Bronchial Responsiveness to Inhaled Histamine in Canine Pulmonary Congestion

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To elucidate the role of bronchial hyperresponsiveness (BHR) in pulmonary congestion, an inhaled histamine provocation test was performed in dogs with acute pulmonary congestion, and the role of vagal nerve activity and arachidonic acid metabolites on bronchial responsiveness was evaluated. We assessed BHR with the provocation concentration of histamine causing a 100% increase in pulmonary resistance (PC_{100}) in an open-chest anesthetized and tracheotomized canine model before and after left atrial balloon inflation. Twenty-two mongrel dogs (8–14 kg) were anesthetized with sodium thiopental (15–20 mg/kg) and mechanically ventilated with positive end-expiratory pressure at 3 cmH₂O. A Foley catheter was inserted into the left atrium to cause pulmonary congestion, in which mean left atrial pressure was increased to 18 mmHg. In 6 dogs, histamine provocation was examined before and after pulmonary congestion was effected. Intravenous indomethacin (1 mg/kg) administration and vagotomy were performed in 5 dogs. In pulmonary congestion, PC_{100} was significantly decreased both before and after vagotomy and after indomethacin administration. We conclude that pulmonary congestion augments bronchial responsiveness to inhaled histamine and that neither vagotomy nor indomethacin administration prevents bronchial hyperresponsiveness in pulmonary congestion. These findings suggest that bronchial hyperresponsiveness in pulmonary congestion is related to another factor such as bronchial edema.

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Key Words: Bronchial hyperresponsiveness; Pulmonary congestion; Vagal nerve; Arachidonic acid metabolite

Bronchial hyperresponsiveness (BHR) has been reported in patients with congestive heart failure^{1–5}. Coughing and wheezing typically occur during the night and thus BHR has also been called cardiac asthma. Although central blood pooling caused by the prolonged supine posture at night has long been postulated, the precise mechanism is still unknown. We have previously found⁶ that BHR is enhanced in patients with mitral valve stenosis after they have been in the supine position for 2 h and that the supine posture may play an important role in the pathogenesis of cardiac asthma, suggesting that pulmonary congestion might be responsible for BHR in mitral valve disease. To our knowledge, however, there has been no experimental study on the effects of pulmonary vascular congestion on BHR, except 1

report⁷ indicating that pulmonary congestion might cause BHR through the vagal reflex. As the model in that study consisted of only 1 min of pulmonary congestion, this was not a true cardiac asthma model. Furthermore, it is not known whether vagotomy prevents BHR completely in more prolonged pulmonary congestion.

In several cardiac disorders, especially myocardial ischemia, thromboxane A₂ is released from platelets⁸ and might influence bronchial tone. In the present study, to clarify the effects of prolonged pulmonary vascular congestion on bronchial responsiveness, the relationship between BHR and the vagal nervous system, and the effect of prostanoids on BHR after pulmonary congestion, we applied an inhaled histamine provocation test to a 1-h canine pulmonary

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congestion model after vagotomy and after indomethacin administration.

Materials and Methods

Animal Preparation

Twenty-two mongrel dogs, weighing 8–14 kg, were anesthetized intravenously with sodium thiopental (15–20 mg/kg); half of this dose was then administered every 30 min to maintain anesthesia. After tracheotomy the dogs were ventilated with a constant-volume ventilator with a tidal volume of 15–20 ml/kg and 17 breaths/min, and with a positive end-expiratory pressure of 3 cmH₂O. Thoracotomy was performed and a Foley catheter was inserted into the left atrium via the left atrial appendage. Before each histamine provocation test, 1 mg/kg propranolol was administered intravenously every 30 min to prevent the effect of intrinsic catecholamine. The use of animals and the experimental protocol were approved by the Animal Welfare Committee of Kobe University School of Medicine.

Pulmonary resistance (RL) was calculated from the airflow determined by connecting a pneumotachometer (Nihon Koden, Tokyo, Japan) to the tracheal cannula, and the intratracheal pressure was measured from a sidehole catheter with the tip placed 5 mm distal to the end of the tracheal cannula, using the subtraction method⁹

Histamine Inhalation Test

To obtain histamine dose-response curves, baseline RL was determined before saline inhalation. Then 5 breaths, each 500 ml of air, of histamine aerosol were delivered to the airways. Although the ventilator was off during histamine inhalation, the dogs were ventilated with the nebulized air. The dose was increased by successively tripling the concentrations of histamine, from an initial concentration of 0.1 mg/ml to a final concentration of 10 mg/ml. We assessed the provocation concentration of histamine causing a 100% increase in RL (PC₁₀₀).

Study Protocol

Effect of Vagotomy on Bronchial Responsiveness in Pulmonary Congestion

In 6 dogs, histamine provocation tests were performed before and 1 h after maintaining pulmonary congestion, in which mean left atrial pressure was elevated to 17–18 mmHg by inflating the intra-atrial balloon. Thereafter, bilateral vagotomy was performed in each dog to assess the role of vagal nerves in BHR with and without pulmonary congestion. In 5

control dogs, histamine provocation was performed in the same protocol except that pulmonary congestion was not maintained.

Effect of Indomethacin Administration on Bronchial Responsiveness in Pulmonary Congestion

Thirty minutes before the first histamine inhalation test, indomethacin (10 mg/kg)¹⁰ was administered intravenously at a concentration of 1 mg/ml every 4 h, and vagal nerves were cut off to assess the role of arachidonic acid metabolites in BHR. A histamine provocation test was performed again in 5 dogs 1 h after causing pulmonary congestion, in which mean left atrial pressure was elevated as in the first study protocol. In the 5 control dogs, histamine provocation was performed according to the same protocol except that pulmonary congestion was not induced.

Histologic Examination

Histologic examinations were carried out in 3 dogs to investigate the effect of pulmonary congestion 60 min after left atrial balloon inflation. Their right lungs were excised for examination by light microscopy. The lungs were inflated via the bronchus with 10% formalin solution at 25 cmH₂O. The samples for light microscopy were embedded in paraffin and stained with hematoxylin and eosin.

Statistical Analysis

Results are presented as mean values $\pm$ SD. Statistical evaluations were performed by Student's *t* test for paired data. A *p* value <0.05 was considered significant.

Results

Effect of Vagotomy on Bronchial Responsiveness to Inhaled Histamine in Pulmonary Congestion

Fig 1 shows the histamine dose-response curves of all 6 dogs with pulmonary congestion. In 4 out of 6 cases, the curves in the presence of pulmonary congestion were shifted to the left compared with those obtained during control provocation, indicating the existence of BHR in pulmonary congestion. The histamine dose-response curves after vagotomy in pulmonary congestion are shown in Fig 2. Three of the 6 curves obtained in the presence of pulmonary congestion were also shifted to the left compared with those obtained during control provocation, indicating the continuation of BHR in pulmonary congestion after vagotomy. On the other hand, the histamine dose-response curves before and after vagotomy without pulmonary congestion indicated tachyphylaxis in the histamine provocation test without pulmonary congestion.

Fig 1. The histamine dose-response curves of all 6 dogs with pulmonary congestion. The broken lines show the dose-response curves in the presence of pulmonary congestion, and the continuous lines show those obtained in the absence of pulmonary congestion.

The results of the histamine inhalation test before and after procedure vagotomy are summarized in Table 1. PC_{100} in the presence of pulmonary congestion was significantly decreased both before and after vagotomy compared with that in the absence of pulmonary congestion. Baseline pulmonary resistance with pulmonary congestion was not significantly different from that without pulmonary congestion.

Intra-atrial balloon inflation resulted in a fall in systemic arterial pressure, but it was not significant (131 ± 22 mmHg and 124 ± 28 mmHg before and after left atrial balloon inflation, respectively). There was no significant difference in heart rate before and after left atrial balloon inflation (150 ± 11 beats/min and 146 ± 10 beats/min, respectively).

Effect of Intravenous Indomethacin Administration on Bronchial Responsiveness in Pulmonary Congestion

Fig 3 shows the histamine dose-response curves in all 5 dogs with pulmonary congestion after vagotomy and indomethacin administration. Three of the 5 curves were shifted to the left compared with those obtained in control animals. In the control study without pulmonary congestion, all curves obtained in the second histamine provocation test were shifted to the right compared with those obtained in the first provocation.

The results of the histamine inhalation test after vagotomy and indomethacin administration are shown in Table 2. In the dogs with pulmonary congestion after vagotomy and indomethacin treatment, the PC_{100} was significantly decreased compared with that obtained in animals without pulmonary congestion,

Fig 2. The histamine dose-response curves after vagotomy in pulmonary congestion. The broken lines show the dose-response curves in the presence of pulmonary congestion, and the continuous lines show those obtained in the absence pulmonary congestion.

Table 1 Histamine Provocation Test Before and After Vagotomy With Pulmonary Congestion

	Before vagotomy		After vagotomy	
	RL (cmH ₂ O/L/sec)	Log PC ₁₀₀ (mg/ml)	RL (cmH ₂ O/L/sec)	Log PC ₁₀₀ (mg/ml)
Pulmonary congestion (n=6)				
Control	2.21±0.63	-0.38±0.39	2.25±0.76	-0.07±0.68
Congestion	2.48±0.83	-0.57±0.45*	2.27±0.72	-0.36±0.57#

RL, pulmonary resistance; PC₁₀₀, provocation concentration of histamine causing a 100% increase in pulmonary resistance.

Values shown are means±SD.

*p<0.05 compared with control before vagotomy using paired t test.

#p<0.05 compared with control after vagotomy using paired t test.

indicating an increase in BHR with no contribution from the vagus and arachidonic acid metabolites. Baseline RL in the pulmonary congestion study after

vagotomy and indomethacin treatment was not significantly different from that without pulmonary congestion after vagotomy and indomethacin treatment.

Fig 3. The histamine dose-response curves of all 5 dogs with pulmonary congestion after vagotomy and indomethacin administration. The broken lines show the dose-response curves in the presence pulmonary congestion, and the continuous lines show those obtained in the absence pulmonary congestion.

Table 2 Histamine Provocation Test After Vagotomy and Indomethacin Administration

	RL (cmH ₂ O/L/sec)	Log PC ₁₀₀ (mg/ml)
<i>Pulmonary congestion study (n=5)</i>		
Control	2.72±0.69	0.12±0.21
Congestion	2.81±0.63	-0.03±0.27*

Abbreviations are defined in Table 1.

Values shown are means±SD.

**p*<0.05 compared with control using paired *t* test.

Results of the Control Study

The results of the histamine inhalation test in the control study are summarized in Table 3. PC₁₀₀ and RL did not change significantly during the first and second histamine provocation tests. However, PC₁₀₀ in the second histamine inhalation test was significantly increased compared with that in the first provocation

after vagotomy and indomethacin administration, indicating the existence of tachyphylaxis to inhaled histamine.

Histologic Analysis

The alveoli and alveolar ducts were well inflated and the airways clear in the control lung. There was no evidence of excess fluid in the perivascular and peribronchial connective tissue spaces and no alveolar exudative fluid was present. The alveolar walls were not thickened. The lungs from dogs subjected to left atrial pressure elevation for 60 min exhibited distended veins and packed red cells in capillaries. The perivascular connective tissue was loose and perivascular lymphatics were dilated.

Discussion

Our results suggest that acute pulmonary congestion

Table 3 Histamine Provocation Test in Control Dogs

	Before vagotomy		After vagotomy	
	RL (cmH ₂ O/L/sec)	Log PC ₁₀₀ (mg/ml)	RL (cmH ₂ O/L/sec)	Log PC ₁₀₀ (mg/ml)
<i>Control studies (n=6)</i>				
first	2.97±0.73	-0.17±0.34	2.84±1.02	-0.13±0.48
second	3.01±0.86	-0.21±0.38	3.04±1.32	0.20±0.48*
<i>Indomethacin administration (n=5)</i>				
first			2.41±0.55	-0.31±0.70
second			2.63±0.65	-0.01±0.83*

RL, pulmonary resistance; PC₁₀₀, provocation concentration of histamine causing 100% increase in pulmonary resistance.

Values shown are means±SD.

**p*<0.05 compared with the first provocation after vagotomy using paired *t* test.

causes BHR in response to inhaled histamine, and that vagotomy and intravenous indomethacin administration do not completely prevent BHR in a canine experimental model of pulmonary congestion.

Kikuchi et al⁷ examined the effect of the vagal nerve on bronchial responsiveness in a canine experimental model of pulmonary congestion. After acute brief pulmonary congestion created by inflating an intra-atrial balloon to raise left atrial pressure to 20–30 cmH₂O for 1 min, bronchial responsiveness to inhaled histamine was enhanced compared with the control study. This phenomenon could not be observed after vagotomy. The authors concluded that pulmonary congestion may cause BHR through the vagal reflex. In our present study, however, vagotomy did not completely prevent BHR in the presence of pulmonary congestion. This discrepancy between their results and ours could be due to the following reasons. The first possible reason is the difference in duration of inflation of the left atrial balloon. Kikuchi et al⁷ inflated the balloon for only 1 min, whereas in our case inflation lasted 1 h. It is possible that pulmonary congestion for 1 min stimulates type J and irritant receptors in the lungs but does not cause airway edema. We maintained pulmonary congestion for 1 h because we intended the present study to be a model of cardiac asthma in congestive heart failure. The second possible reason is the magnitude of elevated left atrial pressure. Kikuchi et al raised left atrial pressure to 20–30 cmH₂O, whereas we raised it to 17–18 mmHg. Nakata et al¹¹ from our laboratory, have previously reported that pulmonary congestion occurs within 15 min but that pulmonary interstitial edema occurs 30–60 min after left atrial pressure is elevated to more than 25 mmHg. In general, alveolar edema could occur after elevation of left atrial pressure to more than 20 mmHg (about 27 cmH₂O). We adopted 17–18 mmHg to prevent alveolar edema.

The mechanisms responsible for BHR in pulmonary congestion are not well determined. Several mechanisms may be considered in the pathogenesis of BHR in pulmonary congestion. The first mechanism is airway edema. The pathologic findings in this study revealed mild bronchial edema 1 h after pulmonary congestion was effected. Although bronchial edema induces an increase in BHR,¹² we could not detect an elevation of baseline RL after pulmonary congestion. In 1972 Hogg et al¹³ showed that pulmonary congestion mainly caused an increase in resistance in the peripheral airway rather than the central airway, because of a passive effect of vascular engorgement. In 1978 Gray et al¹⁴ also reported that dynamic compliance decreased in pulmonary congestion with no increase in RL, suggesting an increase in peripheral airway resistance. Nakata et al¹¹ reported that peribronchovascular edema was evident, especially around the bronchioles and small vessels of the lung, 60 min after pulmonary congestion in a canine experimental model. We have previously reported⁴ that BHR in patients with mitral valve disease might be responsible for the airway caliber, and that improvement in BHR after mitral valve replacement might be due to an improvement in bronchial edema. Therefore, we suggested that airway edema might play an important role in BHR in pulmonary congestion.

The second mechanism is vagally mediated bronchial tone, which is related to the type J receptors and/or pulmonary C fibers in the pulmonary interstitium.^{15,16} We have previously reported⁴ that bronchial responsiveness to inhaled methacholine is related to pulmonary artery wedge pressure. In an experimental study,¹⁷ transient bronchoconstriction mediated by the vagal reflex was shown to occur in the presence of pulmonary congestion caused by raising left atrial pressure to 30 mmHg, as evaluated by a retrograde catheter with forced oscillation. Pulmonary congestion

stimulates type J receptors and raises vagal tone, resulting in aggravation of BHR. Vagotomy, however, did not completely prevent an increase in BHR in our pulmonary congestion model. We also reported previously,¹⁸ in a clinical investigation, that vagal activity might contribute less to BHR in congestive heart failure than in bronchial asthma. We suggest that the vagal pathway does not play a role in the pathogenesis of BHR in our pulmonary congestion model.

The other proposed mechanism is an increase in bronchospasmogens, such as arachidonic acid metabolites released from the endothelium and/or pulmonary parenchymal cells.¹⁸ However, in our pulmonary congestion model intravenous indomethacin administration did not prevent BHR. Kikuchi et al⁷ showed that the increase in RL caused by pulmonary congestion declined rapidly with relief of congestion, suggesting that the increase in RL and bronchial responsiveness in congestion were caused by neural rather than humoral factors. In this study we assumed that arachidonic acid metabolites play a little role in BHR in pulmonary congestion.

As intra-atrial balloon inflation always resulted in a fall in systemic arterial pressure, this reduction in blood pressure could influence airway response through the arterial baroreflex²⁰ and/or through endogenous catecholamines.²¹ In addition, left atrial balloon inflation could cause low cardiac output. As a result of the slow elimination of provoked materials caused by reduced blood flow in submucosal capillaries, the severity of BHR in pulmonary congestion might be higher than in the control study. However, the neural reflex and catecholamine products have bronchodilatory rather than bronchoconstrictory effects, which would underestimate the effect of congestion on RL. On the other hand, as a beta antagonist was employed in the present study, the beta receptor-blocking action of catecholamines might increase bronchial tone. In our experiment, a marked decrease in systemic blood pressure was not observed throughout the procedure and the amount of beta antagonist used was as recommended in a published experimental study.⁷

The limitations of the present study need to be mentioned. First, having a small sample size, we could not exclude a type 2 error. However, we suspect that this is unlikely because the dogs with pulmonary congestion showed a significant decrease in PC₁₀₀, whereas the dogs without pulmonary congestion showed no significant change in bronchial responsiveness. These results strongly suggest that BHR exists in pulmonary congestion both with and without vagotomy. Second, we did not evaluate histologic changes in

the lungs before and after pulmonary congestion, and we speculated on the contribution of bronchial edema to BHR in pulmonary congestion. Third, we performed this study in an open-chest anesthetized and tracheotomized canine model. There might be a difference between anesthetized open-chest and awake closed-chest models. Finally, as the 1 h of pulmonary congestion was repeated twice in each animal in the first protocol, it appears that postvagotomy hyperresponsiveness might be influenced by the previous 1 h of pulmonary congestion. While the second challenge after vagotomy in the control study revealed tachyphylaxis to histamine, postvagotomy hyperresponsiveness with pulmonary congestion is considered to be reliable. Furthermore, PC₁₀₀ after vagotomy without pulmonary congestion was significantly increased in comparison with that before vagotomy without pulmonary congestion (Table 1), whereas PC₁₀₀ did not significantly change before and after vagotomy in the control study. These results indicate the influence of pulmonary congestion on the next procedure. However, bronchial responsiveness after vagotomy without pulmonary congestion was decreased compared with that before vagotomy, suggesting that we should exclude the effects of an increase in the bronchial baseline tone on BHR after vagotomy with pulmonary congestion. If we correct the variables of PC₁₀₀ after vagotomy according to the tachyphylaxis of the control study (the difference between the first and the second provocation after vagotomy in the control study was 0.33; Table 3), bronchial responsiveness with pulmonary congestion increased 4-fold compared with that without pulmonary congestion.

We conclude that acute pulmonary congestion augments bronchial responsiveness to inhaled histamine and that vagotomy and indomethacin do not completely prevent BHR in pulmonary congestion. These findings suggest that BHR in acute pulmonary congestion is related to another factor such as geometric change associated with bronchial edema.

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