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Blunted Ventilatory Response to Hypoxia/Hypercapnia in Mice with Cigarette Smoke-induced Emphysema

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Abstract

It has been reported that the degree of emphysema induced by chronic cigarette smoke (CS) is greater in female C3H/HeN mice as compared to other mouse strains. We hypothesized that these mice would develop the similar major characteristics seen in hypercapnic patients with chronic obstructive pulmonary disease (COPD), including emphysema, pulmonary inflammation, hypercapnia/hypoxemia, rapid breathing, and attenuated ventilatory response (AVR). Mice were exposed either to CS or filtered air (FA) for 16 wk. After exposure, arterial blood gases and minute ventilation were measured before and during chemical challenges in anesthetized and spontaneously breathing mice. We found that as compared to FA, CS exposure caused emphysema and pulmonary inflammation associated with: (1) hypercapnia and hypoxemia, (2) rapid breathing, and (3) AVR to 25 breaths of pure N₂, 5% CO₂ alone, and 5% CO₂ coupled with 10% O₂. The similarity of these pathophysiological characteristics between our mouse model and COPD patients suggests that this model could be effectively applied to study COPD pathophysiology, especially central mechanisms of the AVR genesis.

Keywords

COPD; Inflammation; Ventilatory responses

1. Introduction

Chronic obstructive pulmonary disease (COPD), ~ 85% of it resulting from mainstream and second hand cigarette smoke (CS) (Anto et al. 2001; Siafakas et al. 2002), kills 2.5 million people worldwide every year (Barnes 2000). Chronic hypercapnia often complicates severe COPD and is associated with a worse prognosis (Burrows et al. 1969; Cooper et al. 1991). Epidemiological data showed that in 2000 1.5 million COPD patients were admitted to respiratory intensive care units in the United States, and 8% of those admitted died (Ucgun et al. 2006) predominantly because of respiratory failure (Anto et al. 2001). Surprisingly, mortality increased to 33% in COPD patients with hypercapnia (Khilnani et al. 2004), demonstrating that hypercapnia in COPD patients strongly predicts fatality from the disease.

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Patients with COPD have emphysema, pulmonary inflammation (chronic bronchitis), and pulmonary dysfunction (Pauwels et al. 2001), while hypercapnic patients often have additional three major characteristics. They are hypercapnia ($Pa_{CO_2} > 50$ torr) coupled with hypoxemia ($Pa_{O_2} < 60$ torr) (Pauwels et al. 2001); rapid breathing (Altose et al. 1977; Fahey et al. 1983; Gorini et al. 1990; van de Ven et al. 2002); and an attenuated ventilatory response (AVR) to hypercapnia/hypoxia (Schaefer 1949; Fahey et al. 1983; Franciosi et al. 2006; Ucgun et al. 2006). It is generally accepted that the AVR is correlated with mechanical limitation of ventilatory capacity (Cherniack et al. 1956; Pauwels et al. 2001). However, others reported a possible involvement of an impaired respiratory drive by recording mouth occlusion pressure in the first 100 ms of inspiration ($P_{0.1}$) (Goldring et al. 1971; Fahey et al. 1983).

There have been some clinical limitations to elucidating AVR generation. For example, technically, it is impossible to determine whether an impaired respiratory central drive is involved in the AVR. $P_{0.1}$ can reflect the combined functions of inspiratory muscles and phrenic motor output (an index of respiratory central drive). Because accumulated evidence shows that inspiratory muscle function is abnormal in patients with COPD (Morrison et al. 1989; Marchand et al. 2000), $P_{0.1}$ in these patients cannot precisely represent the respiratory central drive. This abnormality coupled with genetic involvement (Cohen et al. 1975, 1977), high variability of CS duration and intensity, and the safety concerns for hypercapnic patients with COPD has hindered mechanistic studies of AVR genesis. These issues could be addressed if an animal model of hypercapnic COPD is developed. The number of recent reports on the pathophysiology of emphysematous animal models has increased rapidly, but none of them has tested the presence of hypercapnia/hypoxemia ((Mahadeva et al. 2002), see discussion in reference (March et al. 2006b)). The emphysema of C3H/HeN, A/J, and C57BL/6 mice induced by chronic CS exposure (16 wk) has been investigated, and the results shown that the emphysema susceptibility among these strains has been ranked as C3H/HeN > A/J > C57BL/6; female mice are particularly affected (March et al. 1999, 2006a, 2006b; Guerassimov et al. 2004). Thus, we hypothesized that female C3H/HeN mice chronically exposed to CS would display the major characteristics seen in hypercapnic COPD patients, including: (1) emphysema, (2) hypercapnia, (3) rapid breathing at rest, and (4) AVR to hypoxia and hypercapnia.

2. Methods

All procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee in Lovelace Respiratory Research Institute facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

2.1. Chronic CS exposure

Female C3H/HeN mice at 10 wk of age were exposed whole-body to mainstream CS ($n = 26$) or filtered air (FA, $n = 24$) 6 h d^{-1} , 5 d wk^{-1} as previously described (March et al. 2005) with slight modifications. CS was generated from 2R4F filtered research cigarettes (11.7 mg total particulate material (TPM) yield/cigarette; University of Kentucky Tobacco Research and Development Center). Mice were acclimated to exposure during the first week by delivering CS targeted at $100 \text{ mg TPM m}^{-3}$ and then exposed to CS concentration at $250 \text{ mg TPM m}^{-3}$ for the following 15 wk. During exposure water, but not food, was provided for the animals.

2.2. General procedure and measurement of cardiorespiratory variables

After 16 wk of CS exposure, the mice were anesthetized with pentobarbital sodium (Nembutal®, 50 mg kg^{-1} intraperitoneally); supplemental anesthetic (chloralose, 100 mg kg^{-1} , and urethane, 500 mg kg^{-1}) was delivered via a catheterized left femoral vein. The adequacy of anesthesia was assessed every 30 min by monitoring the cardiorespiratory

responses to pinch the hindlimb paw. When any obvious change in arterial blood pressure (≥ 10 mmHg) or heart rate ($\geq 15\%$) or respiratory rate ($\geq 15\%$) was observed during pinching the hindlimb paw, an appropriate supplemental dose of anesthetic was given. If one injection was not enough, more injections were used. The left femoral artery was cannulated for monitoring arterial blood pressure (ABP) and heart rate (HR) and collecting arterial blood samples before and during chemical challenges as described below. The animal was supine and its body temperature was monitored with a rectal probe and maintained at approximately 37.5°C by a heating pad and radiant heat lamp.

2.3. Sampling of arterial blood

Arterial blood sample ($\sim 120\ \mu\text{l}$) was collected before and/or during chemical challenges in 15 CS- and 11 FA-exposed mice. The collection was performed 15 s before the end of the given chemical challenge, and the blood gases were measured immediately by a blood gas analyzer (GEM Premier 3000, Instrumentation Lab., Lexington, MA). An appropriate volume of saline was injected into the circulation after each collection to maintain the blood volume.

2.4. Measurement of $\dot{V}_E$

The trachea was cannulated and connected to a pneumotachograph (Frank's Mfg. Co., Albuquerque, NM) to record airflow in 22 mice, in which 9 mice came from 15 CS-exposed mice that had undergone arterial blood collections as mentioned above. The remaining mice were FA-exposed, 11 of them with and 2 without arterial blood collections. The other end of the pneumotachograph was placed (~ 3 mm depth) in a plastic tube with a diameter fivefold greater than the pneumotachograph. A three-way stopcock was attached to the other side of the plastic tube and connected to a supplemental gases device, by which inhaling room air or mixed gases from the tanks was controlled. The pneumotachograph, as reported before (Wang et al. 2006), was made of stainless steel with a linear flow-pressure relationship in the range of $0\text{--}10\ \text{ml s}^{-1}$ and a flow resistance equal to $0.046\ \text{cm H}_2\text{O ml}^{-1}\ \text{s}^{-1}$ with a dead space of 0.08 ml.

2.5. Acute hypoxic and hypercapnic exposure

The cardiorespiratory variables and arterial blood gases under room air were recorded and collected for 10 min after the baseline variables became stable. To correct the hypoxemia in both groups of anesthetized mice, the animals were exposed to rich oxygen (40% O_2 balanced by N_2) as control. The mice subsequently received the following stimulations: (1) 25 breaths of pure N_2 and 2 min of 10% O_2 (balanced with N_2) to stimulate O_2 chemoreceptors; (2) hyperoxic hypercapnia (5% CO_2 + 40% O_2 + 55% N_2) for 2 min to stimulate CO_2 chemoreceptors; and (3) hypoxic hypercapnia (10% O_2 + 5% CO_2 + 85% N_2) for 2 min to test the interactive capability of both stimuli in respiratory response. The interval between two stimulation-episodes was at least 5 min under hyperoxia.

2.6. Collection of bronchoalveolar lavage fluid (BALF)

The mice were sacrificed with an overdose of Nembutal after completion of the ventilatory tests. The right lobes were lavaged via the tracheal cannula (20 gauge) with $3 \times 0.5\ \text{ml}$ of Dulbecco's phosphate-buffered saline immediately after the lungs were removed. Total cell counts were determined by using a hemocytometer corrected for the total volume of BALF recovered from the mouse, and the differential cell types in the BALF were evaluated by utilizing cytocentrifuge preparation slides stained with Wright-Giemsa. At least 200 cells for each preparation were counted by using morphologic criteria as described in our previous studies (March et al. 2005). Cells were identified as macrophages, neutrophils, lymphocytes, or eosinophils and expressed as the total cells of each type per mouse.

2.7. Lung histopathology

The lung histopathological processes similar to those previously reported (March et al. 2005) were performed in 11 other CS- and FA-exposed mice, respectively. Briefly, lungs were inflated with 10% neutral buffered formalin at a constant hydrostatic pressure of 25 cm H₂O for 6 h. Lungs were fixed further by immersion in fixative for 48–72 h. Whole lung volume was determined by fluid displacement, and the left lobe was trimmed, histoprocessed, sectioned, and stained with hematoxylin and eosin (H&E). Emphysema was subjectively assessed by light microscopy and described as an irregular, multifocal expansion of alveolar air spaces and alveolar ducts due to destruction of parenchymal tissue (Snider 1986). Quantitative, morphometric measurement of alveolar air space expansion (by mean linear intercept [L_m], a morphometric measure of alveolar size, corrected for shrinkage due to histoprocessing) was performed as described in our previous studies (March et al. 2005).

2.8. Data acquisition and analysis

Effects on L_m were tested for statistically significant differences between the two groups by using Student's t-test. The same statistical analysis was used for comparing the difference of cell counts obtained from BALF. All cardiorespiratory raw signals, including ABP, respiratory airflow, and rectal temperature were digitized and recorded by using a PowerLab/8sp (ADInstruments, Colorado Springs, CO) connected to a computer employing the PowerLab Chart 5 software (ADInstruments). Mean arterial blood pressure (MABP), HR, tidal volume (V_T), respiratory frequency (f_R), and $\dot{V}_E$ were derived by the on-line calculation functions of the software. The variables obtained under room air and control (rich oxygen) were collected and averaged for 1 min and represented by absolute values. The cardiorespiratory responses to hypoxia, hypercapnia, and hypoxic hypercapnia were measured for a 10-s period containing the greatest response and presented as $\Delta\%$ change from control. All values in the text and figures are expressed as mean $\pm$ standard error (SE). Comparisons of the cardiorespiratory responses obtained from the two groups were made by using one-way ANOVA with repeated tests followed by Newman-Keuls post-hoc tests. P-values less than 0.05 were considered significant.

3. Results

3.1. Chronic CS produced emphysema and pulmonary inflammation

The lungs from CS-exposed mice were mottled gray-brown and pink, and some did not deflate completely upon thoracotomy. These lungs had irregular enlargement of alveolar and alveolar ductal air spaces as shown in Fig. 1A. The organization of parenchymal acini was often disrupted or unrecognizable. Attenuation, fragmentation, blunting, and folding of alveolar septa were noted in several foci. Inflammatory cells, including macrophages and neutrophils, were numerous among cell debris in air spaces near bronchioalveolar duct junctions. Alveolar septa were sometimes thickened by infiltrates of similar inflammatory cells along with small to moderate numbers of lymphocytes. Infiltrates of macrophages, lymphocytes, and neutrophils were common in the perivascular interstitium; thin-walled venules deep in the parenchyma were often affected. Smaller numbers of mononuclear leukocytes and a few neutrophils were present in the interstitium around terminal bronchioles and associated arterioles and lymphatic vessels. Statistically, CS caused a 36% increase in the L_m and a 46% increase in lung volume over mean control values ($P < 0.05$, Fig. 1B). The total cells from BALF were compared between the FA- and CS-exposed groups. As shown in Fig. 2, total cells were significantly increased approximately threefold by chronic CS exposure with significant increases in macrophages and neutrophils, and lymphocytes tended to increase. Eosinophils were not found in both FA- and CS-exposed mice.

3.2. Chronic CS exposure caused hypercapnia and rapid breathing

During room air breathing, hypercapnia (mean $Pa_{CO_2} = 57.8$ torr) existed in anesthetized CS-exposed mice in which mean Pa_{CO_2} was ~ 11 torr higher than in FA-exposed mice ($P < 0.05$; Fig. 3A). As compared to the latter, a significant decrease of mean Pa_{O_2} ($\downarrow 27$ torr) was observed in CS-exposed mice ($P < 0.05$; Fig. 3B). Arterial blood pH was not significantly different between the two groups (Fig. 3C); however, the values of bicarbonate (HCO_3^-) were significantly higher in the CS- than in the FA-exposed mice ($P < 0.05$; Fig. 3D). In addition, the increase in the hematocrit of CS-exposed mice (Fig. 3E) was significant ($P < 0.05$). Interestingly, rapid breathing with little change in V_T was observed in CS-exposed mice, which led to a rise in $\dot{V}_E$. The typical experimental recordings are illustrated in Fig. 4A, while group data demonstrated that CS increased $\dot{V}_E$ (33%) predominantly by raising f_R (26%; Fig. 4B). Body weight in CS-exposed mice was lower (~18%) than in FA-exposed mice.

3.3. AVR to severe hypoxia and moderate hypercapnia existed in the mice with hypercapnic emphysema

After transfer from room air to 40% O_2 , the reduction of $\dot{V}_E$ in FA-exposed mice was ~35% lower than that in the CS-exposed group ($P < 0.05$). Under this control condition, we tested whether there was AVR to hypoxia and hypercapnia in CS-exposed mice. We found that the $\dot{V}_E$ responses to the initial 10 breaths of pure N_2 were not significantly different between the two groups (Fig. 5A). However, thereafter, $\dot{V}_E$ gradually increased as progressive hypoxia developed in FA-exposed mice, but rapidly reached a plateau in CS-exposed mice. At the end of hypoxia, the $\dot{V}_E$ response in CS-exposed mice was about one-fourth of the response obtained in FA-exposed mice mainly owing to a reduction of f_R response (60% decrease, $P < 0.05$). As compared to FA-exposed mice, the ventilatory response to moderate hypoxia (induced by 10% O_2) was relatively lower in CS-exposed mice, but this change did not reach significance (top of Fig. 5B). Blood gases during hypoxia were not significantly different between the two groups (bottom of Fig. 5B). In response to moderate hypercapnia (induced by 5% CO_2 for 2 min), ventilation was significantly lower (15%) in the CS-exposed mice without differences of arterial blood gases between the two groups (Fig. 6A and 6B).

3.4. Chronic CS exposure attenuated the ventilatory response to hypoxic hypercapnia

As mentioned above, hypercapnic COPD patients often have associated hypoxemia; therefore, we compared the ventilatory response to inhalation of hypoxic hypercapnia between the two groups. As shown in Fig. 7A (upper), the ventilatory response to hypoxic hypercapnia was markedly reduced in the CS-exposed mice (43%) as compared to the FA-exposed mice because of dramatic diminution of f_R (52%; $P < 0.05$). Furthermore, in the CS-exposed mice, there were significantly higher Pa_{CO_2} and lower Pa_{O_2} levels during the stimulation (Fig. 7A, lower), indicating that the blunted respiratory responses existed in the CS-exposed mice even with much stronger respiratory stimulation. To test the effect of chronic CS exposure on the interaction of hypoxia and hypercapnia in ventilatory reflexes, we compared the sum of the ventilatory responses to hypoxia and hypercapnia alone and to hypoxic hypercapnia. As presented in Fig. 7B, the ventilatory response to hypoxic hypercapnia was significantly greater than the sum of responses to hypoxia and hypercapnia alone in FA-exposed mice, strongly suggesting a positive interaction of both stimuli on respiration. However, the hypoxic hypercapnic response became smaller than the sum in CS-exposed mice, suggesting a negative (inhibitory) interaction.

3.5. The effects of chronic CS exposure on cardiovascular activities were limited

We compared cardiovascular activities by measuring systemic MABP and HR before and during chemical challenges in FA- and CS-exposed mice. HR was higher in CS-exposed mice under room air and hyperoxia, and severe hypoxia lowered ABP more in CS-exposed mice as

compared to the FA-exposed mice (Fig. 8). Other than that, the cardiovascular variables under chemical challenges used were not significantly different between the two groups.

4. Discussion

4.1. C3H/HeN mice exposed to chronic CS generate emphysema and pulmonary inflammation

We found significant enlargement of air spaces in CS-exposed mice, including those of alveoli and alveolar ducts, leading to a 36% increase in the L_m . These disorders of parenchymal architecture are similar to those observed in patients with COPD (Sorli et al. 1978). The severity of the emphysema in the present study was much greater than that observed previously in female A/J (25% increase in L_m over controls) and C57BL/6 mice (16% increase in L_m) exposed to CS under similar conditions (Guerassimov et al. 2004; March et al. 2006a, 2006b). Inhalation of CS causes a chronic pulmonary inflammatory infiltration of macrophages, neutrophils and lymphocytes in humans (Mahadeva et al. 2002). In our mouse model, these inflammatory cells were numerous in air spaces near bronchioloalveolar duct junctions, in the perivascular interstitium, and in the interstitium around small conducting airways. Moreover, cell counts from BALF showed that the total number of cells was significantly increased by chronic CS exposure, owing to increases of macrophages and neutrophils as well as lymphocytes. Similar to humans, CS-exposed mice had inflammatory cell infiltrations in the interstitium around the terminal bronchioles, but unlike in human smokers, this was not associated with peribronchiolar fibrosis and/or muscular hypertrophy (“remodeling”) after this relatively short emphysema induction period. Taken together, these results are generally consistent with pulmonary pathological changes in patients with COPD.

4.2. It is novel that the major symptoms of anesthetized C3H/HeN emphysematous mice are similar to those observed in hypercapnic patients with COPD

Aside from emphysema, pulmonary inflammation (chronic bronchitis), and pulmonary dysfunction (Pauwels et al. 2001), hypercapnic patients with COPD often have additional hypoxemic hypercapnia (Pauwels et al. 2001), rapid breathing, and AVR (Altose et al. 1977; Fahey et al. 1983; van de Ven et al. 2002). These features were observed in our CS-exposed mice. First, the averaged Pa_{CO_2} and Pa_{O_2} in CS-exposed mice were 57.8 torr and 45.1 torr, respectively, demonstrating the presence of both hypercapnia and hypoxemia. A significant increase in the hematocrit of these mice further supports the presence of chronic hypoxemia. Our data of blood gases showed that the values of bicarbonate were significantly higher in the CS- than in the FA-exposed mice without remarkable change in arterial blood pH, suggesting a CO_2 retention and renal compensation. This CO_2 retention is likely related to significant ventilation/perfusion mismatching with a relative rise in the dead space/ V_T ratio of each breath as reported in hypercapnic patients with COPD (Calverley 2003). Our blood gases are in agreement with an abnormality of gas exchange based on an alveolar gas equation, i.e., the calculated values of the P_A -a gradient. Second, as compared to FA-exposed mice, the greatest change of ventilation in CS-exposed mice was the rapid breathing, leading to an increase of $\dot{V}_E$. Actually, a rapid breathing rate has often been observed in hypercapnic COPD patients (Altose et al. 1977; Fahey et al. 1983; Gorini et al. 1990; van de Ven et al. 2002), although the baseline ventilation has been reported to be increased (Gorini et al. 1990; Topeli et al. 2001), decreased (Altose et al. 1977), or unchanged (Sorli et al. 1978; Montes de Oca et al. 1998; van de Ven et al. 2002). Additionally, we also found a slightly, but significantly higher HR in CS-exposed mice as compared to FA-exposed mice (Fig. 8). This rapid breathing coupled with hypoxemia/hypercapnia and an increase in HR is somewhat similar to COPD exacerbation. Dyspnea characterized by faster breathing (20% increase), increase in HR and appearance of hypoxemic hypercapnia have been reported in patients with COPD exacerbation (Pauwels et al. 2004; Franciosi et al. 2006). Third, AVR to hypoxia and hypercapnia was observed in CS-

exposed mice. The response to 25 breaths of pure N₂ inhalation (~ 12 s) in CS-exposed mice was reduced to one-fourth of that in FA-exposed mice. These data combined with a greater reduction of $\dot{V}_E$ induced by hyperoxia in FA- than in CS-exposed mice imply an impaired peripheral chemoreceptor-mediated ventilatory response. A recent study indicated that the ventilation at ~ 50 torr P_{aCO_2} in hypercapnic patients with COPD was one third of that observed in normocapnic patients with COPD (van de Ven et al. 2002). We found that chronic CS exposure significantly attenuated the $\dot{V}_E$ response to hyperoxic hypercapnia (40% O₂ + 5% CO₂) by ~ 15%, and this attenuation became much greater during hypoxic hypercapnia (10% O₂ + 5% CO₂; 43%). Taken together, these findings confirm for the first time that C3H/HeN emphysematous mice induced by chronic inhalation of CS display the similarity of the major characteristics observed in hypercapnic patients with COPD.

4.3. Chronic CS exposure produced an abnormal inhibitory interaction of hypoxia and hypercapnia in the ventilatory response

It is generally accepted that human subjects show an increased hypercapnic ventilatory response when the O₂ tension is simultaneously lowered (Tatsumi et al. 1986; Masuda et al. 2001; Sovik et al. 2004). This positive interaction has been confirmed in recent studies carried out on goats, lambs, piglets, cats, rats, and mice (Daristotle et al. 1987; Wolsink et al. 1992; Carroll et al. 1993; Tankersley et al. 1994, 2004; Pepper et al. 1995; Calder et al. 1997), although some early studies showed a negative interaction in cats (Ou et al. 1976). We compared the ventilatory response to hypoxic hypercapnia between CS- and FA-exposed mice. A similar positive interaction of hypoxia and hypercapnia in ventilatory response was observed in our FA-exposed mice. As compared to the sum of the ventilatory responses to hypoxia and hypercapnia alone, the responses to hypoxic hypercapnia were increased by 14%. Interestingly, this positive interaction became negative in CS-exposed mice. In other words, the ventilatory response to hypoxic hypercapnia was 17% smaller than the sum of the ventilatory responses to hypoxia and hypercapnia alone in the CS-exposed mice. This CS-induced impairment (↓31%) of the ventilatory responses may be partially responsible for the respiratory failure and mortality that occur during exacerbation of COPD associated with hypoxemic hypercapnia (Burrows et al. 1969; Cooper et al. 1991; Khilnani et al. 2004). It is worth emphasizing that chronic CS-exposure has little effect on the cardiovascular responses to hypoxia and hypercapnia, compellingly suggesting that the CS-induced AVR is not dependent on cardiovascular alterations.

4.4. There are several limitations in this study

First, as mentioned before, chronic CS produces emphysema to a much greater degree in C3H/HeN as compared to A/J and C57BL/6 mice (Guerassimov et al. 2004; March et al. 2006a, 2006b). However, without comparing other mouse strains, we cannot conclude that C3H/HeN mice are the best model to develop hypercapnic emphysema induced by chronic CS. Nevertheless, the appearance of hypoxemic hypercapnia and the substantial AVR to hypoxia and hypoxic hypercapnia in C3H/HeN emphysematous mice has built a solid basis for further mechanistically studying AVR genesis. In addition, although emphysema can be modeled in other ways, such as exogenous administration of proteinases, chemicals, and particulate materials (Mahadeva et al. 2002), CS-induced emphysema was chosen in the present study because ~ 85% of patients with COPD result from smoking (Anto et al. 2001; Siafakas et al. 2002). Second, we recognized that it would be optimal if the cardiorespiratory (arterial blood gases) variables under room air and in response to chemical challenges could be measured in conscious mice. However, our pilot experiments showed that collection of arterial blood samples in conscious mice without affecting their cardiorespiratory activities and responses to hypoxia and hypercapnia was very difficult. AVR observed in anesthetized emphysematous mice is uniquely important because using this model we will be able to compare the phrenic efferent responses to chemical challenges between FA- and CS-exposed mice to define the

respiratory central involvement of AVR genesis. Third, no attempt has been made in the present study to directly reveal the mechanism(s) underlying the AVR genesis. However, the mechanical limitation of the lungs, the depressed respiratory central drive and impaired respiratory muscles may be attributed to the AVR genesis. Previous studies have demonstrated that the limitation of mechanical ventilatory capacity of COPD patients is the major contributor to AVR generation (Cherniack et al. 1956; Bedell et al. 1961; Pauwels et al. 2001), which may hold true in our emphysematous mice. On the other hand, the enlargement of the lung L_m denoted in our emphysematous mice (36%) is much smaller than that observed in patients with COPD (Verbeke et al. 1992). With this in mind, the AVR in our emphysematous mice that is similar to that in COPD patients implies a greater contribution of the respiratory central drive to AVR genesis in our mouse model. In fact, our data that the $\dot{V}_E$ response to hyperoxia is 35% smaller in CS- than FA-exposed mice suggest a possible reduction of central drive in response to activation of peripheral chemoreceptors, consistent with previous reports (Goldring et al. 1971; Fahey et al. 1983).

In summary, we have developed a mouse model in which chronic CS-exposure produces emphysema, pulmonary inflammation, hypercapnia/hypoxemia, rapid breathing, and AVR. The similarity of these pathophysiological characteristics between our mouse model and COPD patients suggests that this model could be effectively applied to study COPD pathophysiology, especially central mechanisms of the AVR genesis.

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Fig. 1. Histopathology and morphometry of the lungs obtained from mice exposed to FA (filtered air) and CS (cigarette smoke). In panel A, photomicrographs show enlargement of parenchymal air spaces due to CS exposure (right) in comparison to lungs from a FA-exposed control mouse (left; H&E stain, bars = 200 μm). In panel B, group data of the CS effect on mean linear intercept (L_m , left) and lung volume (V_L , right) are presented (n = 11 per group). * $P < 0.05$, comparisons between FA- and CS-exposed mice.

Fig. 2.
The effects of CS exposure on inflammatory cells. Total cells count, macrophages, neutrophils, and lymphocytes are presented in panels A–D, respectively. $n = 6$ per group; mean $\pm$ SE; * $P < 0.05$ and † $P = 0.07$, comparisons between FA- and CS-exposed mice.

Fig. 3. Arterial blood gases and pH obtained under room air in mice exposed to FA or CS. Partial pressure of arterial CO_2 and O_2 (P_{aCO_2} , P_{aO_2}), pH, bicarbonate (HCO_3^-), and hematocrit (Hct) are illustrated in panels A–E, respectively. $n = 15$ and 11 for CS- and FA-exposed mice, respectively; mean $\pm$ SE; * $P < 0.05$, comparisons between FA- and CS-exposed mice.

Fig. 4. Comparison of the cardiorespiratory activity of FA- and CS-exposed mice under room air. Representative traces, including arterial blood pressure (ABP), flow, tidal volume (V_T), respiratory frequency (f_R) and minute ventilation ($\dot{V}_E$), from individual FA- (left) or CS-exposed mice (right) are presented in panel A. Panel B shows the group data of $\dot{V}_E$, V_T, f, and body weight (BW). n = 9 and 13 for CS- and FA-exposed mice, respectively; mean ± SE; * P < 0.05, comparisons between FA- and CS-exposed mice.

Fig. 5.

The ventilatory responses to hypoxia. The comparison of the ventilatory response to 25 breaths of pure N_2 exposure and 10% O_2 for 2 min between CS- ($n = 9$) and FA-exposed mice ($n = 13$) is displayed in panels A and B, respectively. Mean $\pm$ SE; * and † $P < 0.05$, comparisons between control and hypoxia, and between FA- and CS-exposed mice, respectively.

Fig. 6. The respiratory responses to hypercapnia in FA- ($n = 13$) and CS-exposed mice ($n = 9$). Hypercapnia was induced by inhalation of 40% O_2 + 5% CO_2 + 55% N_2 for 2 min. The ventilatory and arterial blood gas responses are depicted in panels A and B, respectively. Mean $\pm$ SE; * $P < 0.05$, comparisons between FA- and CS-exposed mice.

Fig. 7. The respiratory responses to hypoxic hypercapnia in FA- (n = 13) and CS-exposed mice (n = 9). The ventilatory and arterial blood gases responses to hypoxic hypercapnia (10% O₂ + 5% CO₂ + 85% N₂ for 2 min) in these two groups are depicted in panel A, upper and lower, respectively. Panel B presents the interaction of hypoxia and hypercapnia in the ventilatory responses. In each group (FA or CS), the left bar presents the sum of the ventilatory responses to hypoxia and hypercapnia alone, while the right bar represents the responses to hypoxic hypercapnia. * P < 0.05, comparisons between FA- and CS-exposed mice.

Fig. 8.

The cardiovascular variables and their responses to chemical challenges in mice exposed to FA (n = 13) and CS (n = 9). The cardiovascular variables recorded under room air, their response to 40% O₂, 100% N₂, 10% O₂, 5% CO₂, and 10% O₂ + 5% CO₂ are presented in panels A–F, respectively. Mean ± SE; * P < 0.05, comparisons between FA- and CS-exposed mice.