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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) C_4, and prostaglandin (PG) D_2 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 > LTC_4 > serotonin> > histamine = PGD_2. 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 LTC_4 or PGD_2 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, LTC_4 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 LTC_4 or PGD_2, evoke systemic vasodilatation; only serotonin induces airway constriction.

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) C_4 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, LTC_4, and prostaglandin (PG) D_2 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 cmH_2O [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:

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

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

At 20 min after surgery, the baseline measurements were performed. Then PAF, histamine, serotonin, LTC_4, or PGD_2 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, LTC_4, and PGD_2 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, LTC_4, and PGD_2 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 ± 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 cmH_2O.

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 ± 10% and 367 ± 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 ± 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