Acute lung injury augments hypoxic ventilatory response in the absence of systemic hypoxemia

F. J. Jacono, Y.-J. Peng, D. Nethery, J. A. Faress, Z. Lee, J. A. Kern, and N. R. Prabhakar. Acute lung injury augments hypoxic ventilatory response in the absence of systemic hypoxemia. J Appl Physiol 101: 1795–1802, 2006. First published August 17, 2006; doi:10.1152/japplphysiol.00100.2006.—The objective of the present study was to examine the impact of early stages of lung injury on ventilatory control by hypoxia and hypercapnia. Lung injury was induced with intratracheal instillation of bleomycin (BM; 1 unit) in adult, male Sprague-Dawley rats. Control animals underwent sham surgery with saline instillation. Five days after the injections, lung injury was present in BM-treated animals as evidenced by increased neutrophils and protein levels in bronchoalveolar lavage fluid, as well as by changes in lung histology and computed tomography images. There was no evidence of pulmonary fibrosis, as indicated by lung collagen content. Basal core body temperature, arterial PO2, and arterial PCO2 were comparable between both groups of animals. Ventilatory responses to hypoxia (12% O2) and hypercapnia (7% CO2) were measured by whole body plethysmography in unanesthetized animals. Baseline respiratory rate and the hypoxic ventilatory response were significantly higher in BM-injected compared with control animals (P = 0.003), whereas hypercapnic ventilatory response was not statistically different. In anesthetized, spontaneously breathing animals, response to brief hypoxia (Dejours’ test, an index of peripheral chemoreceptor sensitivity) and neural hypoxic ventilatory response were augmented in BM-exposed relative to control animals, as measured by diaphragmatic electromyograms. The enhanced hypoxic sensitivity persisted following bilateral vagotomy, but was abolished by bilateral carotid sinus nerve transection. These data demonstrate that afferent sensory input from the carotid body contributes to a selective enhancement of hypoxic ventilatory drive in early lung injury in the absence of pulmonary fibrosis and arterial hypoxemia.

carotid body; hypoxia; control of breathing

ACUTE LUNG INJURY IS THE RESULT of damage to the epithelial-endothelial barrier of the lung. This disease process begins with an initial insult, which may be due to a variety of agents, both exogenous and endogenous. Regardless of the causative agent, there is an initial inflammatory response with the release of humoral mediators. Left unchecked, damage may progress with the development of chronic lung injury characterized by pulmonary fibrosis, hypoxemia, and minimal active inflammation (13, 17, 20, 22, 28). As this process evolves, disruption of normal pulmonary physiology results in perturbations of ventilatory control. The alterations in lung mechanics and gas exchange in acute and chronic lung injury are complex, and the factors responsible for changes in ventilation will likely vary with time (5, 9, 11, 13, 28).

Respiratory patterns are altered when the lungs are injured. In the latter stages of lung injury, fibrosis predominates (5, 9, 11, 23, 28). Pathologically, the fibrotic lung parenchyma characteristic of chronic lung injury resembles that seen in interstitial lung diseases. With fibrosis, sensory information from vagal afferents contributes to altered breathing patterns. Specifically, sensory information from slowly adapting and rapidly adapting pulmonary stretch receptors promotes a rapid, shallow breathing pattern (19). In contrast, the pathophysiology in the early period of lung injury is different. Shortly after the initial insult but before the progression to pulmonary fibrosis, there is minimal embarrassment of gas exchange or pulmonary mechanics, despite the building inflammatory response (2, 3, 8, 20, 22, 28, 29). Little is known of how breathing patterns are altered or the associated mechanisms responsible for altered control of breathing during early lung injury. Consequently, the objective of the present study was to examine the impact of the early stages of lung injury, before the development of pulmonary fibrosis, on ventilatory control during hypoxia and hypercapnia, and to assess any underlying mechanism involved in ventilatory changes. To investigate this, we used a well-characterized model of bleomycin (BM)-induced lung injury in adult rats.

Our results showed a selective augmentation of hypoxic ventilatory response, but not hypercapnic ventilatory response, in acute lung injury, even in the absence of systemic hypoxemia. Furthermore, our data indicate that the peripheral chemoreceptors rather than vagal sensory afferents contribute to selective augmentation of hypoxic ventilatory response in the early stages of lung injury.

MATERIALS AND METHODS

Experiments were performed on adult male Sprague-Dawley rats (Zivic Laboratories, Pittsburgh, PA) weighing 150–200 g. The Institutional Animal Care and Use Committee of Case Western Reserve University approved the experimental protocols.

Induction of Lung Injury

BM administration. Animals received intratracheal instillation of either BM [1.0 units in 40 μl phosphate-buffered saline (PBS)] or PBS
General surgical anesthesia was established with ketamine, xylazine, and acepromazine, utilizing a weight-based dosing protocol. Animals were placed on a disinfected surgical board, the surgical site was prepared with betadine and 100% alcohol, and the trachea was exposed via a 1-cm midline anterior neck incision. BM or PBS was instilled into an animal’s lungs via a 26-gauge needle inserted between the cartilaginous rings of the trachea, and the incision site was sealed with surgical tissue adhesive. Animals were observed while recovering from anesthesia and then returned to the animal facility and monitored daily.

Assessment of Lung Injury and Inflammation

Collection of bronchoalveolar lavage fluid and lung tissue. Animals were euthanized via anesthetic overdose, a midline thorax to neck incision was made, the ribs removed, and a tracheal cannula was placed. The lungs were lavaged with sterile PBS (2× with 2.5 ml each time), the collected bronchoalveolar lavage fluid (BALF) was centrifuged (1,500 g, 10 min at 4°C), and the supernatant was collected for protein analysis. The cell pellet was resuspended in PBS and processed as described below. After BALF collection, the lungs were removed, frozen in liquid nitrogen, and stored at −80°C for subsequent collagen analysis.

**BALF protein assay.** Protein content was determined using a modified Bradford Protein Assay (Bio-Rad Laboratories, Hercules, CA). Briefly, 5.0 ml of diluted dye reagent were added to 100 μl of BALF supernatant and mixed. Dye-protein complexes were allowed to form during a 10-min incubation at room temperature and then analyzed spectrophotometrically at 595 nm. Protein concentration was determined by comparison to a standard curve constructed using known amounts of bovine serum albumin. The results were expressed as micrograms of protein per microliter of BALF.

**BALF cell count and differential.** The BALF cell pellet was resuspended in PBS, and viable cells were identified by Trypan blue exclusion and counted on a hemocytometer. Nucleated cells (20,000) were applied to a glass microscope slide (Cytospin centrifuge, ThermoShandon, Pittsburgh, PA). Slides were fixed with ethanol, dried overnight, and stained with a modified Wright stain (Diff-Quik, Dade Behring, Deerfield, IL). Cell count and differential was performed by a reader blinded to information about the status of lung injury.

**Lung collagen assay.** Total soluble collagen content of lung tissue was determined using the Sircol Collagen Assay (Biocolor, Newtownabbey, UK). Briefly, frozen lung specimens were thawed, homogenized in 1 ml of complete lysis buffer, and centrifuged (10,000 g) for 10 min at 4°C. Supernatant (50 μl) was added to 50 μl of 0.5 M acetic acid and 1 ml of Sircol dye reagent and mixed for 30 min at room temperature to allow the formation of dye-collagen complexes. Samples were centrifuged to pellet the complexes (10,000 g), and unbound dye was removed. Bound dye was solubilized in 1 ml of 0.5 M NaOH and analyzed spectrophotometrically at 540 nm. A standard curve constructed from known amounts of type I collagen was used to determine the collagen concentration of samples.

**Pulmonary histology.** Lungs from euthanized animals were inflated with 10% formalin for 25 cmH2O for 30 min. The lungs were then removed en bloc, transferred to a cassette, and embedded in paraffin. Subsequently, 5-μm sections were cut and stained with hematoxylin and eosin for histological examination.

**Computed tomography.** Animals were euthanized before imaging to eliminate motion artifacts. Scans were performed by the computed tomography (CT) component of the X-SPECT scanner manufactured by GammaMedica (Northridge, CA). The resolution of CT images is ~100 μm. Single slices through comparable sections of the lungs were examined.

**Arterial Blood-Gas Measurements**

After the induction of sedation [urethane, 1.2 g/kg intraperitoneally (ip)], animals were placed on a heating pad to maintain body temperature, and a tracheostomy tube was inserted to allow delivery of room air supplemented with 100 or 12% O2. To facilitate collection of arterial blood samples, a femoral arterial catheter was inserted, and 0.1 ml of arterial blood was collected in a heparinized capillary tube. Analysis of arterial blood was performed immediately after collection of the sample (ABL5, Radiometer, Copenhagen, Denmark).

**Ventilatory Measurements in Awake Animals**

**Whole body plethysmography.** Ventilation was monitored in unanesthetized, unrestrained, spontaneously breathing animals via temperature-equilibrated whole body plethysmography, as described previously (12). Briefly, animals were placed in a Lucite chamber (diameter of 28 cm, 10.9 liters in volume) connected to a high-gain differential pressure transducer (model MP45, Validyne, North Ridge, CA). Pressure changes within the chamber were converted to signals representing tidal volume (VT), which were amplified (model BMA 830, CWE, Ardmore, PA) and stored in a computer with respiratory acquisition software (PowerLab, ADInstruments, Castle Hill, Australia) for further analysis. The chamber contains gas intake and output ports to allow rapid changes in the gas mixture within the chamber and a continuous flow of gas through the chamber during the testing period (600 ml/min) to prevent CO2 buildup or O2 depletion. O2 consumption (VO2) and CO2 production (VCO2) were determined via the open-circuit method using O2 and CO2 analyzers (models CA-1 and FC-1, Sable Systems). All recordings were obtained between 9 AM and 1 PM to limit circadian effects and at an ambient temperature of 25 ± 1°C. Ventilatory responses were recorded after animals were allowed to acclimate to the recording chamber in room air for 30 min.

**Measurement on Neural Ventilation in Sedated Animals**

**Diaphragm electromyographic activity.** Animals were anesthetized with urethane (1.2 g/kg ip), and the adequacy of anesthesia was assessed regularly by the absence of a withdrawal reflex, blood pressure increase, or heart rate response to paw pinch. Supplemental doses of urethane (15% of the initial dose, ip) were administered as necessary. Body temperature was monitored with a rectal thermometer and maintained between 38 and 39°C with a heating pad. A tracheostomy tube was inserted to allow rapid switching of inspired gas while the rats breathed spontaneously. The femoral artery and vein were catheterized to allow continuous blood pressure recording and administration of fluids as necessary. Diaphragm electromyogram (EMG) activity was recorded with Teflon-coated wire electrodes as an index of neural respiratory output. The bare tips of the electrodes were inserted into the diaphragm, and the distance between the two electrodes was kept to a minimum. A third electrode attached to the ear served as an electrical ground. Diaphragm EMG activity was filtered (band-pass 0.3–1.0 kHz), amplified (AC preamplifier; model P511K, Grass Instruments), and passed through Paynter filters (time constant of 100 ms; CWE, Ardmore, PA) to obtain a moving average signal. The integrated signal and raw EMG activity were stored in a computer via a computer data sampling system (ADInstruments) for subsequent analysis. The contribution of carotid body sensory afferents to the breathing pattern was determined by severing the carotid sinus nerves (CSN) bilaterally. Lidocaine was administered to the glossopharangeal nerves at the site of transection to suppress trauma-related nerve activity. Diaphragm EMG recordings were made after allowing 10 min for the preparation to stabilize. The contribution of vagal afferents to ventilatory responses was reassessed in these same animals after subsequent transection of bilateral vagus nerves. Following local administration of lidocaine and a 10-min stabilization period, integrated diaphragm EMG was again recorded. In a second group of animals, bilateral vagotomy was performed first, followed by later bilateral CSN transection to better identify contributions of vagal afferents to the respiratory patterns.
Experimental Protocols

Based on the anticipated time course of BM-induced lung injury (1, 7), measurements in all groups were made 5 days after intratracheal BM instillation to examine the early inflammatory stage of lung injury, before the development of pulmonary fibrosis. Control animals received intratracheal PBS instillations. In group 1, CT imaging of the lungs (n = 6), lung histology (n = 6), and BALF cell count and differential (n = 6) were examined. In group 2 (n = 16), the effects of lung injury on hypoxic and hypercapnic ventilatory responses, BALF protein concentration, and lung collagen content were examined. Animals were first subjected to plethysmography protocols for measurement of hypoxic and hypercapnic ventilatory responses. Baseline respiratory parameters were collected for 5 min with the animal breathing room air. The gas in the plethysmography chamber was then switched to 12% O2–88% N2, and ventilation was recorded for 5 min before returning to room-air breathing. For hypercapnic ventilatory responses, baseline respiration was monitored while the animals breathed 100% O2 for 5 min, followed by an exposure to 7% CO2–93% O2 for an additional 5 min. V̇O2 and V̇CO2 were measured at the end of each 5-min test period. The protocols for hypoxia and hypercapnia were repeated twice in each animal. Upon completion of plethysmography, BALF was collected for cell count, differential, and protein content. Lungs were then harvested for collagen content determination. In group 3 (n = 12), the impact of early lung injury on arterial blood-gas profiles and neural respiration was examined in urethane-anesthetized rats. Baseline blood pressure and arterial blood pH, Pco2, Po2, and bicarbonate were measured with animals breathing room air supplemented with 100% O2. Hypoxic ventilatory responses to brief (2–3 min) exposures to 12% O2–88% N2 were recorded. Arterial Po2 was measured following the hypoxic exposure (n = 8). Following acclimatization to hypoxia (inspired gas mixture of 12% O2–88% N2), Dejours’ test of respiratory response to brief hypoxia was performed with a 60-s exposure to 100% O2. Neural respiratory parameters collected 15 s after the initiation of hypoxia were analyzed. Next, the contribution of carotid body sensory afferents to the breathing pattern was determined by severing the CN bilaterally and measuring the resultant baseline neural ventilation and response to hypoxia and hyperoxia as described above. Finally, the contribution of vagal afferents to ventilatory responses was reassessed in these same animals. After subsequent transection of bilateral vagus nerves, the protocols described above were repeated. In group 4 (n = 12), the protocol employed in group 3 was repeated, but bilateral vagotomy was performed first, followed later by bilateral CSN transection.

Data Analysis

In unanesthetized animals, the following respiratory variables were analyzed: respiratory rate (RR; breaths/min), V̇t (ml), and minute ventilation (Ve [ml/min] = RR × V̇t). Changes in V̇t and Ve were normalized to body weight (kg). Respiratory variables were averaged over 5 min during baseline and with each O2 or CO2 concentration. V̇O2 and V̇CO2 were measured during each exposure. Changes in respiratory variables were expressed as a percentage of baseline values and as absolute change from baseline values. Based on the fact that RR was the respiratory variable primarily affected by lung injury in the unsedated animals, only neural RR was analyzed in the anesthetized animals. Neural RR (breaths/min) was calculated by considering each burst of EMG activity as a “neural breath” averaged over 1 min during exposures to hypoxia or hyperoxia. Changes in neural RR were expressed as absolute change from baseline values for Dejours’ test and hypoxic ventilatory responses. The data are presented as means ± SE. Statistical evaluations were made using a two-sample t-test or signed-rank test as appropriate. P values of <0.05 were considered significant for most experiments. In the plethysmography experiments where multiple (3) parameters were measured in the same animal, the Bonferroni adjustment was employed, and P values of <0.015 were considered to be statistically significant.

RESULTS

Induction of Lung Injury by BM

Five days after BM administration, the extent of lung injury and fibrosis was examined. As expected, lung injury was present in animals that received intratracheal BM instillations, while lung damage was not present in PBS-injected animals. Figure 1, A and B, depicts representative images of computer-aided tomography (CT) scans of lungs from PBS-injected and BM-injected animals, respectively. Pulmonary infiltrates suggestive of lung injury were present bilaterally in BM-injected animals (white arrow), and areas of injury were primarily localized near major airways, consistent with the intratracheal method of BM administration. Hematoxylin and eosin stained lung sections from control and lung-injured animals are presented in Fig. 1, C and D. Lung histology revealed preserved alveolar architecture in control lungs, while lung sections from BM-exposed animals had areas of significant cellular infiltration (thick arrow), along with areas of relatively undamaged lung parenchyma (thin arrow). BM-injected animals had increased protein concentration in BALF compared with controls (P = 0.001; Fig. 2A), indicating disruption of the alveolar-capillary barrier. Furthermore, total cell counts (P = 0.002; Fig. 2B) and percentage of neutrophils (P < 0.001; Fig. 2B) were elevated in BALF from BM-exposed animals relative to controls, confirming the presence of acute inflammation. In contrast, collagen content was not significantly altered (P = 0.459; Fig. 2C). These observations indicate that, 5 days after BM instillation, lung injury is present, but pulmonary fibrosis has not yet developed.

Effects of Early Lung Injury on Arterial Blood Gases and Body Temperature

Arterial blood gases were performed to determine whether early lung injury altered systemic oxygenation, ventilation, and acid-base status. With animals breathing room air supplemented with 100% O2, arterial pH (BM 7.41 ± 0.02 vs. control 7.43 ± 0.02; P = 0.465), arterial Po2 (BM 183 ± 15 Torr vs. control 186 ± 7 Torr; P = 0.937), arterial Pco2 (BM 38.7 ± 2.5 Torr vs. control 38.8 ± 1.4 Torr; P = 0.955), and arterial bicarbonate (BM 23.3 ± 1.3 mmol/l vs. control 25.2 ± 1.4 mmol/l; P = 0.357) were similar in both groups. Following exposure to 12% O2, arterial Po2 (BM 54.7 ± 2.4 Torr vs. control 55.0 ± 1.8 Torr; P = 0.935) decreased to a similar value in each group. Basal body temperature (BM 37.3 ± 0.2°C vs. control 37.4 ± 0.1°C; P = 0.586) was not different between saline-treated controls and BM-treated rats.

Effects of Early Lung Injury on Breathing

In unanesthetized animals breathing ambient air, RR was significantly greater in the BM-exposed group (P < 0.001; Fig. 3A). There was no difference in V̇t (P = 0.525; Fig. 3B) between the groups. Ve tended to be higher in animals with lung injury, but this difference was not statistically significant (P = 0.169; Fig. 3C). Basal V̇O2 (BM 1.56 ± 0.35 l·kg⁻¹·min⁻¹ vs. control 1.39 ± 0.33 l·kg⁻¹·min⁻¹; P = 1.000) and V̇CO2 (BM 0.47 ± 0.07 l·kg⁻¹·min⁻¹ vs. control 0.46 ± 0.07 l·kg⁻¹·min⁻¹; P = 1.000) were similar in both groups.
Ventilatory Response to Acute Hypoxia

Hypoxic ventilatory response [inspired O2 fraction ($F_{IO2}$) = 0.12] was recorded in unanesthetized animals 5 days after the induction of lung injury. In response to hypoxia, average increases in RR ($P = 0.003$; Fig. 4A) and $\dot{V}_{E}$ ($P = 0.003$; Fig. 4C) were significantly greater in the BM-injected compared with control animals, respectively. Moreover, significant differences persisted after adjusting $\dot{V}_{E}$ for either $V_{O2}$ ($P = 0.009$) or $V_{CO2}$ ($P = 0.011$). Average increase in $V_{T}$ ($P = 0.223$; Fig. 4B) was not significant between the groups. In both animal groups, $V_{O2}$ fell as inspired O2 decreased from 100 to 12% ($P < 0.001$; Fig. 4D). However, the magnitude of the drop in $V_{O2}$ was similar between control (solid bar) and lung-injured (open bar) animals ($P > 0.05$). $V_{CO2}$ was unaffected by hypoxia in both groups ($P > 0.05$; Fig. 4E), and, as a result, changes in $V_{CO2}/V_{O2}$ were comparable between both control and BM-exposed animals ($P > 0.05$; Fig. 4F).

Hypercapnic Ventilatory Response

Ventilatory responses to hyperoxic hypercapnia (7% CO2 + 93% O2) were measured in unanesthetized animals. Average changes in RR ($P >$ Bonferroni-adjusted 0.015; Fig. 5A), $V_{T}$ ($P >$ Bonferroni-adjusted 0.015; Fig. 5B), and $\dot{V}_{E}$ ($P = 0.672$; Fig. 5C) were similar between the groups. Adjusting $\dot{V}_{E}$ for either $V_{O2}$ ($P = 0.185$) or $V_{CO2}$ ($P = 0.560$) did not alter the findings.

Contribution of Peripheral Chemoreceptors

The results described above indicate a selective augmentation of hypoxic ventilatory response but not hypercapnic ventilatory response in early lung injury. Because the hypoxic ventilatory response is primarily mediated by input from peripheral chemoreceptors, the following experiments were performed to assess the contribution of peripheral sensory afferent information to breathing patterns in early lung injury.

Response to Brief Hyperoxia

In anesthetized, spontaneously breathing animals, the average decrease in RR in response to sudden administration of hyperoxia ($F_{IO2}$ change from 0.12 to 1.0, Dejours’ test) was significantly greater in the BM-exposed group compared with control animals ($P = 0.041$; Fig. 6). However, this difference was abolished following bilateral CSN transection ($P = 0.074$; Fig. 6).

Effect of CSN Transection and Vagotomy on Hypoxic Ventilatory Response

Hypoxic ventilatory response ($F_{IO2} = 0.12$) was measured in anesthetized, spontaneously breathing animals, and results are
shown in Fig. 7. On average, increases in RR were more dramatic in the animals with lung injury \((P < 0.001)\). Following bilateral vagotomy, average increases in RR remained significantly greater in BM-injected animals compared with control animals \((P < 0.005)\). In contrast, this difference between control and BM-exposed animals was abolished following bilateral CSN transection \((P = 0.786)\). The magnitude of the hypoxic response was nearly the same with combined CSN transection and bilateral vagotomy compared with the response seen after CSN transection alone \((P = 0.206 \text{ and } P = 0.223, \text{ BM and control, respectively})

Fig. 3. Baseline ventilatory parameters 5 days after BM administration. In unanesthetized animals breathing ambient air, baseline respiratory rate \((A)\) was significantly greater in BM-treated animals \((P < 0.001)\) and control animals \((P < 0.005)\). In contrast, this difference between control and BM-exposed animals was abolished following bilateral CSN transection \((P = 0.786)\). The magnitude of the hypoxic response was nearly the same with combined CSN transection and bilateral vagotomy compared with the response seen after CSN transection alone \((P = 0.206 \text{ and } P = 0.223, \text{ BM and control, respectively})\).

Fig. 2. Lung injury is present in the absence of pulmonary fibrosis 5 days after BM administration. Protein concentration \((A)\) of bronchoalveolar lavage fluid (BALF) is significantly increased in BM-treated animals \(\text{(open bars)}\) compared with controls \(\text{(solid bars)}\), indicating disruption of the alveolar-capillary barrier. Furthermore, BALF total cell count and percent neutrophils \((B)\) are elevated in BM-injected animals, confirming the presence of an acute inflammatory infiltrate. However, lung tissue collagen content \((C)\) is not increased, suggesting that significant fibrosis is not present at this early stage. The data presented are means \(\pm\) SE. \(*P < 0.01. \text{n.s.}, \text{Not significant} (P > 0.05)\).

**DISCUSSION**

The major findings of the present study are as follows: 1) 5 days after BM instillation, patchy lung injury was present, but pulmonary fibrosis had not developed; 2) basal arterial blood gases were not significantly altered; 3) basal RR increased in BM-treated rats; 4) hypoxic but not hypercapnic ventilatory response was selectively augmented; and 5) bilateral sectioning of CSNs but not bilateral vagotomy prevented the respiratory changes in BM-treated rats.
Our results demonstrate that early lung injury was present 5 days after BM instillation. The pattern of lung injury manifested as patchy areas of acute inflammatory cell infiltration (primarily adjacent to the central airways) along with other areas of normal, uninjured lung parenchyma. The fact that baseline arterial blood gases were unchanged suggests that the areas of normal lung compensated for areas of injury, preventing the development of systemic hypoxemia at this stage. The absence of significant alterations in lung collagen content suggests that, at this time point of early lung injury, there was no development of pulmonary fibrosis.

We examined the impact of early stages of lung injury on ventilation in unsedated rats to exclude the confounding influence of anesthesia on breathing. Despite the absence of systemic hypoxemia, we observed a consistent increase in basal RR in BM-treated compared with control rats. This observation demonstrates that even as short as 5 days of lung injury can profoundly impact basal breathing manifested as tachypnea. Similar increases in RR were also reported in late stages of lung injury with pulmonary fibrosis. The tachypnea seen in the present study was not associated with pulmonary fibrosis, implying that the mechanisms contributing to tachypnea in the early vs. late stages of lung injury differ. Not only did the BM-treated rats manifest tachypnea, more importantly they exhibited an exaggerated ventilatory response to acute hypoxia. In striking contrast, the ventilatory response to hypercapnia was not significantly altered, demonstrating that early stages of acute lung injury selectively affect the hypoxic ventilatory response. An exaggerated drop in PO2 in response to hypoxic challenge in the animals with lung injury could lead to an augmented hypoxic ventilatory response relative to control animals. However, P02 levels were comparable between both groups following brief hypoxia. Hypoxia is known to affect VO2 and VCO2, which, in turn, can influence the hypoxic ventilatory response. However, changes in VO2 and VCO2 between both groups (i.e., injured and uninjured, Fig. 3) were of similar magnitude, indicating that the augmented hypoxic ventilatory response was not secondary to metabolic alterations. Injury and inflammation, as well as hypoxia, can affect body temperature, and hypo- or hyperthermia can subsequently influence ventilatory patterns. However, basal body temperature was comparable between the groups studied, excluding its contribution to the augmented hypoxic ventilatory response.

Fig. 4. Hypoxic ventilatory response is enhanced 5 days after BM administration. In response to hypoxia (FIO2 = 0.12), average increase in respiratory rate (A) in unanesthetized animals was significantly greater in the BM-exposed group (open bars) compared with control animals (solid bars). In contrast, average increase in tidal volume (B) was not statistically significant between the groups. Average increase in minute ventilation normalized to body weight (C) was also significantly augmented in animals with lung injury. In both groups, oxygen consumption (VO2) fell (D) with decreasing inspired O2 fraction (FIO2), while carbon dioxide production (VCO2) was unaffected (E) by changes in inspired O2. Consequently, changes in the respiratory quotient (VCO2/VO2) were similar (F) in both BM-exposed and control animals. The data presented are means ± SE. **P < 0.005 relative to control. *P < 0.001 relative to 100% O2 baseline. #No difference relative to 12% O2 control (P > 0.05), but P < 0.001 relative to 100% O2 baseline. n.s. (P > Bonferroni-adjusted 0.015).
with control rats. Ventilatory responses to brief hyperoxia were abolished following bilateral CSN transection, demonstrating that sensory information from the carotid bodies contributes to the hyperoxia-evoked respiratory depression. A role for carotid bodies in the enhanced hypoxic ventilatory response in BM-treated rats is further supported by the finding that the respiratory response to hypoxia was abolished by bilateral transection of the sinus nerves. The fact that bilateral vagotomy had minimal impact on the hypoxic response in BM-treated rats suggests that vagal afferents contribute little, if any, to the basal as well as to the hypoxic response during early stages of lung injury. These observations also highlight the fact that distinct afferent systems are recruited to affect breathing as lung injury advances. For instance, in early lung injury, breathing is predominantly influenced by carotid body afferents, as shown in the present study, whereas influences of vagal afferents predominate in late stages of lung injury with pulmonary fibrosis, as reported previously (19).

How do the carotid bodies mediate the increase in basal ventilation in the absence of systemic hypoxia or hypercapnia in BM-treated rats? One possibility is that BM might have a direct effect on the carotid body. While we cannot rule this out completely, we believe this is an unlikely contributor, as intratracheal instillation of BM limits systemic distribution. Other factors, such as ageing, obesity (21), and hypoxic conditioning (12, 15), are known to modulate carotid body responses to hypoxia. All of the animals were of similar age and weight, but our findings may be ascribed to intermittent hy-

Fig. 5. Hypercapnic ventilatory response is not dramatically altered 5 days after BM administration. In response to hyperoxic hypercapnia (7% CO₂ + 93% O₂), unanesthetized animals had average increases in respiratory rate (A), tidal volume (B), and minute ventilation normalized to body weight (C) that were similar between control animals (solid bars) and BM-treated animals (open bars). The data presented are means ± SE. n.s. (P > Bonferroni-adjusted 0.015).

Fig. 6. Augmented response to Dejours’ test in BM-exposed animals is abolished by CSN transection. In sedated, spontaneously breathing animals, average decreases in respiratory rate in response to sudden administration of hyperoxia (FIO₂ change from 0.12 to 1.0) were significantly greater in the BM-exposed group (open bars) compared with control animals (solid bars). In contrast, this difference was abolished following bilateral transection of CSNs. The data presented are means ± SE. bpm. Breaths per minute. *P < 0.05. n.s. (P > 0.05).

Fig. 7. Enhanced hypoxic ventilatory response in BM-exposed animals is abolished by bilateral CSN transection but not by bilateral vagotomy. In response to hypoxia (FIO₂ change from 1.0 to 0.12), average increase in respiratory rate in sedated, spontaneously breathing animals was significantly greater in the BM-exposed group (open bars) compared with control animals (solid bars). This difference remained significant following bilateral vagotomy. The magnitude of the hypoxic response was nearly the same with combined CSN transection and bilateral vagotomy compared with the response seen after CSN transection alone. The data presented are means ± SE. *P < 0.05. n.s. (P > 0.05).
poxic exposures (possibly during sleep or exercise?). We did not monitor these variables in our experiments due to technical limitations; however, their contribution is unlikely, as blood-gas samples from sedated animals were similar in control and lung-injured animals. Another possibility is that lung injury might have resulted in elevated circulating levels of ATP and/or its metabolites, which are known to exert a stimulatory influence on the carotid body (10, 16, 18, 24, 30). Alternatively, inflammatory mediators (such as proinflammatory cytokines) released systemically in the setting of lung injury may be responsible for sensitization of oxygen sensing by peripheral chemoreceptors. The adult rat carotid body expresses interleukin receptors (25, 26); however, their importance to carotid body physiology is not completely understood. The potential mechanisms contributing to carotid body sensitization during early stages of lung injury require further investigation and are beyond the scope of the present study.

In summary, the present study demonstrates that carotid body chemoreceptors are sensitized during early stages of lung injury, leading to increased RR and enhanced ventilatory response to hypoxia. Lung injury is a frequently encountered clinical problem, and many patients suffer with significant morbidity related to shortness of breath. It has been proposed that stimulation of peripheral chemoreceptors may promote dyspnea beyond that arising from respiratory-mechanical factors (27). Thus a better understanding of the mechanisms responsible for oxygen-independent sensitization of the carotid body may lead to better approaches for limiting dyspnea in patients with lung injury.

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