Respiratory-related bronchial rhythmic constrictions in the dog with extracorporeal circulation

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Kondo, Tetsuri, Ichiro Kobayashi, Naoki Hayama, Gen Tazaki, and Yasuyo Ohta. Respiratory-related bronchial rhythmic constrictions in the dog with extracorporeal circulation. J Appl Physiol 88: 2031–2036, 2000.—Respiratory-related bronchial rhythmic constriction was quantitatively analyzed in eight paralyzed dogs. The caliber of the fifth-generation bronchus was continuously measured as the pressure (Pbr) of a balloon-tipped catheter under the condition of complete immobilization due to extracorporeal oxygenation. Pbr changed rhythmically in synchrony with phrenic nerve activity (PNA) bursts. Rhythmic bronchial constriction started at 1.4 ± 0.49 (SD) s after onset of PNA, reached a maximum level at 2.8 ± 1.6 s after termination of PNA, and then decreased exponentially with a time constant of 6.9 ± 2.5 s. When the respiratory rate of dogs increased at hypercapnia, the various bronchial contractions fused to behave like a tonic contraction. The rhythmic component of this constriction was separated and quantitatively analyzed. Each rhythmic Pbr amplitude linearly increased with increases in PNA amplitude, whereas the end-expiratory Pbr level was not significantly changed. Bilateral efferent nerve transection did not decrease the end-expiratory Pbr level. In response to electric stimulation of efferent nerve fibers, the bronchus did not maintain tonic constriction. We concluded that vagally mediated commands contract bronchial smooth muscle only intermittently and that most of bronchial resting tension may thus be attributed to the summation of rhythmic contractions.

vagus nerve; airway resistance; airway smooth muscle

MATERIALS AND METHODS
This study was approved by the Animal Ethics Committee of the Tokai University School of Medicine. Eight beagle dogs (5–10 kg) were deeply anesthetized with a short-acting barbiturate (Thyamal, 5–10 mg/kg), and decerebration was achieved by transection of the midbrain at the rostral margin of the superior colliculi (16, 17). To avoid decerebration-induced brain stem edema, prednisolone succinate (1.2 mg/kg) was intravenously administered 2 h before decerebration. The dogs were placed in a supine position, and a tracheostomy was made low in the neck. A catheter was placed in the left femoral artery for monitoring arterial pressure and for blood-gas analyses. Another catheter was placed in the right radial vein for intravenous administration of drugs. The anterior halves of the 8th–15th tracheal cartilaginous rings were surgically removed, and four silk strings were tied to either side of the remaining cartilaginous rings. The strings on the left side of the trachea were connected to a rod fixed to the experimental platform. The strings on the right side were connected to an isometric force transducer (model TB611T, Nihon Kohden, Tokyo, Japan) for recording tension of the tracheal smooth muscle (Ttr). Then, the right C3 phrenic nerve was bisected at the neck. Activity of the proximal end of the phrenic nerve (PNA) was continuously measured and was processed by a leaky integrator with time constant of 0.3 s. Bronchial diameter was measured as the pressure (Pbr) in a balloon-tipped catheter inserted through the tracheal tube. The bronchial balloon was donut shaped and surrounded a silicon tube (4 mm OD and 14 mm length). This shape allowed airflow between the peripheral and central airways. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
bronchial catheter was implanted in the fifth-generation bronchus of the right lower lobe under observation by fiberoptic bronchoscopy.

A partial arteriovenous bypass was performed in each dog after cannulation of venous and arterial vessels. Blood was withdrawn from the bilateral femoral veins and right subclavian vein, oxygenated with a blood oxygenator (model Mediflo D-705, Dideco, Mirandra, Italy), and then projected by a pulsating pump (model 1421, Harvard, South Natick, MA; stroke volume 25 ml, pulse frequency 80–100 beats/min) into the aortic arch through the right subclavian artery.

Recordings were started at least 3 h after decerebration, and the dogs were paralyzed with intravenous pancuronium bromide (2.0 mg·kg\(^{-1}\)·h\(^{-1}\)). Mechanical ventilation (model 613, Harvard) was performed with 100% O\(_2\), and then extracorporeal circulation was started immediately. Systemic arterial pressure was maintained at a physiological level throughout the experiment. If necessary, dextran sulfate was added into the extracorporeal circuit. After 10 min of mechanical ventilation, arterial Pa\(_{O_2}\) (Pa\(_{O_2}\)) became higher than 450 Torr, and the ventilator was removed from the dogs for 20 min. Arterial blood-gas analyses were performed several times in this complete apneic interval, and we confirmed that Pa\(_{O_2}\) did not fall below 150 Torr throughout the experiment. Five minutes after removal of mechanical ventilation, PNA, Ttr, and Pbr were recorded (n = 8). We increased or decreased Pa\(_{CO_2}\) by passing gas mixtures with several levels of CO\(_2\) concentration through the extracorporeal oxygenator. The vagus nerves were then transected bilaterally (n = 4). Vagotomy was performed at the CO\(_2\) level where the end-expiratory level of bronchial constriction could be discriminated, but arterial blood gases of the dogs were not measured. Finally, the distal end of the transected vagus nerve was mounted on bipolar platinum electrodes. The nerve was intermittently stimulated with pulse trains (40 Hz, 0.5 ms, 20 V, 60 pulses) with 1.5- to 4.5-s intermissions (n = 6).

All the data are expressed as means ± SD. Statistical analyses were performed by repeated-measures ANOVA. A P value < 0.05 was considered to indicate statistical significance.

RESULTS

Immediately after cessation of mechanical ventilation, periodic bronchial dilation due to ventilatory pressure disappeared. The tracheal smooth muscle contracted gradually and reached a plateau level of contraction in a few minutes (16). Because the plateau level of Ttr was generally higher than 100 g, we had to reset the resting Ttr to observe tracheal rhythmic contractions. Recordings of Ttr, Pbr, and PNA were started after Ttr reached this plateau level.

Figure 1 shows changes in Ttr and Pbr at normocapnia, acute hypocapnia, and acute hypercapnia produced by passing 5, 0, and 10% CO\(_2\) in O\(_2\) through the oxygenator. During both normocapnia and hypercapnia, Ttr and Pbr increased rhythmically and decreased in synchrony with PNA bursts. The PNA bursts and bronchial constrictions (Pbr) at hypercapnia developed over shorter intervals than those at normocapnia. Consequently, the rhythmic changes of Pbr at hypercapnia fused as if the tonic constriction were superimposed on the rhythmic one. With regard to tracheal tension, as our laboratory has reported previously (16, 17), there were rhythmic and tonic components of contraction. The mean levels of each parameter are listed in Table 1.

The mean of maximum Pbr at hypercapnia in the eight dogs (mean PA\(_{CO_2}\) 66.3 ± 12.7 Torr) was ~2.5 times that at normocapnia (mean PA\(_{CO_2}\) 35.8 ± 4.8 Torr). As can be seen in Fig. 1, the PNA burst disappeared at hypocapnia, and the respiratory-related Pbr changes in constriction also disappeared under this condition. At hypocapnia (mean PA\(_{CO_2}\) 21.9 ± 1.75 Torr), PNA disappeared in five dogs. In the other three dogs, the respiratory rate slowed considerably, but complete apnea was not achieved. In a comparison of the traces of Ttr and Pbr at hypocapnia, it can be seen that the bronchus dilated promptly during the expiratory phase, whereas the tracheal smooth muscle relaxed gradually, suggesting that the time constant of bronchial dilation was much shorter than that of the trachea. When the dog was made acutely hypercapnic, tracheal smooth muscle began to contract before development of the Pbr burst, suggesting that there is a tonic component to the contraction of the trachea and that this component is dependent on the PNA burst.

Although rhythmic Pbr decreased during the late expiratory phase, it did not reach a complete plateau even at end expiration in normo- or hypercapnia (Fig. 1). However, we were able to find an almost complete pattern of rhythmic Pbr at hypocapnia (i.e., “hypocapnic Pbr”).

We measured parameters of this particular rhythmic Pbr in the eight dogs (Fig. 2). The duration of PNA bursts was 3.0 ± 0.5 s. Rhythmic constriction of Pbr started 1.4 ± 0.49 s after the onset of PNA, reached a maximum value at 2.8 ± 1.6 s after the peak of PNA, and then decreased exponentially. The time constant of the exponential decay of Pbr was 6.9 ± 2.5 s. We measured the rhythmic contraction of Ttr as well. It started 3.08 ± 0.52 s after the onset of PNA and always reached its maximum value later than Pbr did, although the precise duration between the PNA onset and Ttr peak...
was not determined because Ttr remained at a maximum level for a long period.

The Pbr trace in Fig. 3 is identical to that in Fig. 1. The trace reconstructed by repeatedly placing one bronchial constriction at hypocapnia (Rec-Pbr trace) consists of only one particular bronchial constriction at hypocapnia (i.e., “hypocapnic Pbr” in Fig 1). We repeatedly placed this particular bronchial constriction trace along the time axis by referring to the onset of phrenic bursts, and then all the template traces were summed. The Rec-Pbr trace closely resembles the Pbr trace, and the close resemblance of Rec-Pbr to Pbr was found in all dogs. This led us to wonder whether Pbr consisted of only one particular pattern of bronchial constriction. We therefore subtracted Rec-Pbr from Pbr, resulting in the Sub-Pbr. It was found that the Sub-Pbr was still rhythmically augmented with the phases of PNA bursts at hypercapnia. Thus the Pbr was not a simple repetition of one template bronchial constriction, but the amplitude of bronchial rhythmic constriction was augmented at hypercapnia.

There was another question as to whether the tonic component developed during hypercapnia. To investigate this matter, we amplified the trace of hypocapnic Pbr according to the increase in PNA amplitude and arrayed each amplified trace as Rec-Pbr. This trace was then subtracted from Pbr trace (i.e., Mod-Pbr). As can be seen from the Mod-Pbr in Fig. 3, no tonic component seemed to exist at hypercapnia.

Figure 4 shows the representative relationship between the bronchial amplitude (i.e., Sub-Pbr amplitude added to the hypocapnic Pbr amplitude) and the PNA amplitude during progressive hypercapnia in one dog. Both the bronchial constriction and PNA amplitude are normalized as relative values to those at hypocapnia. Figure 4 shows that increases in amplitude of bronchial constriction were linearly related to those of PNA amplitude. The relationship between the constriction levels at end expiration in the trace generated with subtraction of the amplified Rec-Pbr from the Pbr (Mod-Pbr trace) and the PNA amplitude during progressive hypercapnia in the same dog is shown. The level of the bronchial constriction does not seem to change with changes in PNA amplitude, suggesting that no tonic constriction developed at hypercapnia.

The findings in Fig. 4 were quantitatively analyzed in the six dogs. Table 2 shows the parameters [slope, intercept of the y-axis, correlation coefficient (r), and P value] of the linear regression lines of the amplitude vs. PNA amplitude and the baseline level (i.e., the end-expiratory level) vs. PNA amplitude. The mean slope of the amplitude regression lines was 2.18 ± 1.89, the r values ranged between 0.89 and 1.00, and all the P values were <0.004. Thus it can be said that the amplitude of bronchial rhythmic constriction was linearly related to the amplitude of PNA during progressive hypercapnia. In Table 2, the mean slope of the baseline regression lines was −0.11 ± 0.80; the r values ranged between 0.07 and 0.90, and the P values were widely distributed. These findings suggested that there were no consistent tendencies of baseline level to increase or decrease during progressive hypercapnia. In other words, no tonic constriction developed at hypercapnia.

Figure 5 shows Ttr, Pbr, and PNA before and after bilateral vagotomy during extracorporeal circulation. After vagotomy, Ttr lost both rhythmic and tonic tensions. Pbr also lost rhythmic changes, and the level of Pbr after vagotomy was almost equal to that at end expiration. This finding suggested that bronchial smooth muscle lacks tonic resting tension. A similar finding, i.e., no changes or slight reduction in end-expiratory Pbr, was observed in the other three dogs examined.

![Fig. 2. Parameters of bronchial rhythmic constriction. TC, time constant.](image1)

![Fig. 3. Raw data of Pbr and Pbr generated from a template pattern (indicated by bar; Rec-Pbr), subtraction of Rec-Pbr from Pbr (Sub-Pbr), and subtraction of individual amplitude-matched Sub-Pbr from Pbr (Mod-Pbr).](image2)
bronchial rhythmic constrictions with complete elimination of respiratory motions. Our experimental setup allowed us to quantitatively analyze the time course and amplitude of bronchial rhythmic constriction. In addition, we revealed that tonic constriction was almost absent in the bronchus in response to the vagally mediated descending commands.

In a previous study from our laboratory (17), we recorded bronchial rhythmic constrictions by means of a V-shaped intrabronchial strain gauge in the paralyzed and mechanically ventilated dog. We observed spontaneous bronchial constriction over the course of several breaths, whereas PNA bursts were unsynchronized with the rhythm of mechanical ventilation. When the ventilator was briefly removed, several bronchial rhythmic constrictions were observed. However, even under such a condition, influences of accumulated CO2 (13) in arterial blood and of reflexes from pulmonary mechanoreceptors are inevitable (6). In the present study, we measured bronchial rhythmic constrictions using a balloon-tipped catheter over a 20-min apneic interval by using an extracorporeal oxygenator. During this apneic interval, the lung volume was fixed at functional residual capacity and the arterial blood gases were fixed at near-hypoxic levels (≥150 Torr). It has been suggested that hyperoxia (19) and/or elimination of phasic input from pulmonary receptors (18) may cause the respiratory rate to slow considerably. In our dogs, neither arterial O2 nor CO2 fluctuated in one breath cycle (7, 8), and thus we can say that bronchial rhythmic constrictons did not arise either from pulmonary receptors input or respiratory related fluctuation of blood gases.

Decerebration may be important for provoking bronchial responses. Iscoe and Fisher (13) have reported that bronchial responses to hypoxia and hypercapnia were significantly reduced by anesthesia but were maintained in decerebrated cats. We also experienced a better elucidation of tracheal rhythmic constrictions in decerebrated dogs in our previous study (16). It has not been proved whether rhythmic bronchial constriction in decerebrated animals is physiological. However, the analogy of bronchial rhythmic constriction to rhythmic contraction of tracheal smooth muscle, both of which are observed in both anesthetized and decerebrated animals (15, 23, 26), suggests that the bronchus has the potential for rhythmic constriction in phase with respiration.

### Table 2. Parameters of linear regression lines between the bronchial constriction and PNA amplitude during progressive hypercapnia

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Amplitude vs. PNA</th>
<th>Baseline vs. PNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope, y-intercept, r, P</td>
<td>Slope, y-intercept, r, P</td>
</tr>
<tr>
<td>1</td>
<td>0.41, 0.71, 0.94, &lt;0.001</td>
<td>-0.04, 0.01, 0.48, 0.14</td>
</tr>
<tr>
<td>2</td>
<td>0.18, 0.87, 0.89, 0.004</td>
<td>0.13, -0.17, 0.49, 0.011</td>
</tr>
<tr>
<td>3</td>
<td>4.15, -2.94, 0.99, &lt;0.001</td>
<td>0.72, -0.63, 0.90, &lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>4.65, -3.00, 0.94, 0.003</td>
<td>-0.11, 0.21, 0.07, 0.497</td>
</tr>
<tr>
<td>5</td>
<td>1.30, -0.17, 0.89, 0.002</td>
<td>-1.63, 1.93, 0.26, 0.238</td>
</tr>
<tr>
<td>6</td>
<td>2.37, -1.70, 1.00, 0.003</td>
<td>0.26, -0.31, 0.48, 0.126</td>
</tr>
</tbody>
</table>

Mean ± SD: 2.18 ± 1.89*, -1.04 ± 1.75

*P < 0.05 compared with baseline data.
The respiratory-related rhythmic constriction started \( \sim 1.4 \) s after the onset of phrenic burst and reached the maximal level \( \sim 2.8 \) s after termination of phrenic burst. The bronchus then relaxed almost exponentially with a relatively long time constant. Fisher et al. (9) also have measured tracheal diameter by using a balloon-tipped catheter. They calculated airway diameter as \( (C_1 - (C_2 - (\text{balloon volume}/C_3))^{2/3})^{1/2} \) where \( C_1 \), \( C_2 \), and \( C_3 \) are constants. This equation suggested that bronchial diameter vs. balloon pressure relationship was not linear. However, this equation also suggested that their relationship was almost linear if the changes of balloon pressure were small.

In a previous study from our laboratory (17), we observed that the bronchus started constriction with a short delay interval \( (1.0 \pm 0.9 \) s) when vagal efferent fibers were electrically stimulated. Thus delayed development of bronchial constriction to development of the PNA burst may not originate from the conduction of the efferent pathway or the features of muscular contraction. Presumably, the relationship between bronchial rhythmic constriction and PNA burst is programmed in the central neuronal structures. The time parameters of bronchial rhythmic constriction did not change when respiratory frequency was increased at hypercapnia. As a result, the individual constrictions fused during hypercapnia to produce a tonic tension. However, this observation raises a question as to whether the bronchial tone at hypercapnia is exclusively made up of summation of rhythmic constrictions.

By subtracting a particular template from the bronchial rhythmic trace, we discriminated the net increases of bronchial constriction from the Pbr trace. We found that there was a linear relationship between changes in phrenic amplitude and in the amplitude of bronchial rhythmic constriction. This finding suggested that the amplitude of bronchial rhythmic constriction is dependent on augmentation of phrenic amplitude in acute hypercapnia. Therefore, at least two components, i.e., summation of individual constriction and augmentation of amplitude of each constriction, contributed to the increase in Pbr during hypercapnia. Compared with the summation component, the augmentation of amplitude played a much smaller role in hypercapnia-induced bronchial tonic constriction. On the other hand, the end-expiratory Mod-Pbr level, i.e., the tonic component of bronchial constriction, did not increase at hypercapnia. The end-expiratory level of the bronchial constriction was almost equal to the level after vagotomy, although the subject number was very small. These findings further suggest that bronchial smooth muscle is almost devoid of tonic contraction. Instead, the resting tension may consist of the summation of rhythmic constriction.

The hypothesis that bronchial smooth muscle lacks tonic contraction does not contradict the classic notion that bronchial tonic tension is maintained by vagally mediated continuous descending commands (1, 5). For example, just as in most of the previous studies (3, 4, 11, 12), in the present study, we predict a reduction of bronchial tone after cervical vagotomy. In our study, vagotomy caused only the disappearance of each rhythmic constriction, and the decrease in end-expiratory bronchial tone was not significant. However, when the changes in Pbr during hyper- or normocapnia are considered, it may be predicted that bronchial tone decreases after vagotomy in hyper- or normocapnia. Interestingly, in some of the previous reports, in which airway resistance was measured as the mean of several breaths, vagotomy reduced airway resistance slightly (4), not consistently (3), or insignificantly (11, 22). These observations may support our hypothesis. Further support may be given by our present findings on the stimulation of vagal efferent fibers. As in a previous study from our laboratory (17), the bronchus constricted during vagal stimulation, but this constriction was not sustained when the stimulus frequency was high. In most of the previous studies, vagus nerve stimulation was performed with a frequency similar to that used here, but the time course of airway constriction was not analyzed (2, 21). However, several descriptions (4, 10, 11) have suggested that the increased airway resistance gradually decreased during stimulation.
tension. Thus our speculation that vagally mediated descending commands do not maintain bronchial tonic tension is not in conflict with previous reports.

We should also discuss another classic notion, namely, that bronchial smooth muscle continuously contracts in response to intravenous acetylcholine (25). Our laboratory also applied acetylcholine intravenously in a previous study (15) and confirmed that the fifth-order bronchus of the dog contracted tonically. In the present study, bronchial smooth muscle did not maintain tonic contraction while vagal efferent nerves were intermittently stimulated with short intermissions. Thus bronchial smooth muscle tonically contracted when muscarinic receptors were pharmacologically stimulated, but this tonic contraction was not maintained when muscarinic receptors were stimulated through efferent nerve fibers. This finding suggests that tonic descending commands do not pass through the vagus nerve and/or neuromuscular junction. It has been suggested that fatigue at neuromuscular transmission may be responsible for early cessation of bronchial constriction (15). In Fig. 6, electrically elicited bronchial constriction is always followed by bronchial relaxation. Similar relaxation was frequently seen in the electric stimulation experiments. Leff and Munoz (20) have reported that electric stimulation of the cervical vagus nerve leads to undesirable sympathetic effects on airway tone. Using an experiment of adrenalectomy, they speculated that circulating catecholamines from the adrenal gland are responsible for airway relaxation (20).

The physiological role of bronchial rhythmic constriction has not yet been clarified and will require further analysis.

In conclusion, bronchial smooth muscle rhythmically contracted at end inspiration and early expiration was almost completely lacking in tonic tension. At acute hypercapnia, these respiratory-related rhythmic contractions fused to produce a tonic constriction.

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