Pulmonary Vasoconstrictive and Bronchoconstrictive Responses to Anaphylaxis Are Weakened *via* β_2-adrenoceptor Activation by Endogenous Epinephrine in Anesthetized Rats

Wei Zhang, M.Sc.,* Toshishige Shibamoto, M.D., Ph.D.,† Yuhichi Kuda, B.Sc.,‡ Chieko Ohmukai, B.Sc.,‡ Yasutaka Kurata, M.D., Ph.D.§

ABSTRACT

Background: Patients treated with propranolol, a nonselective β-adrenoceptor antagonist, have increased incidence and severity of anaphylaxis. We determined whether β_1- or β_2-adrenoceptor antagonist modulated pulmonary vasoconstriction and bronchoconstriction in rat anaphylactic hypotension.

Methods: Anesthetized ovalbumin-sensitized male Sprague-Dawley rats were randomly allocated to the following pretreatment groups (n = 7/group): (1) sensitized control (nonpretreatment), (2) propranolol, (3) the selective β_2-adrenoceptor antagonist ICI 118,551, (4) the selective β_1-adrenoceptor antagonist atenolol, and (5) adrenalectomy. Shock was induced by an intravenous injection of the antigen. Mean arterial pressure, pulmonary arterial pressure, left atrial pressure, central venous pressure, portal venous pressure, airway pressure, and aortic blood flow were continuously measured.

Results: In either sensitized control or atenolol-pretreated rats, mean arterial pressure and aortic blood flow decreased substantially, whereas pulmonary arterial pressure and airway pressure did not increase soon after antigen injection. In contrast, in rats pretreated with either propranolol, ICI 118,551, or adrenalectomy, airway pressure significantly increased by 14 cm H_2O, and pulmonary arterial pressure by 7.5 mmHg after antigen injection. At 2.5 min after antigen injection, the plasma concentration of epinephrine increased 14-fold in the sensitized rats except for the adrenalectomy group. Portal venous pressure after antigen injection increased by 16 mmHg similarly in all sensitized rats. All of the sensitized control group and two of the atenolol group were alive for 60 min after antigen injection, whereas all rats of the propranolol, ICI 118,551, and adrenalectomy groups died within 50 min after antigen injection.

Conclusions: The pulmonary vasoconstrictive and bronchoconstrictive responses to systemic anaphylaxis were weakened *via* β_2-adrenoceptor activation by epinephrine endogenously released from the adrenal gland in the anesthetized Sprague-Dawley rats.

What We Already Know

- Patients with cardiovascular disease and/or treated with β-adrenoceptor antagonist have an increased risk for development of anaphylactic reactions.

What Is New

- β2-adrenoceptor blockade augments pulmonary hypertension and bronchoconstriction during anaphylaxis.
- These side effects are attenuated by endogenously released epinephrine resulting in activation of β2-adrenoceptors.
- Anaphylactic hepatic vasoconstriction is not modulated by β-adrenoceptors.

IT is reported that patients with preexisting cardiovascular diseases and those treated with propranolol, a β-adrenoceptor antagonist, have increased incidence and severity of anaphylaxis.[1,2] Propranolol is a nonselective β-adrenoceptor antagonist and can inhibit both β_1- and β_2-adrenoceptor. However, it remains unknown which subtype of β-adrenoceptor, β_1- or β_2-adrenoceptor, is primarily involved in the detrimental effect of the β-adrenoceptor antagonists on anaphylactic shock. We have recently demonstrated that inhibition of the β_2-adrenoceptor rather than β_1-adrenoceptor exerts the detrimental action on systemic anaphylaxis of conscious Sprague-Dawley (SD) rats: the survival rate of the β_2-adrenoceptor antagonist ICI 118,551-pretreated rats was much smaller than that of the β_1-adrenoceptor antagonist atenolol-pretreated rats.[3] However,

* Graduate Student, Department of Physiology II, Kanazawa Medical University, Uchinada, Japan, and Associate Professor, Department of Pathophysiology, Medical College of Qinghai University, Xining, China; † Professor, ‡ Research Fellow, § Associate Professor, Department of Physiology II, Kanazawa Medical University.

Received from the Department of Physiology II, Kanazawa Medical University, Uchinada, Japan. Submitted for publication September 7, 2010. Accepted for publication November 10, 2010. This study was supported by a Grant-in-Aid for Scientific Research (20592131) (to Dr. Shibamoto) from the Japan Society for Promotion of Science, Tokyo, Japan, and a Grant for Collaborative Research (C2010-5) (to Dr. Shibamoto) from Kanazawa Medical University, Ishikawa, Japan.

Address correspondence to Dr. Toshishige Shibamoto: Department of Physiology II, Kanazawa Medical University, Uchinada Ishikawa 920-0293, Japan. shibamo@kanazawa-med.ac.jp. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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the mechanism underlying the survival rate decrease is not known. In this study, we investigated the possible contribution of the pulmonary vasoconstriction and bronchoconstriction to the detrimental action of β_2-adrenoceptor antagonists on systemic anaphylaxis in anesthetized SD rats.

Systemic anaphylaxis is sometimes accompanied by pulmonary vasoconstriction and bronchospasm in humans[4] and animals.[5–9] Constriction of the pulmonary vessels and bronchi could increase right heart load, and then augment anaphylactic hypotension. However, it is not well known whether pulmonary vasoconstriction and bronchoconstriction accompany anaphylactic hypotension in *in vivo* SD rats, although they were reported to occur in the anaphylactic response of isolated perfused rat lung.[10] On the other hand, constriction of the pulmonary artery and bronchi can be modulated by β_2-adrenoceptor in rats; activation of β_2-adrenoceptor attenuates contraction of smooth muscle cells of the pulmonary arteries[10] and trachea-bronchial trees.[11] Indeed, β-adrenoceptor agonists are administered in favor of inhibiting pulmonary vasoconstriction and bronchoconstriction in canine systemic anaphylaxis.[12,13] However, the roles of subtypes of β-adrenoceptors in the rat pulmonary vascular and airway responses to systemic anaphylaxis have not been reported. In this study, we hypothesized that inhibition of the β_2-adrenoceptor, rather than of the β_1-adrenoceptor, augments anaphylactic pulmonary hypertension and bronchoconstriction, resulting in the death of the rats suffering from systemic anaphylaxis.

The initial decrease of systemic arterial pressure during anaphylactic shock in rats may be caused by arteriolar dilatation induced by anaphylactic chemical mediators such as histamine, serotonin, and platelet-activating factor.[14,15] The vascular tone of the systemic circulation, or the state of systemic arteriolar constriction, could be reflected by the total peripheral resistance (TPR) in whole animals *in vivo*. However, the investigation on serial changes in TPR by continuously measuring cardiac output during rat systemic anaphylaxis was limited, although cardiac output has been measured by thermodilution[16] or the microsphere method[17] in the rat. These methods might have missed the initial changes in cardiac output immediately after the challenge of the antigen injection. Determination of sequential changes in pulmonary vascular resistance (PVR) as well as in TPR requires continuous measurement of cardiac output at the main pulmonary artery or the ascending aorta with magnetic or ultrasonic blood flow meters.

The first purpose of this study was to determine the PVR and airway pressure (P_{aw}), as well as hemodynamic variables of the systemic circulation such as TPR and portal venous pressure (PVP), during anaphylactic hypotension in anesthetized rats sensitized with ovalbumin. The second purpose was to investigate the effects of β_1- and β_2-adrenoceptor antagonists on the responses of the lung and systemic circulation to anaphylactic hypotension. Mean arterial pressure (MAP), mean pulmonary arterial pressure (PAP), left atrial pressures (LAPs), central venous pressure (CVP), PVP, aortic blood flow (ABF), and heart rate were directly measured in sensitized open-chest rats when the ovalbumin antigen was challenged under the pretreatment with β_1- and/or β_2-adrenoceptor antagonists. We used open chest rats because we directly measured LAP and ABF by putting the blood flow probe at the ascending aorta.

Materials and Methods

Animals

Fifty-seven male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 398 ± 5 g were used in this study. Rats 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 current study were approved by the Animal Research Committee of Kanazawa Medical University.

Sensitization

Rats were actively sensitized by the subcutaneous injection of an emulsion made by mixing complete Freund adjuvant (0.5 ml) and 1 mg ovalbumin (grade V; Sigma Chemical Company, St. Louis, MO) containing physiologic saline (0.5 ml).[18] In the nonsensitized rats, the mixture without ovalbumin was injected. Two weeks after injection, rats were used for the following experiments.

Surgical Preparation

Rats were anesthetized with pentobarbital sodium (50 mg · kg^{-1} IP) and placed supine on a heating pad (ATC-101B; Unique Medical, Tokyo, Japan) that maintained body temperature at 36–37°C. The trachea was cannulated with a stainless steel tube (2 mm ID), and rats were mechanically ventilated (model 683, Harvard Apparatus, South Natick, MA) with a tidal volume of 7 ml/kg, a respiratory rate of 60 breaths/min and a positive end-expiratory pressure of 2.5 cm H_2O. Based on the preliminary study, under this artificial ventilation, Pa_{CO_2} and Pa_{O_2} were kept at 35–40 mmHg and 100–110 mmHg, respectively, throughout the experimental period of 1 h. The P_{aw} was measured through the T-type tube set in the inspiratory line. Every 15 min, to prevent the resorption atelectasis, the lung was transiently inflated by occluding the expiratory line for a short time so that the end-expiratory airway pressure increased by 2.5 cm H_2O. A polyethylene catheter (ID 0.35 mm, OD 0.55 mm) was inserted into the left femoral artery for measurement of MAP and another polyethylene catheter (ID 0.5 mm, OD 0.9 mm) was inserted into the left femoral vein for injections of drugs and saline (4 ml · kg^{-1} · h^{-1}). After a midline incision of the chest, a Micro-Tip Pressure Transducer 2F Catheter (Millar Instruments, Houston, TX) was advanced into the main pulmonary artery through the right ventricle for measurement of PAP. A polyethylene catheter was also placed into the left atrium for LAP measurement. The pulsed Doppler flow probe (MC2PSS, Transonic Systems, Ithaca, NY) was placed on the ascending aorta for continuous mea-

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surement of cardiac output or ABF. After a midline incision of the abdominal wall, a polyethylene catheter (ID 0.4 mm, OD 0.6 mm) was advanced into the portal vein *via* the cecal vein with the tip of the catheter just protruding into 2–3 cm, for continuous measurement of PVP. In the experiment with adrenalectomy, adrenal glands were removed bilaterally before the cecal vein cannulation. Then the abdomen was closed by sutures.

Experimental Protocol

All experimental protocols were carried out in anesthetized open-chest rats. MAP, LAP, CVP, and PVP, as well as P_{aw}, 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 displayed on a thermal physiograph, and also digitally recorded at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia).

The TPR and PVR were calculated using the following equations:

$$TPR = (MAP - CVP)/ABF \quad (1)$$

$$PVR = (PAP - LAP)/ABF \quad (2)$$

The following experimental protocol was used: either the nonselective β-adrenoceptor antagonist propranolol (1 mg/kg; 100 μl; n = 7), the selective β_1 adrenoceptor antagonist atenolol (2 mg/kg; 100 μl; n = 7) or the selective β_2-adrenoceptor antagonist ICI 118,551 (0.5 mg/kg; 100 μl; n = 7) was intravenously administered at 10 min before an intravenous injection of the antigen (0.6 mg) in 300 μl saline. In the sensitized control rats (n = 7), the adrenalectomized rats (n = 7), and the nonsensitized control rats (n = 7), saline alone (100 μl) was injected before administration of the antigen. The doses of the antagonists were previously shown to be effective in inhibiting relevant β-adrenoceptors[19–21]; the doses of ICI 118,551 0.5 mg/kg[19] and atenolol 2 mg/kg[20] were reported to effectively antagonize the action of β_1- and β_2-agonists, respectively, in rats. The dose of propranolol, 1 mg/kg, is known to inhibit both β_1- and β_2-adrenoceptors in rats.[21] The sensitized rats were randomly assigned to one of the aforementioned five groups, except the nonsensitized control group. The variables measured were observed for 1 h after administration of antigen.

All drugs were purchased from Sigma Chemical Company, St Louis, MO. All drugs were dissolved in saline.

Plasma Catecholamine Concentrations

To determine the plasma concentrations of epinephrine and norepinephrine during systemic anaphylaxis, the ICI 118,551-pretreated rats (n = 5), the adrenalectomized rats (n = 5), and the intact rats (n = 5) were used in the separate experiments, in which PAP, MAP, and P_{aw} were also measured in the same way as previously described. At baseline and 2.5 min after antigen administration, blood (2 ml) was sampled from the right jugular vein, and the same volume of saline was intravenously injected for replacement. Blood samples were transferred immediately to chilled tubes containing EDTA, and then centrifuged (1,200*g*, 10 min, 4°C). The plasma samples were separated and stored at −80°C. Plasma catecholamine concentrations were determined by high-performance liquid chromatography with a trihydroxyindole reaction.

Statistics

Results are expressed as mean ± SEM and survival as percentage. The Student *t* test was used to compare the basal variables before and after pretreatment with β-adrenoceptor antagonists. For the analysis of the variables after the antigen injection, intragroup and between-group comparisons were performed using one-way and two-way ANOVA for repeated measures. When a significant difference was observed with the two-way ANOVA, paired comparisons were made within a group and also between groups by using a Bonferroni posttest. Comparison of the plasma catecholamine concentrations before and after the antigen administration was made by the Wilcoxon signed-rank test. Kaplan–Meier survival curves were analyzed by log-rank test. Significance was assumed when *P* value was less than 0.05 (two-tailed). All statistical analyses were performed by StatView, version 5.0 (SAS Institute Inc., Cary, NC).

Results

Table 1 shows the effects of β-adrenoceptor antagonists on the basal levels of the variables. Heart rate decreased significantly after administration of atenolol and propranolol. An injection of ICI 118,551 significantly decreased ABF.

Figure 1 shows representative recordings of the changes in hemodynamic variables and P_{aw} during the first 10 min after an antigen challenge in the sensitized control, propranolol, and adrenalectomy groups, and figure 2 shows the averaged changes in the variables for all groups for 1 h. In the sensitized control group, MAP decreased from the baseline of 102 ± 3 mmHg to 67 ± 4 mmHg at 1 min after the antigen injection, along with decreases in ABF and LAP, as shown in figures 1A and 2. PAP did not change for 2 min after the antigen injection and then decreased significantly as ABF progressively decreased. In contrast, in the propranolol group, PAP significantly increased from the baseline of 14.7 ± 0.2 mmHg to the peak level of 21.8 ± 2.1 mmHg at 2.5 min after antigen administration (figs. 1B and 2). PAP returned to the baseline concentrationat 6 min when ABF decreased progressively to 6.6 ± 0.6 ml/min from the baseline of 34.0 ± 1.6 ml/min. The similar increase in PAP was also observed in the ICI 118,551-pretreated rats, as shown in figure 2C. In parallel with an increase of PAP, PVR significantly increased fivefold in the propranolol and ICI 118,551 groups, at 2.5 min and 4 min, respectively, and then remained increased until 6 min. In figures 2 and 3, when death

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Table 1. The Basal Level of the Variables before and after Administration of β-Adrenoceptor Antagonists in Sensitized Rats

	Sensitized Control (n = 7)		Propranolol (n = 7)		ICI 118,551 (n = 7)		Atenolol (n = 7)		Adrenalectomy (n = 7)	
Groups	Before	After	Before	After	Before	After	Before	After	Before	After
Airway pressure (cm H_2O)	8.4 ± 0.4	8.5 ± 0.5	9.3 ± 0.6	9.9 ± 0.7	9.3 ± 0.4	9.4 ± 0.4	9.3 ± 0.6	9.7 ± 0.9	8.9 ± 0.4	9.0 ± 0.2
Pulmonary arterial pressure (mmHg)	16.2 ± 0.3	15.8 ± 0.3	14.9 ± 0.4	14.7 ± 0.2	15.8 ± 0.5	15.2 ± 0.6	15.6 ± 0.8	14.5 ± 0.5	13.2 ± 1.1	14.6 ± 1.8
Mean arterial pressure (mmHg)	106.4 ± 2.8	101.5 ± 3.1	104.1 ± 7.9	104.8 ± 5.0	108.0 ± 4.5	102.6 ± 3.6	108.1 ± 6.7	110.3 ± 5.3	114.0 ± 4.1	105.7 ± 2.5
Mean aortic blood flow (ml/min)	36.8 ± 2.0	35.4 ± 1.7	36.2 ± 2.2	34.0 ± 1.6	36.3 ± 1.1	32.7 ± 0.9*	36.1 ± 1.9	33.9 ± 0.9	24.6 ± 2.0#	23.0 ± 1.6#
Central venous pressure (mmHg)	3.4 ± 0.5	3.0 ± 0.4	5.0 ± 0.6	4.0 ± 0.4	3.1 ± 0.3	2.6 ± 0.3	3.9 ± 0.5	3.7 ± 0.3	2.1 ± 0.2	2.1 ± 0.2
Left arterial pressure (mmHg)	3.3 ± 0.2	3.1 ± 0.2	3.2 ± 0.2	3.5 ± 0.1	3.2 ± 0.2	3.1 ± 0.2	3.1 ± 0.1	3.3 ± 0.1	2.5 ± 0.1	2.5 ± 0.2
Portal venous pressure (mmHg)	7.2 ± 0.3	6.4 ± 0.3	7.2 ± 0.5	6.3 ± 0.6	6.8 ± 0.2	5.4 ± 0.2	6.6 ± 0.6	5.6 ± 0.4	6.4 ± 0.3	6.0 ± 0.2
Heart rate (beats/min)	399 ± 9	398 ± 10	386 ± 13	333 ± 14*#	414 ± 13	387 ± 13	393 ± 14	338 ± 7*#	418 ± 10	398 ± 18

Values are means ± SEM. In the Sensitized control group and the Adrenalectomy group, saline was injected instead of β-adrenoceptor antagonists.

* $P < 0.05$ *vs.* Before.

# $P < 0.001$ *vs.* the Sensitized control group.

was imminent, the data of rats with low levels of ABF (less than 5 ml/min) and MAP (less than 40 mmHg) were not adopted because of inaccuracy in calculation of vascular resistance. In contrast, at the early time within 6 min after antigen injection, PVR did not change significantly in either the sensitized control or atenolol group, but it gradually increased twofold and fourfold, respectively, at the end of the experimental period as the ABF decreased markedly (fig. 2B). After antigen injection, LAP decreased significantly and similarly in all sensitized rats of any groups. Similar to LAP response, CVP also decreased, although the decrease in CVP was smaller in the propranolol and ICI 118,551 than in other groups.

The P_{aw} response to the antigen was similar to that of pulmonary artery in that P_{aw} did not change significantly in the sensitized control and atenolol groups, whereas it reached peak levels of 23 ± 3 and 24 ± 1 cm H_2O in the propranolol and ICI 118,551 groups, respectively (figs. 1 and 3A). In the adrenalectomy group, the pulmonary hemodynamic and airway responses to antigen were similar to that of the propranolol and ICI 118,551 groups: PAP and P_{aw} increased significantly after antigen injection, whereas the basal ABF was significantly smaller than that of the other groups (figs. 2 and 3).

In all sensitized groups, TPR transiently decreased to 78% of the baseline at 1 min after antigen administration, as shown in figure 3B. Then, TPR increased to the peak of twofold the baseline in both the propranolol and ICI 118,551 groups, and 1.3-fold in the atenolol group, whereas it remained around the baseline levels in the sensitized control group.

In contrast to the pulmonary vascular response, PVP began to increase as early as 20 s after antigen injection, reaching a peak level of 22.4 ± 1.1 mmHg at 2.5 min from the baseline of 6.4 ± 0.3 mmHg. Any β-adrenoceptor antagonists did not significantly affect the antigen-induced increases in PVP, as shown in figure 3D.

In any sensitized rats, heart rate did not change significantly after antigen administration, although the basal heart rate in the atenolol and propranolol groups was significantly smaller than that in the other groups (table 1). In the nonsensitized control group, no variables changed significantly during the experimental period.

Figure 4 shows the survival rate for individual groups. All rats of the propranolol, ICI 118,551, and adrenalectomy groups died within 50 min after antigen, whereas two rats of the atenolol groups, and all seven rats of both the nonsensitized and sensitized control groups, were alive throughout the experimental period of 60 min after antigen injection. The survival rate was significantly smaller in the propranolol, ICI 118,551, and adrenalectomy groups than in the atenolol or sensitized control group.

To clarify the possible roles of endogenous catecholamine in attenuation of the antigen-induced pulmonary vasoconstriction and bronchoconstriction, as previously described, the plasma concentrations of epinephrine and norepinephrine were measured 2.5 min after antigen challenge, when P_{aw} and PAP reached each peak level, in the separate experiments. As shown in table 2, the epinephrine concentrations after antigen increased 14-fold in both sensitized control and ICI 118,551-pretreated rats, whereas those measured before and after antigen were less than 5 pg/ml in

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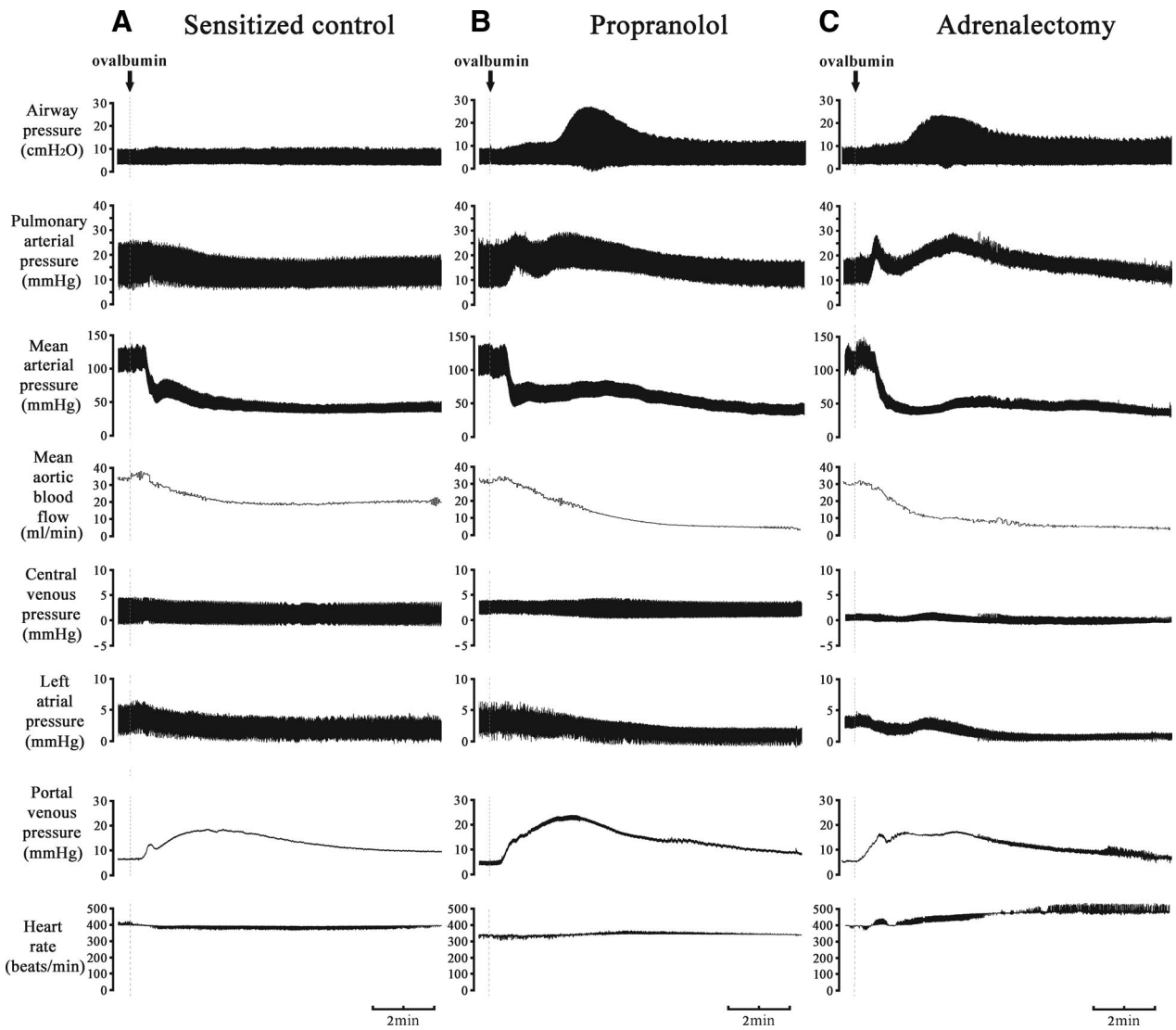

Fig. 1. The representative recordings of airway pressure, pulmonary arterial pressure, mean arterial pressure, aortic blood flow, central venous pressure, left atrial pressure, portal venous pressure, and heart rate after an intravenous injection of the antigen of ovalbumin (0.6 mg) in a sensitized control rat (*A*), a propranolol-pretreated rat (*B*), and an adrenalectomized rat (*C*).

adrenalectomized rats. Norepinephrine similarly increased fourfold in the sensitized control and ICI 118,551 groups, and twofold in the adrenalectomy group. Figure 5 shows the hemodynamic responses of these rats, which were essentially the same as those of the rats without blood sampling: Pretreatment with ICI 118,551 or adrenalectomy caused the antigen-induced increase in PAP and P_{aw}.

Discussion

In the current study, we examined the pulmonary vascular and bronchial responses to anaphylaxis in comparison with the responses of systemic circulation in anesthetized open-chest SD rats. We also determined the role of β-adrenoceptors in these responses. There are three main findings in the current study. First, after the antigen administration either PAP, PVR, or P_{aw} did not significantly increase in the sensitized control rats, whereas these variables substantially increased in the rats pretreated with propranolol, ICI 118,551, or adrenalectomy, but not atenolol. Furthermore, the plasma concentrations of epinephrine increased immediately after antigen injection in the sensitized rats without adrenalectomy, but not with adrenalectomy. These findings clearly indicate that activation of β_2-adrenoceptors, rather than β_1-adrenoceptors, attenuates the antigen-induced pulmonary vasoconstriction and bronchoconstriction, and that epinephrine endogenously released from the adrenal gland is involved in the activation of β_2-adrenoceptors. Second, PVP increased by approximately 20 mmHg in all sensitized rats with and without β-adrenoceptor antagonists, whereas PAP increased only slightly by 7–8 mmHg even in the rats pretreated with propranolol, ICI 118,551, or adrenalectomy. This finding suggests that anaphylactic hepatic venoconstriction is not modulated by β-adrenoceptor, and that the vascular responsiveness of the pulmonary artery to anaphylaxis

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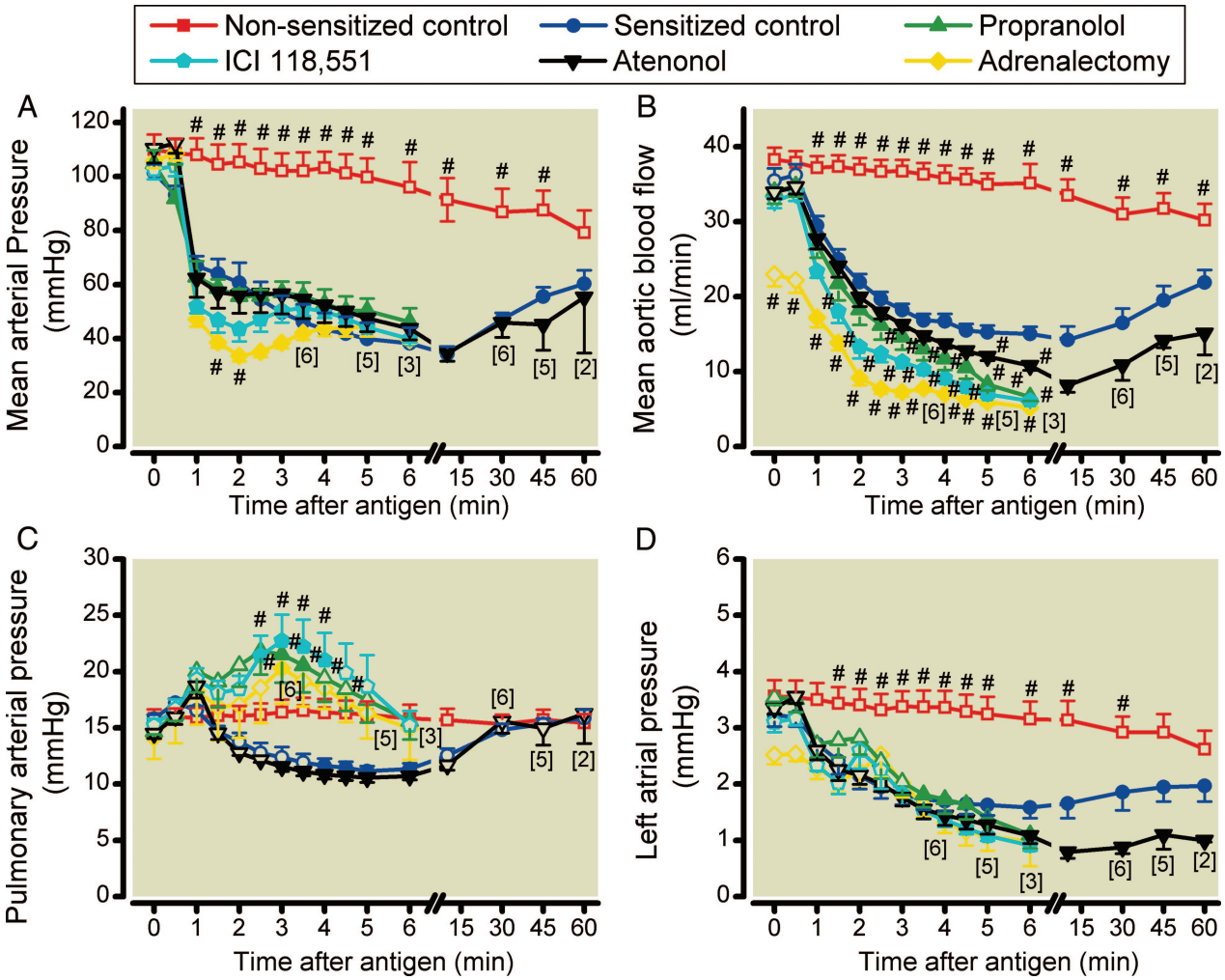

Fig. 2. The summary of the changes in mean arterial pressure (*A*), mean aortic blood flow (*B*), pulmonary arterial pressure (*C*), and left atrial pressure (*D*) after an injection of the ovalbumin antigen. Square, Nonsensitized control group (n = 7); circle, Sensitized control group (n = 7); triangle, Propranolol group (n = 7); pentagon, ICI 118,551 group (n = 7); inverted triangle, Atenolol group (n = 7); diamond, Adrenalectomy group (n = 7). Values are means ± SEM; # $P < 0.001$, *versus* the Sensitized control group; Closed symbols, $P < 0.002$, *versus* the baseline. The numbers in the parentheses indicate the sample numbers of the animals.

is much weaker than that of the portal vein. Finally, continuous measurement of ABF along with MAP and CVP revealed that TPR decreased to 78% of the baseline immediately after antigen, followed by a quick return to the baseline. This finding suggests that the initial decrease of MAP is accounted for at least in part by vasodilatation of the systemic arterioles in rat anaphylactic hypotension.

In the anesthetized SD rats of the current study, we did not find an increase in PAP or pulmonary vasoconstriction in the sensitized control group after antigen injection, although pretreatment with the nonselective β- and selective β_2-adrenoceptor antagonists revealed substantial anaphylactic pulmonary vasoconstriction, as reflected by increases in PAP and PVR (figs. 1 and 2). This finding is in disagreement with that reported in isolated perfused lungs of ovalbumin-sensitized Brown Norway rats, in which PAP increased significantly at a constant flow perfusion in response to the antigen.[22] One of the reasons for this discrepancy may be the rat strain difference: the Brown Norway rat is well known to be very sensitive to anaphylaxis.[23] Another reason is the presence of the sympathetic nervous system in the current anesthetized rats, but not in the isolated perfused lungs[22]: our *in vivo* rats were substantially influenced by the adrenal gland-derived epinephrine, which can exert a vasodilator effect *via* β_2-adrenoceptor. Actually, the plasma epinephrine concentration increased after antigen, and the adrenalectomy clearly caused the antigen-induced pulmonary vasoconstriction in the current study.

However, even in the rats with β_2-adrenoceptor blockade or adrenalectomy, the increase in PAP was as small as 7.5 mmHg. Moreover, in the isolated perfused Brown Norway rat lung, PAP increased only by 1.7–2.2 mmHg.[22] These findings contrast with those of other experimental *in vivo* models of anaphylactic shock in dogs,[7,8] monkeys,[6] rabbits,[9] and sheep,[5] where PAP increased by 11–16 mmHg after antigen administration. This indicates that the anaphylactic pulmonary vasoconstriction is much weaker in rats than in other mammals.

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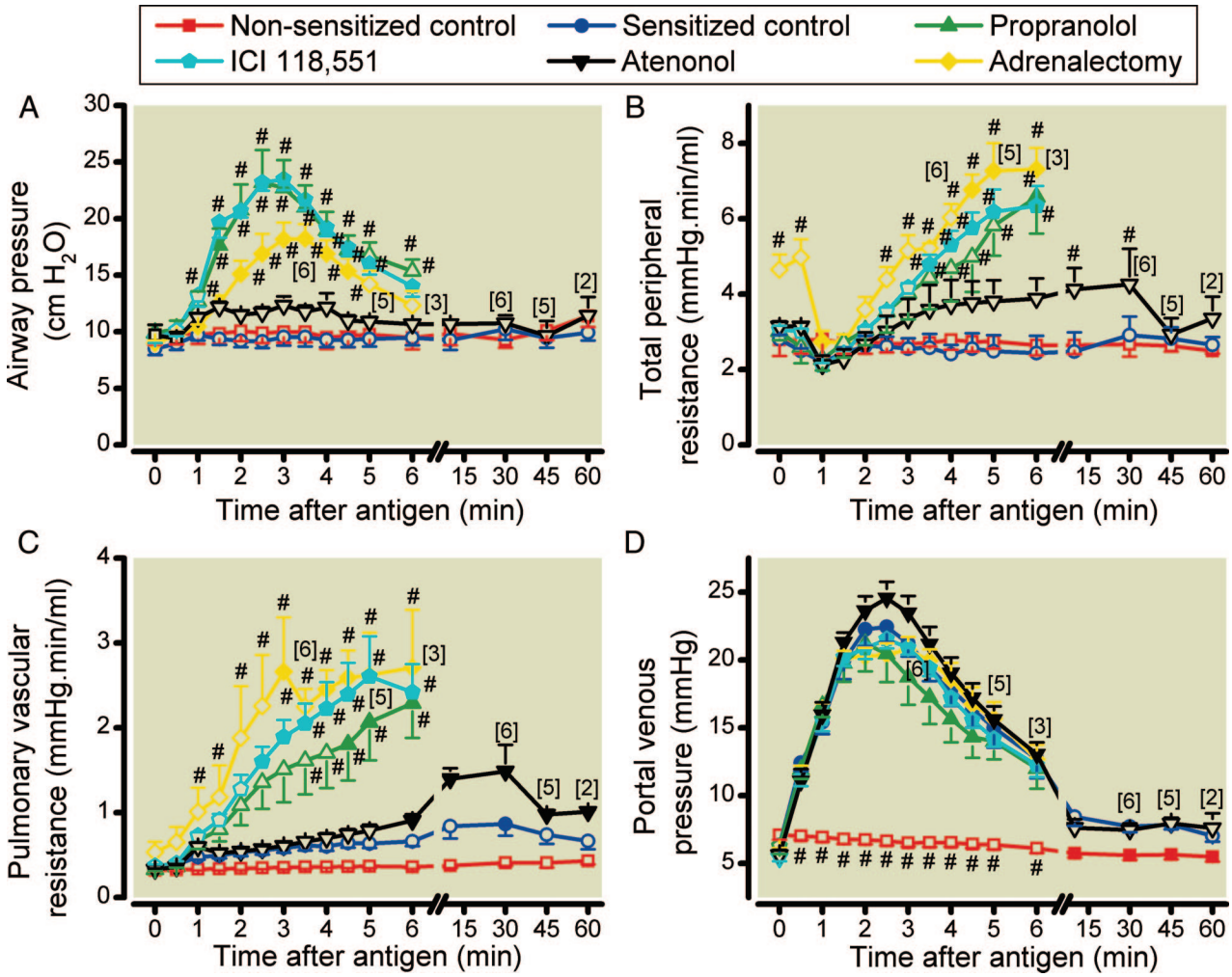

Fig. 3. The summary of the changes in airway pressure (*A*), total peripheral resistance (*B*), pulmonary vascular resistance (*C*) and portal venous pressure (*D*) after an injection of the ovalbumin antigen. Square, Nonsensitized control group (n = 7); circle, Sensitized control group (n = 7); triangle, Propranolol group (n = 7); pentagon, ICI 118,551 group (n = 7); inverted triangle, Atenolol group (n = 7); diamond, Adrenalectomy group (n = 7). Values are means ± SEM; # $P < 0.001$, *versus* the Sensitized control group; Closed symbols, $P < 0.002$, *versus* the baseline. The numbers in the parentheses indicate the sample numbers of the animals.

Substantial increase of PAP, PVR, and P_{aw} was evident after blockade of β_2-adrenoceptors and adrenalectomy. This indicates that β_2-adrenoceptors might have been inherently activated by epinephrine, resulting in relaxation of smooth muscle cells of the pulmonary vessels and airway. We found that the plasma concentration of epinephrine increased 14-fold after antigen injection in the sensitized control and ICI 118,551-pretreated rats and that epinephrine was not detected in the plasma of the adrenalectomized rats. This finding strongly suggests that this increased plasma epinephrine are released from the adrenal glands. Potas *et al.* reported that the sympathetic nervous system was activated as evidenced by increased renal sympathetic nerve activity in SD rat with anaphylactic hypotension.[24] Plasma norepinephrine concentrations also increased after antigen injection. However, the affinity of norepinephrine to β-adrenoceptors is much weaker in comparison with epinephrine.[25] Furthermore, in the adrenalectomized rats, norepinephrine increased albeit to a lesser degree, whereas increases in PAP and P_{aw} were observed, as in the propranolol and ICI 118,551 groups. Thus, it seems unlikely that norepinephrine plays a significant role in activation of β_2-adrenoceptor in the current study.

The mechanism for the increase in PAP and PVR as observed in the adrenalectomized rats and β_2-adrenoceptor antagonist-pretreated rats is not known. We think that the most likely explanation is related to a direct effect of mediators on the pulmonary circulation: Pulmonary vasoconstriction can be induced in rats by anaphylactic chemical mediators such as serotonin,[26] leukotrienes,[27–29] and platelet-activating factor,[29] but not histamine.[30] As another explanation, the increases in PVR observed in our anaphylactic hypotension model might be simply due to a passive effect of lower flows.[31,8] During allergen challenge, as LAP fell, zone II conditions of West *et al.*[31] (PAP > alveolar pressure > LAP) would evolve even in the middle and lower lung regions. PVR would increase due to zonal changes as flow decreased

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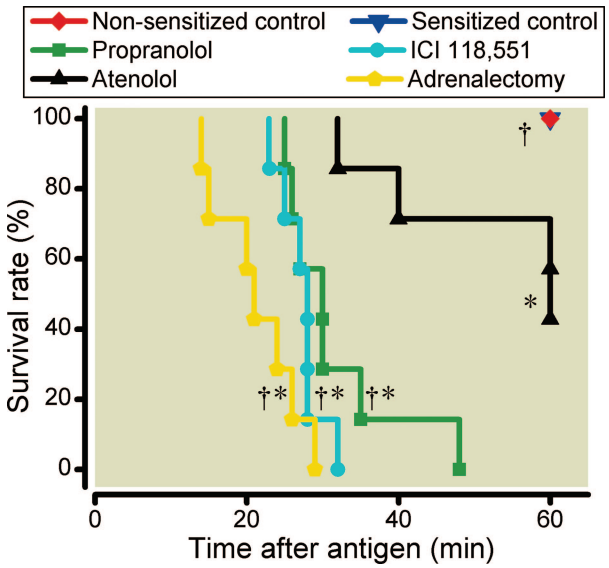

Fig. 4. The survival rates after an injection of the ovalbumin antigen. * $P < 0.05$, *versus* the Sensitized control group; † $P < 0.05$, *versus* the Atenolol group.

during shock. However, this flow-related mechanism seems unlikely because ABF decreased similarly in almost sensitized rats, whereas PVR increased exclusively in rats treated with ICI 118,551, propranolol, and adrenalectomy.

Bronchospasm sometimes accompanies systemic anaphylaxis in humans.[32,33] However, the current study clearly showed that the antigen-induced increase in P_{aw} of the anesthetized SD rats in the sensitized control group was so small that it did not reach statistical significance. This finding was in agreement with the results reported by Sun *et al.*[34] However, allergic bronchoconstriction is definitely observed in the Brown Norway rat,[35] the rat strain most sensitive to anaphylaxis. Based on the experiments of β_2-adrenoceptor antagonist and adrenalectomy, the same process as observed in the pulmonary vasoconstriction might have occurred: endogenous catecholamines from the adrenal glands may attenuate the antigen-induced contraction of bronchial smooth muscles *via* the β_2-adrenoceptor activation. Indeed, catecholamines, especially epinephrine, cause bronchodilatation *via* β_2-adrenoceptor activation.[36] The anaphylactic bronchoconstriction was reported to be mediated mainly by serotonin and leukotrienes in Brown Norway sensitized rats.[35,37]

In this study, the order of the detrimental effects on survival rate was adrenalectomy = propranolol = ICI 118,551 > atenolol. This finding is consistent with that in conscious rats.[3] Similarity between propranolol and ICI 118,551 may be reasonable, because although propranolol is a nonselective β-adrenoceptor antagonist, this agent is actually more β_2-selective rather than β_1-selective.[25]

The mechanism for the deterioration of the survival in anaphylaxis of the rats in the current study by β_2-adrenoceptor inhibition is not known. We assumed that β_2-adrenoceptor antagonists exert detrimental effects on the antigen-induced pulmonary vasoconstriction and bronchoconstriction, as evidenced by the greater increases in PVR and P_{aw} after antigen injection in the ICI 118,551-pretreated rats. Furthermore, β_2-adrenoceptor antagonists may induce substantial vasoconstriction of the heart and skeletal muscles,[38] as evidenced by the increase in TPR. It is known that β_2-adrenoceptors are distributed in the coronary artery and are involved in coronary vasospasm that results in decreased cardiac contractility. The other mechanism, as evoked by β_2-adrenoceptor inhibition, is increased vascular permeability resulting in reduction of effective circulating blood volume and then anaphylactic hypotension.[39]

We clearly demonstrated that TPR decreased immediately after the antigen injection. This suggests that the initial decrease of MAP after antigen is due to arteriolar dilatation. This finding is consistent with the results that the initial ovalbumin-induced hypotension in rat could be ascribed to peripheral vasodilatation caused by combined actions of histamine, serotonin, and nitric oxide.[14] These vasodilating mediators can be released from mast cells, which are mainly responsible for the initial stage of rat anaphylactic hypotension.[40]

There were no significant differences in the antigen-induced increase in PVP among any sensitized rat groups. These findings indicate that any β-adrenoceptor antagonists studied in the current study did not affect acute anaphylactic portal hypertension. This finding in anesthetized rats is consistent with our previous findings in conscious rats.[3] Thus, we confirmed that rat hepatic venoconstriction induced by anaphylactic chemical mediators is not modulated by β-adrenoceptors.

The current study has limitations. The first is related to the open-chest procedure. The open-chest condition eliminates the beneficial pump action of respiration to facilitate

Table 2. The Plasma Concentrations of Epinephrine and Norepinephrine before and after Administration of the Ovalbumin Antigen

	Epinephrine (pg/ml)		Norepinephrine (pg/ml)	
Groups	Before	After	Before	After
Sensitized control (n = 5)	501 ± 351	6,940 ± 3,251*	148 ± 46	563 ± 228*
ICI 118,551 (n = 5)	651 ± 248	8,848 ± 3,548*	100 ± 32	432 ± 135*
Adrenalectomy (n = 5)	N.D.	N.D.	105 ± 19	199 ± 22

Values are means ± SEM.
After = at 2.5 min after an intravenous administration of the ovalbumin antigen; N.D., not detected, *i.e.*, the values are less than 5.0 pg/ml.
* $P < 0.05$ *vs.* Before.

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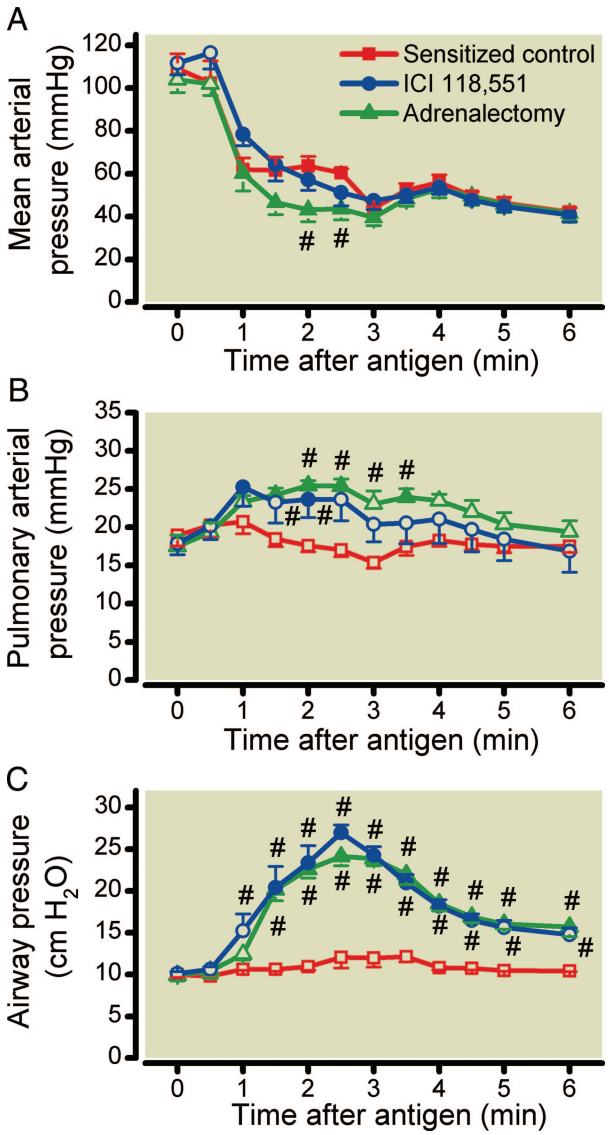

Fig. 5. The summary of the changes in mean arterial pressure (*A*), pulmonary arterial pressure (*B*), and airway pressure (*C*) after an injection of the ovalbumin antigen. Square, Sensitized control group (n = 5); circle, ICI 118,551 group (n = 5); triangle, Adrenalectomy group (n = 5). Values are means ± SEM; # $P < 0.02$, *versus* the Sensitized control group; Closed symbols, $P < 0.02$, *versus* the baseline.

venous return, resulting in a decrease in cardiac output. The negative intrathoracic pressure during inspiration should have increased the flow of blood from the veins into the right heart, because the right atrium is located within the thoracic cavity. Furthermore, for open-chest conditions, positive end-expiratory pressure, which could decrease cardiac output, was required to achieve well ventilation in the current study. However, in this study, the open-chest condition was inevitable for continuous measurement of the cardiac output and LAP, and all rats used were examined in the same condition. In addition, the basal level of cardiac output in the current study was comparable to that of the previous study,[41] which was measured by the same method of an ultrasound flowmetry. The second limitation is that the rat strain used might have influenced the results. In experiments of systemic anaphylaxis in rats, Brown Norway rats are frequently used because of their high sensitivity to anaphylactic reaction,[23] as previously described. The responses of sensitized Brown Norway rats to the antigen were so strong that almost all rats of this strain died within 1 h after antigen administration.[14] In contrast, the sensitized SD rats were alive 1 h or longer after antigen administration, although a substantial decrease in blood pressure was transiently observed,[3,16,18,40] as shown in the current study. We did not measure serum IgE or IgG in this study, which might have helped to define the severity of the allergic reaction more precisely in comparison with other studies.

In summary, we determined the pulmonary vascular and airway responses to anaphylaxis in anesthetized open-chest SD rats, with special references to the influences of the β_1- and β_2-adrenoceptor antagonists. No significant increases in either PAP, PVR, or P_{aw} were found in the presence of increased plasma catecholamine concentration after antigen in the sensitized control rats, whereas all these variables substantially increased after the β_2-adrenoceptor blockade and adrenalectomy. These findings clearly indicate that activation of β_2-adrenoceptors by catecholamines endogenously released from the adrenal gland attenuates the antigen-induced pulmonary vasoconstriction and bronchoconstriction. PVP increased by approximately 20 mmHg in all sensitized rats, whereas PAP increased only slightly by 7–8 mmHg even in the β_2-adrenoceptor antagonist-pretreated rats, again suggesting that the responsiveness of the pulmonary artery to anaphylaxis is much weaker than that of the portal vein.

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