Attenuation of the pulmonary chemoreflex following acute cervical spinal cord injury

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Tsai IL, Lee KZ. Attenuation of the pulmonary chemoreflex following acute cervical spinal cord injury. J Appl Physiol 116: 757–766, 2014. First published February 20, 2014; doi:10.1152/japplphysiol.01370.2013.—Bronchopulmonary C fibers are the primary chemosensitive afferents in the lung. The activation of bronchopulmonary C fibers evokes the pulmonary chemoreflex, which is characterized by apnea, hypotension, and bradycardia and is a critical reflex that modulates cardiorespiratory responses under physiological and pathological conditions. The present study was designed to investigate whether the pulmonary chemoreflex is altered following acute cervical spinal injury. A unilateral hemisection (Hx) or laminectomy (uninjured) in the second cervical spinal cord was performed in adult male Sprague-Dawley rats. The pulmonary chemoreflex induced by intrajugular capsaicin administration was evaluated by measuring respiratory airflow in spontaneously breathing rats and phrenic nerve activity in mechanically ventilated rats. Capsaicin treatment evoked a cessation of respiratory airflow and phrenic bursting in uninjured animals, but not in C2Hx animals. To clarify whether the attenuation of the pulmonary chemoreflex in C2Hx animals is restricted to capsaicin-induced stimuli, or generally applied to other stimuli that excite bronchopulmonary C fibers, another bronchopulmonary C-fiber stimulant (phenylbiguanide) was used to evoke the pulmonary chemoreflex in spontaneously breathing rats. We observed that phenylbiguanide-induced apnea was also blunted in C2Hx animals, suggesting that the respiratory response induced by bronchopulmonary C-fiber activation was attenuated following acute cervical spinal Hx. The blunted inhibitory respiratory response may represent a compensatory respiratory plasticity to preserve the breathing capacity and may also reduce the capability of preventing inhaling irritants in this injured condition.

Pulmonary chemoreflex; cervical spinal cord injury; phrenic; bronchopulmonary C fiber

HIGH CERVICAL SPINAL CORD injury usually interrupts bulbo spinal respiratory pathways and causes changes in the breathing pattern and pulmonary mechanics (9, 14, 15, 21, 37, 41, 44, 45). Impairment of the pulmonary defense reflex following spinal cord injury may result in several pulmonary complications (e.g., acute lung injury, atelectasis, and pneumonia) and in turn lead to associated morbidity and mortality in patients with cervical spinal cord injury (2, 11, 48). The pulmonary chemoreflex is a critical type of pulmonary defense reflex characterized by apnea/tachypnea, hypotension, and bradycardia (32, 33). This complex pattern of cardiorespiratory responses can protect the respiratory tract from inhaled irritants and/or limit the distribution of harmful substances in the bloodstream (5, 32, 33). It is well established that the pulmonary chemoreflex is mainly evoked by activation of vagal bronchopulmonary C fibers, which are the primary chemosensitive afferents in the lung and can be excited or sensitized by various inhaled stimuli (e.g., cigarette smoke and ozone) and internal inflammatory mediators (e.g., tumor necrosis factor-α and interleukin-1) (18, 30, 34, 52). However, whether the pulmonary chemoreflex is altered after cervical spinal cord injury remains unclear. Our previous study showed that a vagally mediated lung inflation reflex was changed following high cervical spinal cord injury (29). Specifically, the inhibitory effect of positive end-expired pressure (PEEP; 6 and 9 cmH2O) on phrenic and hypoglossal bursting was attenuated in C2 spinal hemisection (C2Hx) rats. Additionally, the respiratory response to hypocapnia appears to be blunted following cervical spinal cord injury (22). Golder et al. (13) also observed that the CO2 apneic threshold (i.e., the end-tidal CO2 value when the inspiratory phrenic bursting ceased) reduced following cervical contusion, indicating that cervical spinal injured animals can still maintain rhythmic phrenic bursting under lower end-tidal CO2 value compared with control animals. Moreover, several airway protective behaviors (e.g., cough and augmented breath) and respiratory reflex during respiratory challenge (i.e., hypercapnia) are impaired in humans and/or animals with cervical spinal cord injury (1, 2, 12, 20). These results suggest that respiratory motor outputs become more resistant to inhibitory inputs and insensitive to the stimuli that can evoke the pulmonary defense reflex following cervical spinal cord injury. Accordingly, we hypothesized that animals with cervical spinal cord injury have a lower capability to produce the pulmonary chemoreflex in response to bronchopulmonary C-fiber activation. Three experimental protocols were conducted to test our hypothesis. First, the pulmonary chemoreflex evoked by intrajugular administration of capsaicin was compared between uninjured and C2Hx spontaneously breathing animals. Second, the phrenic nerve response to intrajugular capsaicin injection was evaluated in mechanically ventilated and paralyzed animals. Third, pulmonary chemoreflexes induced by phenylbiguanide (PBG) were assessed in uninjured and C2Hx animals.

MATERIALS AND METHODS

Animals

A total of 53 adult male Sprague-Dawley rats (7−8 wk of age) purchased from the BioLasco Taiwan were used in the present study. The animals were divided into uninjured (i.e., C3 laminectomy only) (n = 27) and C2Hx (n = 26) groups. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the National Sun Yat-sen University.

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Spinal Cord Injury

C2 laminectomy or C2Hx surgery was performed at 9 wk of age (65 ± 1 day, mean ± SE). Animals were anesthetized with xylazine (10 mg/kg sc, Rompun, Bayer) and ketamine (140 mg/kg ip, Ketalar, Pfizer). A dorsal cervical incision was made from the C1 to C3 spinal cord followed by C2 laminectomy in both the uninjured and C2Hx groups. A left C2 hemisection was then performed in C2Hx animals by microscalpel with gentle aspiration. The dura and overlying muscles were sutured with 10–0 nylon and 4–0 chronic sutures (UNIK), respectively. The skin was subsequently closed with 4–0 nylon sutures (UNIK). Following the surgery, the animals were given yohimbine injections (1.2 mg/kg sq, Tocris), to reverse the effect of the xylazine, and lactated Ringer solution (5 ml sq, Nang Kuang Pharmaceutical) to prevent dehydration. An analgesic (buprenorphine, 0.03 mg/kg sq, Shinlin Sin-seng Pharmaceutical) was administered to all of the animals for analgesia. The postsurgical care protocol, including the daily oral supply of Nutri-cal (1–3 ml, EVSCO Pharmaceuticals) and the injection of lactated Ringer’s solution (5 ml sc), was applied until the recovery of adequate volitional eating and drinking.

General Animal Preparation

At 1 day post-C2 laminectomy or C2Hx, the animals were anesthetized with urethane (1.6 g/kg ip, Sigma) and placed in a supine position. The rectal temperature was monitored by an electrical thermometer and maintained at 37 ± 1°C by a servo-controlled heating pad (model TC-1000, CWE). The following surgical operation was only performed when the toe pinch withdrawal reflex was no longer present. The trachea was cannulated below the larynx with the endotracheal tube (PE-240, Clay Adams). The femoral artery was catheterized (PE-50) for blood pressure measurement (transducer: DTX-1; amplifier: TA-100, CWE). Another PE-100 catheter was inserted near the right atrium via the right jugular vein for drug administration, as previously described (23–25, 27).

Pharmacological Agent Preparation

Capsaicin and 1-phenylisquinoamide hydrochloride (PBG) purchased from Tocris Bioscience were used to activate bronchopulmonary C fibers and to evoke the pulmonary chemoreflex. A stock solution of capsaicin (500 μg/ml) was prepared in 80% saline, 10% alcohol (95%), and 10% polysorbate 80 (Tweeen 80, Alfa Aesar). A stock solution of PBG (400 μg/ml) was prepared in saline. Both solutions were stored at −20°C and diluted to the desired concentration with saline based on the weight of the animal on the day of experiment. The bolus injection volume was 0.2 ml, which was injected into the right jugular catheter and then flushed into the right atrium by injecting 0.35–0.5 ml saline.

Respiratory Airflow Measurement In Spontaneously Breathing Rats

Respiratory airflow was measured in spontaneously breathing animals at 1 day post-C2 laminectomy or C2Hx in the first and third experimental protocols (see below). After general animal preparations, the endotracheal tube was connected to a respiratory flow head (MLT1L, ADInstruments), which was connected to a spirometer (FE141, ADInstruments). A hyperoxic gas mixture (50% O₂, balance N₂; flow rate: 2 l/min) was delivered to the endotracheal tube via a “T-piece” design (9). Both respiratory airflow and blood pressure signals were digitized using the CED Power 1401 (Cambridge Electronic Design, Cambridge, UK) and the PowerLab data acquisition system (ADInstruments). All data were recorded in a computer using Spike 2 and LabChart software.

Phrenic Nerve Recording In Mechanically Ventilated Rats

Bilateral phrenic nerve activity was recorded in the second experimental protocol (see below) at 1 day post-C2 laminectomy or C2Hx.

After general animal preparation, the animals were mechanically ventilated (KDS 35, KD Scientific) with an oxygen/nitrogen mixture (50% O₂, balance N₂; volume = 7 ml/kg; frequency = 60–70/min) and paralyzed with pancuronium bromide (2.5 mg/kg iv, Fresenius Kabi). The partial pressure of end-tidal CO₂ was analyzed with a Capnogard CO₂ monitor (Novametrix Medical Systems) by placing a CO₂ sensor on the expiratory line of the ventilator circuit. The partial pressure of end-tidal CO₂ was maintained at 50 Torr by adjusting the ventilator rate and/or inspired CO₂ throughout the experiment. A PEEP was maintained at 2 cmH₂O by placing the outlet tube of the ventilator 2 cm under the water surface. Bilateral phrenic nerves were isolated and cut distally in the cervical region by a ventral approach, as previously described (22, 29). Both phrenic nerves were placed over monopolar silver electrodes (no. 782500, A-M Systems), which were connected to a differential A/C amplifier (model 1700, A-M Systems). Neural signals were amplified (×1,000), band-pass filtered (0.3–10 kHz) by the amplifier, and digitized by the CED Power 1401. Both neural and blood pressure signals were recorded and stored in a computer using Spike 2 software.

Experimental Protocol

Three experimental protocols were conducted to investigate whether the pulmonary chemoreflex is altered following acute spinal cord injury (i.e., 1 day post-sham or C2Hx surgery). Capsaicin is an active pungent ingredient of hot pepper and serves as a potent agonist of transient receptor potential vanilloid type 1, which is expressed within the nodose and jugular ganglia that innervate the lungs (53). Intravenous administration of capsaicin has been widely used to activate bronchopulmonary C fibers and to evoke the pulmonary chemoreflex in our, and others’, studies (23–25, 27). In the first protocol, capsaicin-induced pulmonary chemoreflexes were compared between uninjured (n = 8) and C2Hx (n = 7) animals under spontaneous breathing conditions. Three doses of capsaicin (0.5, 1.0, and 1.5 μg/kg) were randomly injected into the jugular vein catheter after stable recording of respiratory airflow and blood pressure. At least 20 min were allowed to elapse between two successive capsaicin treatments to prevent possible tachyphylaxis.

C2Hx usually results in the paralysis of the ipsilateral hemidiaaphragm and decreased tidal volume (9, 49). To evaluate whether the alteration of the pulmonary chemoreflex is due to changes inafferent inputs from the chest wall and diaphragm after C2Hx, the second protocol used the paralyzed and mechanically ventilated animals (uninjured, n = 7; C2Hx, n = 7) to investigate the phrenic nerve response to intragastric capsaicin administration (1.0 and 1.5 μg/kg). This experimental model can standardize the setting of ventilator parameters (e.g., frequency, volume, and PEEP) between uninjured and C2Hx animals to reduce the differential mechanical inputs across groups. In addition, capsaicin treatment was also performed after bilateral vagotomy to examine the role of vagus nerves on the capsaicin-induced pulmonary chemoreflex.

Bronchopulmonary C fibers are polymodal sensitive and express various pharmacological receptors (e.g., transient receptor potential vanilloid type 1, 5-HT₃ receptor, and P2X receptor) (7, 17, 42, 47). To examine if altered pulmonary chemoreflexes following spinal cord injury are merely induced by capsaicin application, or also by other bronchopulmonary C-fiber stimulants, a 5-HT₃ receptor agonist (PBG, 20 and 40 μg/kg; Tocris) was used to evoke the pulmonary chemoreflex in anesthetized and spontaneously breathing animals (uninjured, n = 12; C2Hx, n = 12) before and after bilateral cervical vagotomy in the third study.

Spinal Cord Histology

At the end of the experimental protocol, C2Hx animals were systemically perfused with heparin-saline followed by 4% paraformaldehyde (Alfa Aesar). The cervical spinal cord was removed, cryoprotected, and sectioned at 40 μm via the Vibratome (Series AP900, Leica Microsystems). The sections were mounted on slides and stained with Toluidine blue for examination and morphometric analysis.

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1000, Vibratome). A subset of animals was perfused with heparin-saline followed by 4% paraformaldehyde, and then 10% sucrose in 4% paraformaldehyde. The cervical spinal cord tissue was removed and placed in 30% sucrose in phosphate buffer solution and then cut into 20-μm sections using a Cryostat (CM 1850, Leica). The spinal cord tissue sections were serially mounted on glass slides and stained with crystal violet (Acros Organics). A representative example of a cervical spinal cord hemisection is presented in Fig. 1.

Data Analysis and Statistics

Respiratory airflow measurement in anesthetized and spontaneously breathing rats. The inspiratory, expiratory, and respiratory cycle duration and respiratory frequency were calculated based on the respiratory airflow trace (9). The tidal volume was derived by integrating the inspiratory airflow. Respiratory parameters averaged over 10 s before capsaicin or PBG treatments were defined as the baseline value. A t-test and two-way repeated-measures analysis of variance (RM-ANOVA) were used to compare the breathing pattern between uninjured and C2Hx animals using protocols 1 and 3, respectively. To evaluate the intensity of the pulmonary chemoreflex, the immediate response of the expiratory duration following capsaicin or PBG administration was determined. The expiratory duration was expressed in second (s) and normalized as a percentage of the baseline value (%BL). A two-way RM-ANOVA (factor 1: uninjured vs. C2Hx group; factor 2: capsaicin dosage), followed by the Student-Newman-Keuls post hoc test, was used to compare the pulmonary chemoreflex between uninjured and C2Hx animals in the first protocol. PBG induced two distinct types of immediate responses: tachypnea (i.e., the increase in respiratory frequency) and apnea in the third protocol; therefore, these data were separated and analyzed independently using two-way ANOVA (factor 1: uninjured vs. C2Hx group; factor 2: PBG dosage).

Phrenic nerve recording in anesthetized and ventilated rats. The inspiratory, expiratory, and respiratory cycle durations were calculated by the rectified and smoothed phrenic neurogram contralateral to the spinal lesion. The inspiratory duration was determined as the period between inspiratory phrenic onset and the time point when the rectified and smoothed phrenic amplitude declined by 50% of the peak value, as previously described (22, 28, 29). The expiratory duration is defined as the interval between the end of inspiration and the onset of the subsequent inspiratory burst. The respiratory frequency is calculated as 60/(inspiratory + expiratory duration). The phrenic burst amplitude is defined as the difference between the maximal and minimum value of the processed phrenic neurogram within a single neural breath. The immediate respiratory response after capsaicin treatment was defined as the longest expiratory duration (i.e., cessation of rhythmic phrenic bursting) and was expressed in seconds (s) and converted into %BL.

Mean arterial blood pressure and heart rate. The mean arterial blood pressure and heart rate data averaged over 10 s before the drug treatment was defined as the baseline value. The immediate cardiovascular response was analyzed in 1-s bins and compared by two-way RM-ANOVA.

All data are expressed as the mean ± standard error. A P value of <0.05 is considered statistically significant.

RESULTS

Capsaicin-induced Pulmonary Chemoreflex in Spontaneously Breathing Rats

Representative examples of the capsaicin-induced pulmonary chemoreflex at 1 day postinjury are presented in Fig. 2. The expiratory duration, mean arterial blood pressure, and heart rate were similar between uninjured (n = 8) and C2Hx (n = 7) animals before capsaicin treatment (Fig. 3); however, the tidal volume was significantly lower in the C2Hx animals (0.9 ± 0.1 ml) compared with the uninjured animals (1.2 ± 0.0 ml, P < 0.01). Intrajugular capsaicin administration evoked a dose-dependent elongation of expiratory duration (i.e., apnea) in the uninjured animals (Figs. 2 and 3). Specifically, the expiratory duration was significantly increased from 2.6 ± 0.6 s during the baseline to 0.25 ± 0.01 s following a high dose (1.5 μg/kg) of capsaicin (P < 0.01, Fig. 3). When the data were normalized to %BL, the expiratory duration extended to 366 ± 111% BL and 1,109 ± 229% BL after moderate (1.0 μg/kg) and high (1.5 μg/kg) doses of capsaicin, respectively (Fig. 3). In addition, significant bradycardia and hypotension were also induced by 1.5 μg/kg capsaicin treatment (P < 0.01, Fig. 3). In contrast, no significant changes were observed in the respiratory pattern and the cardiovascular response following capsaicin administration in the C2Hx animals (Figs. 2 and 3).

Phrenic Nerve Response Following Intrajugular Capsaicin Administration in Mechanically Ventilated Rats

Representative examples depicting bilateral phrenic neurograms recorded in an uninjured and C2Hx animal at 1 day postsurgical injury are presented in Fig. 4. The bilateral phrenic nerve exhibited rhythmic inspiratory bursting in uninjured animals; however, only the phrenic nerve contralateral to the lesion (i.e., right side) displayed inspiratory activity in C2Hx animals. The phrenic burst frequency and contralateral phrenic burst amplitude are similar between uninjured (n = 7) and C2Hx (n = 7) animals during the baseline condition (Table 1). Intrajugular capsaicin administration evoked significant pulmonary chemoreflexes in uninjured animals. In particular, moderate (1.0 μg/kg) and high (1.5 μg/kg) doses of capsaicin resulted in a significant elongation of the expiratory duration to 2.2 ± 0.6 s (369 ± 90% BL) and 3.3 ± 0.3 s (588 ± 64% BL), respectively (P < 0.01, Fig. 5, A and B). The mean arterial blood pressure and heart rate also decreased in response to the high dose of capsaicin (P < 0.05, Fig. 5, C and D). In C2Hx animals, neither dose of capsaicin induced significant changes in the expiratory duration; however, a significant reduction in

Fig. 1. A representative histological example of a C2 spinal hemisection (C2Hx).
the heart rate was observed following capsaicin treatment ($P < 0.01$, Fig. 5D), and the intensity of bradycardia was similar between uninjured and C2Hx animals.

To evaluate whether cardiorespiratory reflexes induced by intrajugular capsaicin administration result from the activation of vagal afferents, the same doses of capsaicin were delivered into the right atrium after bilateral vagotomy (Fig. 4). The phrenic burst frequency was reduced, and the burst amplitude of the right side phrenic nerve increased following vagotomy in both groups ($P < 0.01$, Table 1). The elongation of expiratory duration was significantly elongated in uninjured ($n = 8$) animals, but not in C2Hx ($n = 7$) animals, when data were presented in seconds (A) or as percent baseline (%BL; B). The mean arterial BP (C) and heart rate (D) decreased in response to capsaicin treatment only in uninjured animals. **$P < 0.01$, significance from the BL value. ***$P < 0.01$, significance from the uninjured animals.
duration (i.e., apneic response) of vagotomized uninjured rats was significantly attenuated to 147 ± 19% BL and 237 ± 32% BL following moderate and high doses of capsaicin treatment, respectively (P < 0.01 compared with the value before vagotomy). No significant changes were observed in the expiratory duration of C2Hx animals following capsaicin treatment under the vagotomized condition (Fig. 4 and Table 2). However, a significant reduction in the heart rate occurred in response to both doses of capsaicin in vagotomized uninjured and C2Hx animals (Table 2), suggesting intrajugular capsaicin administration-induced bradycardia is not specifically due to the activation of vagal afferent inputs in mechanically ventilated and paralyzed animals.

**PBG-induced Pulmonary Chemoreflex in Spontaneously Breathing Rats**

The respiratory pattern, mean arterial blood pressure, and heart rate of uninjured (n = 12) and C2Hx (n = 12) animals

Table 1. *Contralateral phrenic bursting pattern of uninjured animals and C2Hx animals during the baseline condition*

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<tr>
<th></th>
<th>Vagal-intact</th>
<th>Vagotomized</th>
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<tbody>
<tr>
<td>Frequency, bursts/min</td>
<td>64 ± 1</td>
<td>45 ± 2*</td>
</tr>
<tr>
<td>Phrenic Burst Amplitude, AU</td>
<td>0.16 ± 0.03</td>
<td>0.20 ± 0.02*</td>
</tr>
<tr>
<td>Uninjured</td>
<td>Vagal-intact</td>
<td>62 ± 1</td>
</tr>
<tr>
<td></td>
<td>Vagotomized</td>
<td>0.12 ± 0.03</td>
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Values are means ± SE. The phrenic burst amplitude is calculated from the right side phrenic nerve. C2Hx, C2 spinal hemisection rats; AU, arbitrary units.

*P < 0.01 compared with the value under vagotomized conditions.

before PBG treatment at 1 day postspinal surgery are presented in Table 3 and Figs. 6 and 7. Intrajugular PBG administration induced two distinct respiratory responses in both groups (Fig. 6): 1) tachypnea (i.e., the expiratory duration is shorter than the 75% BL value); and 2) apnea. The apneic response following 20 μg/kg PBG administration was observed in 58% (7/12) and 67% (8/12) of the uninjured and C2Hx animals, respectively. The proportion of the apneic response increased to 92% (11/12) in uninjured animals and 75% (9/12) in C2Hx animals in response to a high dose (40 μg/kg) of PBG delivery. Both doses of PBG induced a significant elongation of the expiratory duration in uninjured animals (P < 0.01, Fig. 7A), but C2Hx animals only demonstrated significant apnea in response to a high dose of PBG administration (P < 0.01, Fig. 7A). When the data were normalized to %BL, PBG-induced apnea was significantly attenuated in C2Hx animals compared with uninjured animals (P < 0.05, Fig. 7B). The sample size of the tachypneic response group was low (n = 1 in uninjured group; n = 3 in C2Hx group) following the high PBG dose (40 μg/kg); therefore, we only compared the tachypneic response between the uninjured and C2Hx animals after a moderate dose of PBG (20 μg/kg) treatment. Figure 7, C and D, demonstrates that the expiratory duration was significantly reduced after PBG treatment, and that the tachypneic response was similar between the uninjured and C2Hx animals.
distinct respiratory responses were combined and analyzed by two-way RM-ANOVA. The result indicated that intrajugular administration of PBG induced a significant reduction in the mean arterial blood pressure and heart rate in both uninjured and C2Hx animals ($P < 0.05$, Figs. 7, E and F); however, the intensity of hypotension and bradycardia was similar between both groups. Following the vagotomy, both doses of PBG no longer induced significant changes in the cardiorespiratory pattern (Table 4), suggesting that PBG-induced pulmonary chemoreflexes under the vagal-intact condition are primarily induced by the activation of vagal afferents.

**DISCUSSION**

The present study demonstrated that an acute cervical spinal cord injury not only changed the breathing pattern but also modulated the pulmonary chemoreflex. Specifically, the pulmonary chemoreflex induced by intrajugular capsaicin administration was abolished in spontaneously breathing animals at 1 day postinjury. In addition, capsaicin-induced phrenic cessation was significantly attenuated in mechanically ventilated C2Hx animals compared with uninjured animals. PBG-evoked apnea was also blunted in the C2Hx animals. These data suggest that the pulmonary chemoreflex induced by the activation of bronchopulmonary C fibers is attenuated following acute cervical spinal cord hemisection. The blunted respiratory reflex may enable animals with spinal cord injury to maintain essential ventilation in the face of inhibitory respiratory inputs; however, the reflex may be accompanied by a decreased capability of preventing inhaled irritants.

**Critique of the Method**

Several aspects of our experimental method should be discussed. First, intrajugular administration of capsaicin or PBG has been widely used to activate bronchopulmonary C fibers and to evoke the pulmonary chemoreflex (10, 23–25, 27, 32, 34, 35, 54). However, Ho et al. (17) indicated that both capsaicin and PBG excite a small portion of the rapidly adapting pulmonary receptors. The activation of rapidly adapting pulmonary receptors usually induces tachypnea (6, 43); therefore, the elongation of the expiratory duration (i.e., apnea) following intrajugular capsaicin or PBG injection may be underestimated in the present study. In addition, intrajugular injection enables capsaicin and PBG to enter the systemic circulatory system after passing through the pulmonary ciru-

![Table 2. The cardiorespiratory pattern following intrajugular capsaicin treatment in vagotomized ventilated animals](http://jap.physiology.org/)

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<th>Uninjured</th>
<th>C2Hx</th>
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<tr>
<td></td>
<td>BL</td>
<td>Capsaicin (µg/kg)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Expiratory duration, s</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>96 ± 7</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>465 ± 6</td>
<td>412 ± 15*</td>
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Values are means ± SE. BL, baseline condition. *$P < 0.01$ compared with the BL value.
latory system. Accordingly, the excitation of nonvagal C fibers may also evoke cardiorespiratory responses (27, 31). However, the effect of capsaicin and PBG was greatly attenuated following bilateral vagotomy, suggesting that the cardiorespiratory reflex induced by the intrajugular administration of capsaicin/PBG is primarily due to the activation of vagal afferents in the present study. Second, the spontaneously breathing animals were under hyperoxic and poikilocapnic conditions in protocols 1 and 3. Although we did not measure blood gases in the present study, our previous report has demonstrated that arterial PCO2 levels in rats with spinal cord injury are relatively higher than in the uninjured controls at the acute injury phase (1–3 days postinjury) (8, 9). Therefore, the pulmonary chemoreflex of spontaneously breathing C2Hx animals may be modulated by the potential interaction between the central chemoreceptor and the vagal afferent inputs. Third, the paralytic agent (i.e., pancuronium bromide) used in the present study also blocks cardiac muscarinic receptors (38). The bradycardia component of the pulmonary chemoreflex may be masked or underestimated in mechanically ventilated animals.

Attenuation of the Pulmonary Chemoreflex Following Acute Cervical Spinal Cord Injury

The present study showed that intrajugular administration of capsaicin evoked the typical pulmonary chemoreflex, including apnea, bradycardia, and hypotension, in spontaneously breathing uninjured animals. In contrast, the breathing pattern, blood pressure, and heart rate of C2Hx animals were not significantly changed following capsaicin injection, indicating that the capsaicin-induced reflexes were almost eliminated following acute cervical spinal cord injury. Our laboratory’s previous study reported that the Hering-Breuer inflation reflex was attenuated at 2 wk post-C2Hx (29). In addition, the hypercapnic ventilatory response was blunted following cervical spinal cord injury in both human and animal studies (1, 12, 20). The impairment of swallow and cough was also observed in patients with cervical spinal cord injury (50, 51). These results indicate that cervical spinal cord injury not only changes the baseline breathing pattern, but also influences respiratory reflexes induced by mechanical and/or chemical stimuli.

Lee et al. (26) and Nicaise et al. (40) observed that the respiratory frequency of an unanesthetized rat significantly elevated to compensate for the decreased tidal volume at 1 day postinjury. In addition, the reduced tidal volume of a cervical contused rat can return to the normal value at 4 day postinjury (40). Our present study demonstrated that the inhibitory respiratory response was blunted at 1 day post-C2Hx. This evidence

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<th>Frequency, bursts/min</th>
<th>Tidal Volume, ml</th>
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<tr>
<td></td>
<td>Vagal-intact</td>
<td>Vagotomy</td>
</tr>
<tr>
<td>Uninjured</td>
<td>110 ± 7</td>
<td>49 ± 3†</td>
</tr>
<tr>
<td>C2Hx</td>
<td>93 ± 8</td>
<td>47 ± 4†</td>
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Values are means ± SE. *P < 0.01 compared with uninjured animals. †P < 0.01 compared with the value during the vagal-intact condition.

Fig. 6. Representative examples of phenylbiguanide (PBG)-induced apnea and tachypnea in spontaneously breathing animals. PBG induced two distinct respiratory frequency responses. Left: airflow was ceased (i.e., apnea) after PBG treatment. Right: respiratory frequency increased in response to PBG administration. The apneic response is weaker in C2Hx than in uninjured animals; however, the tachypneic response is similar between two groups.
implies that compensatory respiratory responses could be evoked to maintain the essential ventilator capacity within a short period after spinal injury-induced respiratory insufficiency. Although Sperry and Goshagarain (46) demonstrated that the morphological changes in the phrenic nucleus ipsilateral to the lesion can be induced as early as 2 h after cervical hemisection, the spontaneous recovery of injured side hemidiaphragm activity did not occur until 6 wk postinjury (39). These studies lead us to speculate that there is a differential expression time course between functional compensatory (i.e., increase of uninjured supraspinal and/or spinal respiratory activity) vs. restorative (i.e., recovery of injured side spinal respiratory activity) respiratory plasticity following cervical spinal hemisection.

The mechanism underlying the blunted pulmonary chemoreflex remains unclear. We proposed that both peripheral and central mechanisms are involved in this compensatory respiratory response following acute cervical spinal cord injury. The sensitivity of the bronchopulmonary C fibers may be regulated by several endogenous mediators, such as tumor necrosis factor-α, cannabinoids, and adenosine (16, 34, 36). Changes in the level of endogenous mediators following cervical spinal cord injury may modulate bronchopulmonary C-fiber sensitivity and, in turn, influence the pulmonary chemoreflex. In

Table 4. The cardiorespiratory pattern in response to intrajugular PBG administration following vagotomy

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<th></th>
<th>Uninjured</th>
<th></th>
<th>C2Hx</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>PBG (µg/kg)</td>
<td>BL</td>
<td>PBG (µg/kg)</td>
</tr>
<tr>
<td>Expiratory duration, s</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>87 ± 4</td>
<td>84 ± 5</td>
<td>79 ± 5</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>490 ± 8</td>
<td>459 ± 12</td>
<td>482 ± 8</td>
<td>483 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SE. PBG, phenylbiguanide.
addition, Holmes (19) proposed that the reduction of vagal afferent sensitivity to gastrointestinal chemicals (e.g., neuroactive peptide, neurotransmitters, and macronutrients) may contribute to gastric dysmotility after spinal cord injury. Therefore, the blunted responsiveness of bronchopulmonary C fibers to the stimuli used in the present study (i.e., capsaicin and PBG) may also result in the attenuation of the pulmonary chemoreflex in C2Hx animals. However, if the reduction in bronchopulmonary C-fiber sensitivity is the primary contributor to the attenuation of the pulmonary chemoreflex following acute cervical spinal hemisection, all characteristics of the pulmonary chemoreflex (e.g., apnea, bradycardia, and hypotension) should be attenuated in C2Hx animals. Our present data demonstrated that the PBG-induced reduction in the mean arterial blood pressure and heart rate is similar between uninjured animals and C2Hx animals, suggesting that another mechanism, in addition to the alteration of vagal afferent sensitivity (e.g., supraspinal plasticity), may also be involved in the regulation of the pulmonary chemoreflex following acute cervical spinal cord injury.

The anatomy of ascending spinobulbar pathways and/or descending bulbospinal projections following spinal cord injury may influence the supraspinal activity. Several studies have indicated that spinal cord injury can alter central respiratory motor outputs. For example, Zimmer and Goshgarian (55) demonstrated that the respiratory response (i.e., burst duration of C4 ventral root) to pH is opposite between neonatal rats that are uninjured and those with spinal cord injury in in vitro brain stem–spinal cord preparations. Golder et al. (14) showed that the hypercapnic response of the hypoglossal output was attenuated at 2 mo postinjury (55). Our laboratory’s previous study also reported that the inhibitory effect of lung inflation on the hypoglossal nerve activity is blunted at 2 wk post-C2Hx (29). Moreover, the bronchopulmonary C fibers are terminated in the caudal nucleus tractus solitarius (NTS) of the brain stem, which not only is a relay site, but can integrate various peripheral and central input signals to initiate a proper pulmonary chemoreflex (3, 4). The NTS has the capacity to regulate the reflex output by altering both intrinsic and extrinsic properties in response to certain experimental conditions (3); therefore, spinal cord injury may induce changes in NTS neuronal properties and in turn may modulate the pulmonary chemoreflex. Overall, the blunted pulmonary chemoreflex may partially result from the neuroplastic changes in these central regions following cervical spinal cord injury.

Physiological Significance

The pulmonary chemoreflex is an important pulmonary defense reflex to modulate the cardiorespiratory patterns following bronchopulmonary C-fiber activation. The adequate expression of the reflex can enable subjects to prevent the inhalation of respiratory irritants and reduce the distribution of the harmful substance in the circulation. Our present results demonstrated that the pulmonary chemoreflex was attenuated in C2Hx animals, suggesting cervical spinal cord injury not only influences the resting breathing pattern, but also has a substantial impact on the respiratory reflexes. Although the blunted pulmonary chemoreflex may sustain breathing in the face of inhibitory respiratory inputs, this phenomenon may unfavorably impact the ability to prevent the inhalation of irritants and result in the pulmonary pathological condition following cervical spinal cord injury. We suggested that future studies investigating spontaneous or induced recovery of the pulmonary chemoreflex after cervical spinal cord injury are warranted, and evaluation of recovery of the pulmonary defense reflex may be a critical index to examine the therapeutic effectiveness on respiratory functional recovery.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


