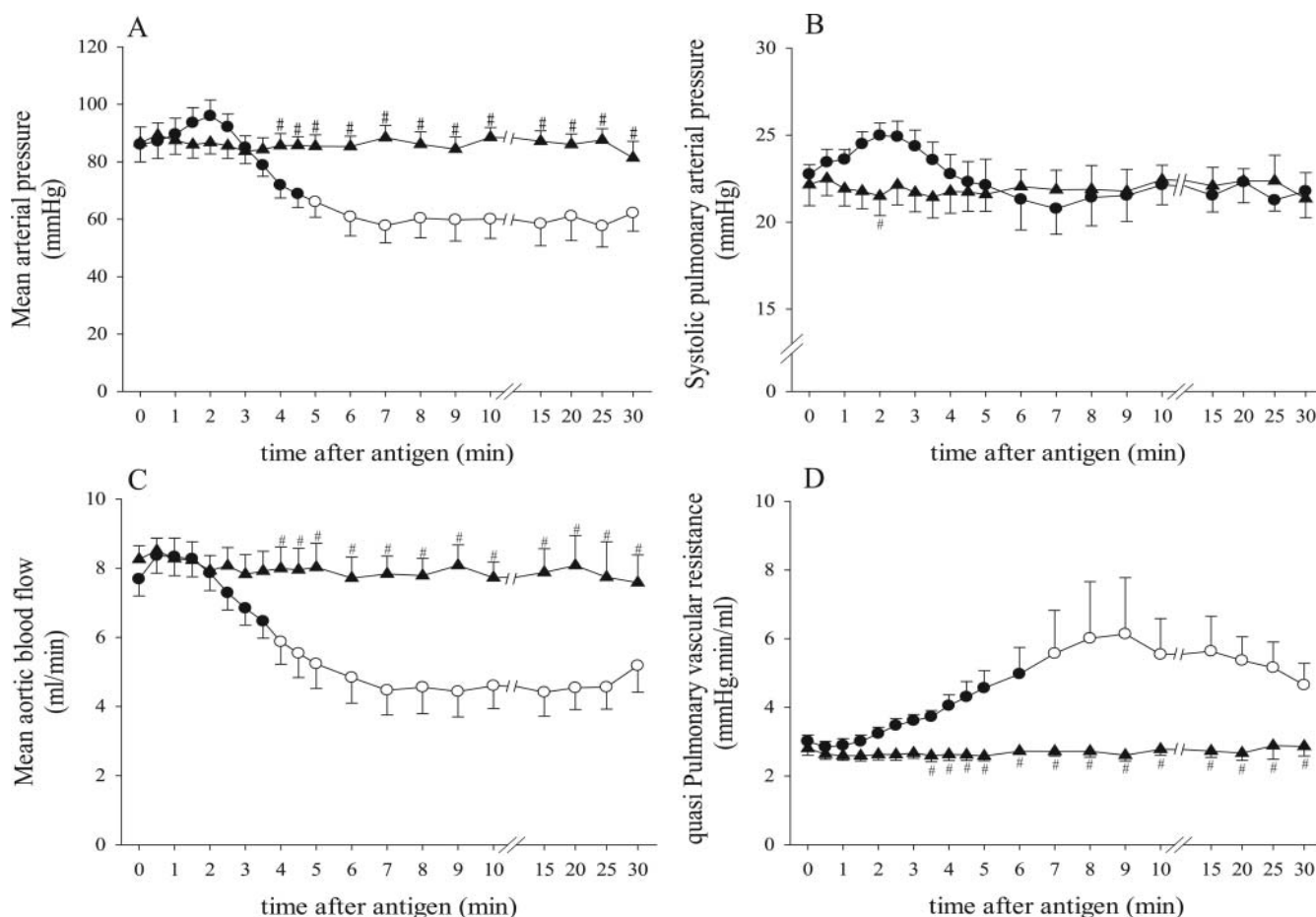


FIGURE 2. The summary of the changes in mean arterial pressure (A), systolic pulmonary arterial pressure (B), mean aortic blood flow (C), and quasi pulmonary vascular resistance (D) after an injection of the ovalbumin antigen. Circle, sensitized control group ($n = 6$); triangle, non-sensitized control group ($n = 6$). Values are means $\pm$ S.E.M.; open symbols, $P < .05$, versus the baseline; # $P < .05$, versus the sensitized control group.

afterload, and then a decrease in venous return, which finally contributes to anaphylactic hypotension. Indeed, it is reported that PAP increased during systemic anaphylaxis in dogs [7, 14], monkeys [5], rabbit [8], and sheep [4]. In contrast, rats do not show any increase in PAP during systemic anaphylaxis [9]. The present study is the first report to demonstrate that systolic PAP did not increase during anaphylactic hypotension in anesthetized mice. This finding indicates that there is no possibility for increased PAP to contribute to the decreased venous return during anaphylactic hypotension in mice. The reason why systolic PAP did not increase in mouse anaphylactic hypotension remains unknown. We speculate that pulmonary vasoconstriction occurred to a small degree so that the anaphylaxis-associated considerable decrease in pulmonary blood flow might have resulted in lack of an increase in systolic PAP. The enhancement of anaphylactic responses by L-NAME and ICI 118,551 indicates that anaphylactic pul-

monary vasoconstriction was attenuated by NO and activation of β_2 -adrenoceptors, as described below.

Bronchospasm sometimes accompanies systemic anaphylaxis in humans [26, 27]. Bronchoconstriction, if occurs in the lower airway during anaphylaxis, might have resulted in regional hypoxia, which could cause hypoxic pulmonary vasoconstriction, and then might have contributed in part to anaphylactic pulmonary vasoconstriction. However, this assumption could not be applied to the mouse anaphylaxis because anaphylactic bronchoconstriction did not occur in mice, as evidenced by the lack of an increase in the airway pressure. The lack of bronchoconstriction also may, in part, account for the absence of increased PAP in mouse anaphylactic hypotension.

We determined the role of β -adrenoceptors in the pulmonary vascular response to anaphylactic hypotension in mice. Significant increases in MAP and systolic PAP were found soon after antigen in the ICI 118,551-pretreated mice, but not atenolol-pretreated

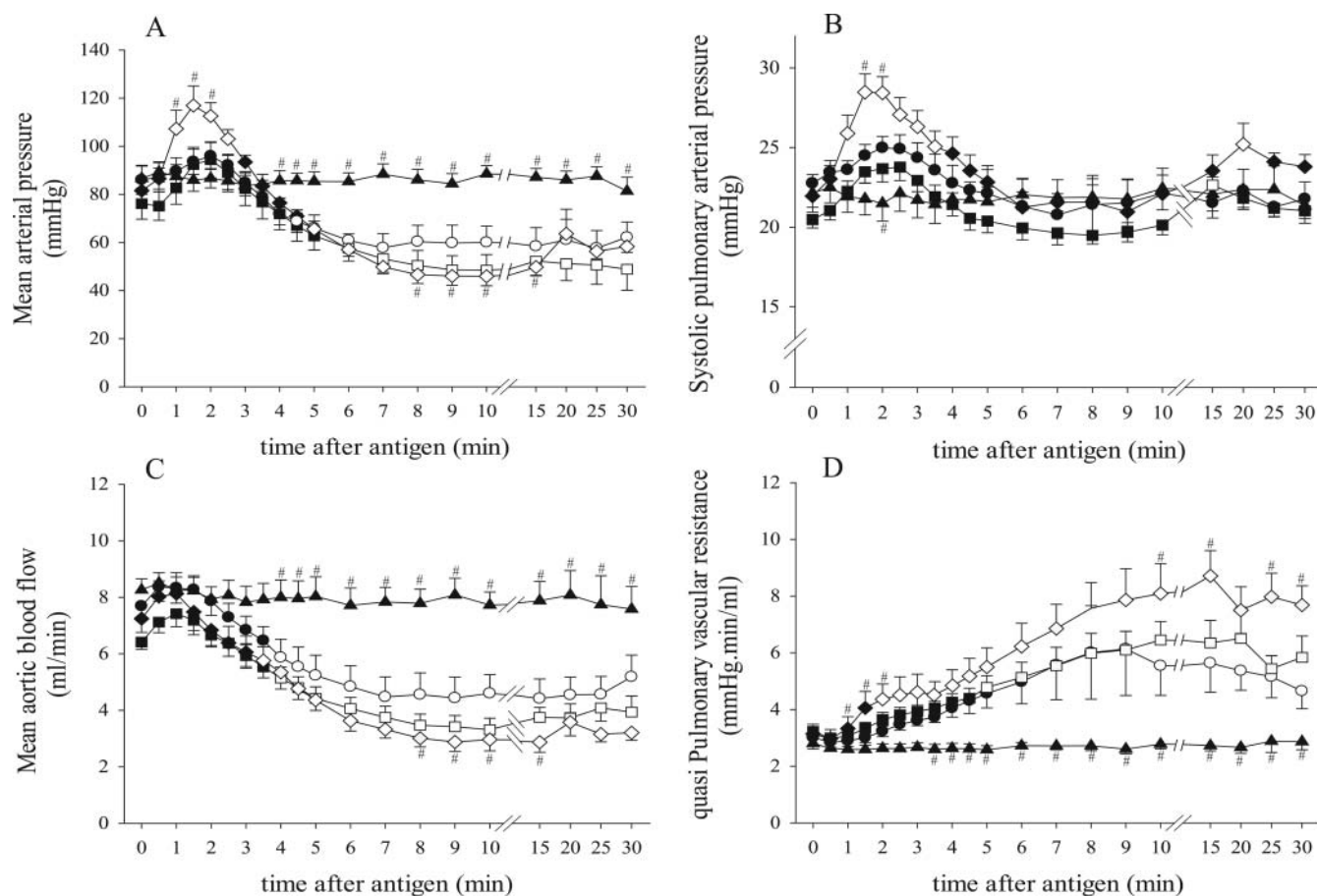


FIGURE 3. The summary of the changes in mean arterial pressure (A), systolic pulmonary arterial pressure (B), mean aortic blood flow (C), and quasi-pulmonary vascular resistance (D) after an injection of the ovalbumin antigen. Circle, sensitized control group ($n = 6$); triangle, non-sensitized control group ($n = 6$); diamond, ICI 118,551-pretreated group ($n = 6$); square, atenolol-pretreated group ($n = 6$). Values are means $\pm$ S.E.M.; open symbols, $P < .05$, versus the baseline; # $P < .05$, versus the sensitized control group.

mice. This result clearly indicates that activation of β_2 -adrenoceptors, rather than β_1 -adrenoceptors, attenuates the antigen-induced pulmonary vasoconstriction. Actually, a pulmonary vasodilator action of β_2 -adrenoceptor activation was reported in mice [10]. This finding is in agreement with our previous report on the effects of β_2 -adrenoceptor antagonists on rat pulmonary circulation during anaphylaxis [9].

Although attenuation of anaphylactic hypotension with L-NAME has been reported previously [16, 18, 28], the present study provided new observation that L-NAME pretreatment enhances anaphylactic pulmonary vasoconstriction, as evidenced by the greater increases in systolic PAP and quasi-PVR in the L-NAME-pretreated group than the control group. This finding indicates that the contribution of activated NO synthesis to attenuation of anaphylactic pulmonary vasoconstriction.

The exact mechanism for an activation of NO synthesis during anaphylaxis in this study remains unclear. However, chemical mediators could increase

NO production during anaphylaxis; most mediators of anaphylaxis, such as histamine [29], leukotrienes [30], thromboxane A_2 [29], and platelet-activating factor [31], all stimulate NO release from the vascular endothelium. Indeed, it has been reported that NO released during mouse systemic anaphylaxis was not produced by iNOS, but by the constitutive isoform eNOS, which may be rapidly phosphorylated and activated via the PI3K/Akt pathway to produce large amounts of NO [16].

The mechanism for the early increases in systolic PAP and quasi-PVR in the ICI 118,551- and L-NAME-pretreated mice is not known, but may be related to a direct effect of anaphylactic chemical mediators on the pulmonary circulation: pulmonary vasoconstriction can be induced by anaphylactic chemical mediators such as serotonin [32], leukotrienes [33–35], platelet-activating factor [35], but not histamine [36, 37]. On the other hand, the late increases in quasi-PVR, as observed in any sensitized groups studied, might be simply due to a

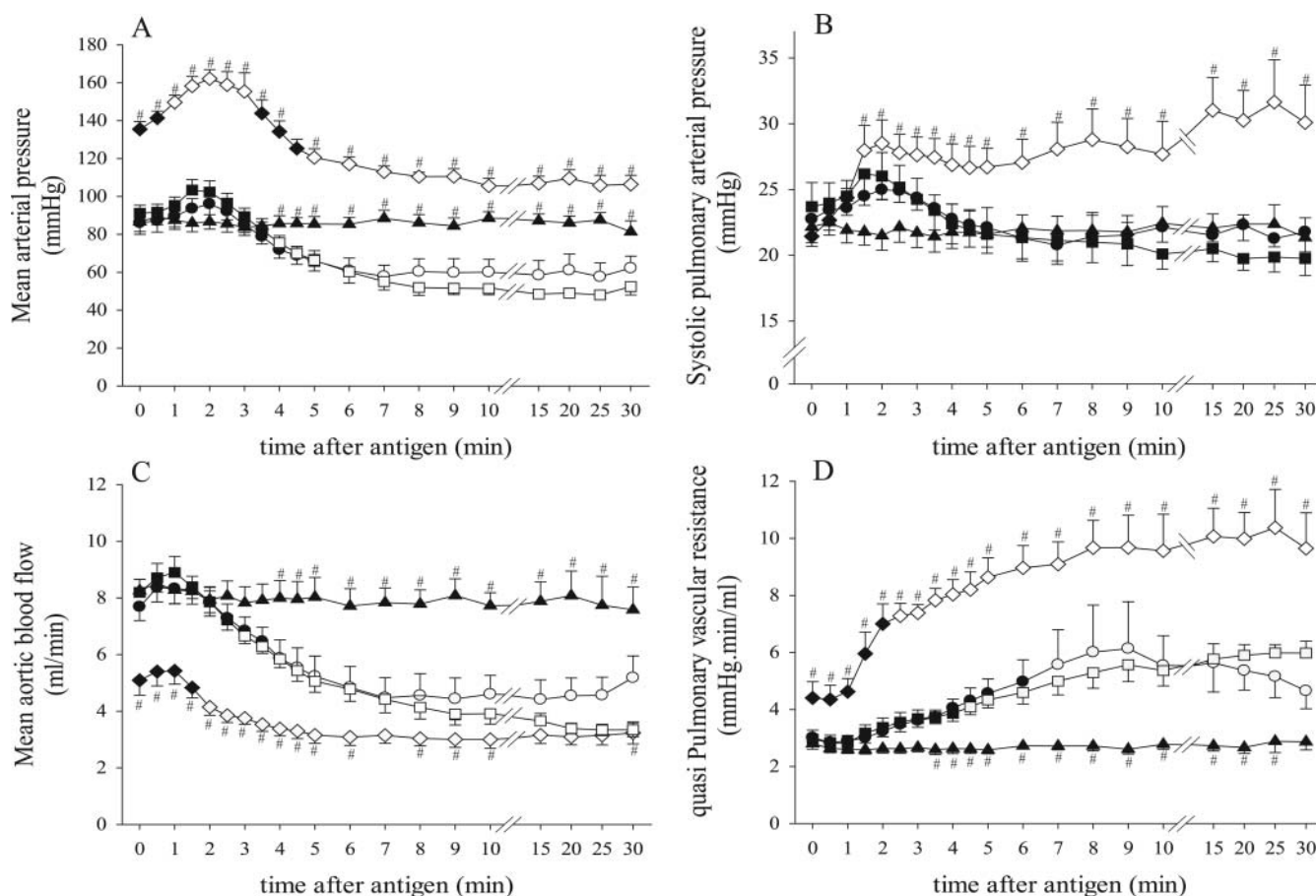


FIGURE 4. The summary of the changes in mean arterial pressure (A), systolic pulmonary arterial pressure (B), mean aortic blood flow (C), and quasi pulmonary vascular resistance (D) after an injection of the ovalbumin antigen. Circle, sensitized control group ($n = 6$); triangle, non-sensitized control group ($n = 6$); diamond, L-NAME-pretreated group ($n = 6$), square, D-NAME-pretreated group ($n = 6$). Values are means $\pm$ S.E.M.; open symbols, $P < .05$, versus the baseline; # $P < .05$, versus the sensitized control group.

passive effect of lower blood flows [7, 38]. As ABF progressively decreased after antigen, zone II conditions of West *et al.* [38] (PAP > alveolar pressure > left atrial pressure) would evolve even in the middle and lower lung regions, resulting in collapse of pulmonary vessels and finally increased quasi-PVR.

It is notable that the increase in PAP for the ICI 118,551- and L-NAME-pretreated mice was small in magnitude: systolic PAP increased only by 7 mmHg after antigen in these two groups. This finding contrast with those of other experimental *in vivo* models of anaphylactic shock in dog [6, 7], monkey [5], rabbit [8], and sheep [4], where PAP increased by 11–16 mmHg after antigen administration. Thus, the anaphylactic pulmonary vasoconstriction is much weaker in mice than in other mammals except rats. The reason why the pulmonary vasoconstrictor response in mice was weak is not known. There is a possibility that the contractile property, or vasoreactivity of mouse pulmonary artery may be inherently weak. It

is well known that hypoxic pulmonary vasoconstriction and vasoconstrictive response to the thromboxane mimetic U46619 was relatively weak in mice [39]. Further experiments are required to examine whether the pulmonary vasoreactivity of mice is weak to other vasoactive substances.

With the roles of cyclooxygenase metabolites in mouse anaphylaxis, we found that inhibition of prostaglandin production did not affect pulmonary vascular response to the antigen. This result was unexpected because the cyclooxygenase inhibitor indomethacin attenuates the anaphylactic pulmonary vasoconstriction in dogs [40], rabbits [41], and sheep [4]. In addition, these previous studies demonstrated that the thromboxane A_2 plays a major role among the cyclooxygenase metabolites in anaphylaxis-related pulmonary vasoconstriction [4, 40, 41]. Species differences may account for the disagreement of the effect of indomethacin in pulmonary anaphylaxis.

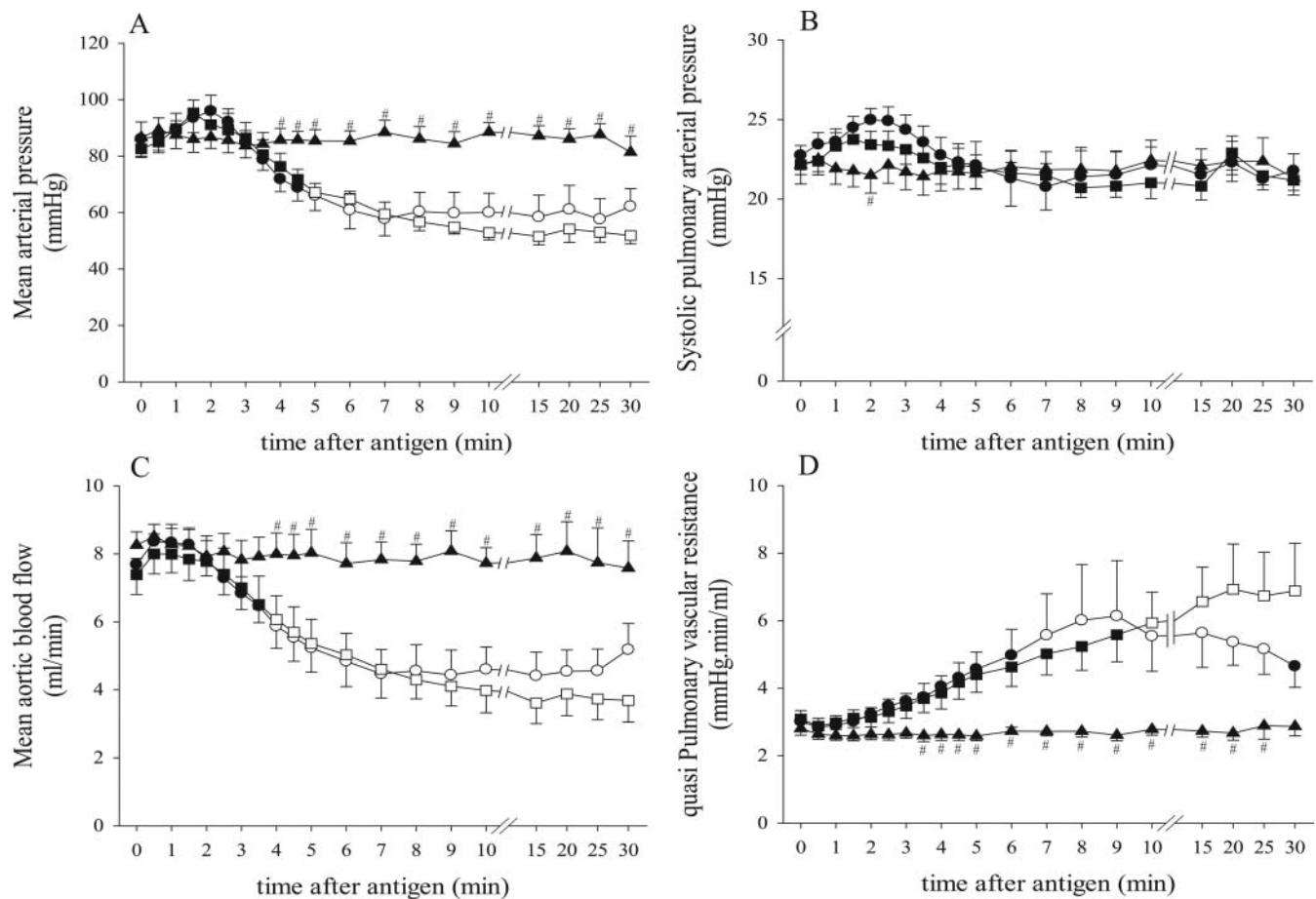


FIGURE 5. The summary of the changes in mean arterial pressure (A), systolic pulmonary arterial pressure (B), mean aortic blood flow (C), and quasi pulmonary vascular resistance (D) after an injection of the ovalbumin antigen. Circle, sensitized control group ($n = 6$); triangle, non-sensitized control group ($n = 6$); square, Indomethacin-pretreated group ($n = 6$). Values are means $\pm$ S.E.M.; open symbols, $P < .05$, versus the baseline; # $P < .05$, versus the sensitized control group.

There are methodological limitations in the present study. First, we substituted the right ventricular pressure as PAP, because the systolic pressure of right ventricle is nearly equal to that of pulmonary artery. Although the systolic PAP is not PAP, the continuous measurement of PAP in mice was difficult in the presence of low ABF of anaphylactic shock. Second, quasi-PVR, which we reported here, was not the correct PVR, for which measurements of mean PAP and left atrial pressure were required. However, it was also too difficult to measure the left atrial pressure or pulmonary arterial wedge pressure in mice. Instead, we assumed the left atrial pressure to be zero (as presented in Equation 1) throughout the experimental period. In mice suffering anaphylaxis, ABF markedly decreased, indicating reduced blood flow to the left atrium and resultant decreased left atrial pressure, if the left heart function was well preserved [2]. Thus, the pressure difference along the pulmonary

artery, i.e., PVR might have been underestimated during anaphylactic hypotension in the present study. Nonetheless, we believe that quasi-PVR determined in the present study, albeit incompletely measured, could provide more useful information on pulmonary vasoconstriction than PAP alone in the presence of decreased pulmonary blood flow of anaphylactic hypotension.

In summary, the antigen-induced increase in PAP was not observed in the control sensitized BALB/c mice, but was clearly observed in those pretreated with either ICI 118,551 or L-NAME, but not atenolol or indomethacin. Airway pressure did not significantly increase in any sensitized mice studied. These findings suggest that pulmonary response to systemic anaphylaxis does not increase the right heart afterload and, therefore, may not contribute to the initial decrease in venous return and anaphylactic hypotension in anesthetized mice. Furthermore,

β_2 -adrenoceptor activation and NO, but not β_1 -adrenoceptor activation or cyclooxygenase metabolites, attenuates the antigen-induced pulmonary vasoconstriction.

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