Cervical spinal cord injury alters the pattern of breathing in anesthetized rats

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Golder, Francis J., Paul J. Reier, Paul W. Davenport, and Donald C. Bolser. Cervical spinal cord injury alters the pattern of breathing in anesthetized rats. J Appl Physiol 91: 2451–2458, 2001.—The mechanisms by which chronic cervical spinal cord injury alters respiratory function and plasticity are not well understood. We speculated that spinal hemisection at C2 would alter the respiratory pattern controlled by vagal mechanisms. Expired volume (VE) and respiratory rate (RR) were measured in anesthetized control and C2-hemisected rats at 1 and 2 mo postinjury. C2 hemisection altered the pattern of breathing at both postinjury time intervals. Injured rats utilized a higher RR and lower VE to maintain the same minute ventilation as control rats. After bilateral vagotomy, the pattern of breathing in injured rats was not different from controls. The frequency of augmented breaths was higher in injured rats at 2 mo postinjury before vagotomy; however, the VE of augmented breaths was not different between groups. In conclusion, C2 hemisection alters the pattern of breathing at 1 and 2 mo postinjury via vagal mechanisms.

augmented breath; vagal afferents

CERVICAL SPINAL CORD INJURY (SCI) in people is associated with high mortality and morbidity (5, 12, 33) in part because of interruption of respiratory motor output to the diaphragm. The adult rat is often used as an experimental model to investigate the effects of cervical SCI on respiratory function and plasticity (8, 15, 16, 24, 32, 34). However, the effects of chronic cervical SCI on the pattern of spontaneous breathing in this species remain unknown.

In humans, pulmonary function tests often remain impaired beyond the acute postinjury period (17, 20, 21, 39). Despite this, many cervically injured patients in the supine position can maintain minute ventilation with a normal respiratory rate and tidal volume (29, 36, 37, 45). In contrast, these patients will often ventilate with an elevation in respiratory rate and decrease in tidal volume when in the sitting position (10, 23, 25, 26). This altered pattern of breathing has been associated with changes in pulmonary volumes (9, 25, 26), but it may also be related to the decreased lung or chest wall compliance that can follow SCI (9, 11, 14, 41).

Pulmonary vagal afferents, including slowly adapting receptors (SARs), rapidly adapting receptors (RARs), and bronchopulmonary C fibers, modulate the eupneic pattern of breathing in humans and other animals (6, 22, 40, 42). SARs are mechanoreceptors that are primarily stimulated by tensile forces acting on airway smooth muscle cells. Stimuli that most commonly alter SAR activity include changes in lung volume and decreased compliance. In general, stimulation of SARs shortens the inspiratory phase of the respiratory cycle, producing a rapid shallow breathing pattern (42). RARs are polymodal, responding to both mechanical and chemical stimuli. These receptors mediate various airway protective reflexes (i.e., cough, augmented breaths), hyperventilation, and mucus secretion (40). Bronchopulmonary C fibers are chemosensitive, and their stimulation induces apnea, a rapid shallow breathing pattern, and cough (22). In the rat, vagal afferent feedback has been demonstrated to mediate the rapid shallow breathing pattern in various models of respiratory disease (27, 35, 43, 47).

The role of vagal afferents in mediating the altered pattern of breathing after SCI in humans is unknown. Many respiratory sequelae after injury have the potential to stimulate pulmonary vagal afferents, and these include atelectasis, pneumonia, bronchitis, bronchospasm, chest wall spasticity (18), decreased lung volumes (17, 20, 21, 39), and reduced lung and chest wall compliance (9, 11, 14, 41).

We speculated that chronic cervical SCI would alter the pattern of breathing in the anesthetized rat. In addition, considering the potential respiratory sequelae to injury identified in humans, we hypothesized that the effects of injury on the pattern of breathing in the rat would be mediated by vagal mechanisms.

METHODS

Animals

Thirty-five specific-pathogen-free female rats (Harlan Sprague Dawley, Indianapolis, IN) from colony K63317, ranging in mass from 225 to 318 g, were used in this study. Animals were divided into normal (n = 11), sham-operated...
(n = 12), and C2-hemisected (n = 12) groups. The animals were subsequently evaluated at either 1 or 2 mo postinjury. Animal husbandry and all procedures were in compliance with the Institutional Animal Care and Use Committee at the University of Florida.

Spinal Cord Hemisection

Rats were anesthetized with medetomidine (75 μg/kg im) and isoflurane in oxygen. After orotracheal intubation, anesthesia was maintained with isoflurane in oxygen, and rats were mechanically ventilated. A laminectomy was made at the second cervical vertebral level, and the second cervical spinal segment and the cranial segment of the third cervical spinal segment were exposed. A 1-mm-long left-sided hemisection was made in the cranial segment of C2, and the section was aspirated with a fine-tipped glass pipette. The dura and arachnoid mater were closed with 10-0 suture. All animals were allowed to recover and received atipamezole (0.1 mg/kg iv) to antagonize the anesthetic effects of medetomidine. Buprenorphine (50 μg/kg iv) and carprofen (5 mg/kg iv) were administered for postsurgical pain control. Analgesics were repeated as required over the next 2 days. One to two months were allowed to elapse before the rats were placed in terminal studies. In rats that were sham operated, the procedure was the same but the spinal cord was left intact after the meninges were incised and sutured.

Protocol

After a 1- or 2-mo postsurgical period, rats were anesthetized with urethane (1.4 g/kg ip) and allowed to breathe room air. A femoral arterial catheter was placed to allow monitoring of direct arterial blood pressure and collection of arterial blood for blood-gas analysis (iSTAT, Waukesha, WI). Each blood-gas measurement required 0.15 ml of blood, and a maximum of four blood samples was taken from each rat. A femoral vein catheter was placed to administer drugs and fluids. Atropine sulfate (0.1 mg/kg iv) was administered to decrease upper respiratory secretions. The trachea was cannulated at the midcervical level, and rats were allowed to breathe spontaneously throughout the study. Rectal temperature at the time of sampling. When surgical preparation was completed, rats were placed in a supine position and a pneumotachometer (Hans Rudolph, Kansas City, MO) was attached to the tracheal cannula. The pneumotachometer was calibrated before and at intervals during the study using a square-wave pulse of known volume and duration. Airflow was recorded using a differential pressure transducer (model MP45-14-871, Validyne, Northridge, CA) attached to the pneumotachometer. Airflow was electrically integrated to derive volume. Each breath phase (inspiration and expiration) was integrated separately, providing continuous display of both inspiratory volume (Vi) and expiratory volume (Ve).

After the pneumotachometer was attached to the tracheotomy tube, rats were allowed to breathe room air for 30 min before the first measurement of airflow was recorded, and this time represented spontaneous ventilation on room air. At the end of this period, both vagi were isolated in the midcervical region and cut. Fifteen minutes after bilateral vagotomy, airflow was again recorded. Arterial blood was sampled at the end of each 15-min recording period. After each sample was taken, 0.4 ml of 0.9% saline was administered intravenously to replace the lost blood volume.

Histological Confirmation of C2 Hemisection

All rats that received a spinal hemisection were exsanguinated after the study and transectively perfused with 4% paraformaldehyde solution in phosphate-buffered saline. The cervical spinal cord was removed, and the C2 spinal segment was sectioned at 40-μm thickness and stained with cresyl violet. The extent of cervical spinal cord injury was assessed under light microscopy.

Data Analysis

Arterial blood pressure, inspiratory and expiratory airflow, Vi, and Ve were recorded on videocassette recorder tape and digitized on-line by a computer-based data analysis system (CED 1401) and chart recorder. Ve, inspiratory time (T1), and expiratory time (T2), were averaged over five consecutive breaths immediately before blood-gas analysis. Ve was measured as the peak integrated Ve. T1 and T2 were measured from the airflow signal using zero airflow as the point of phase transition. Respiratory rate was calculated by dividing 60 s by (T1 + T2 in s). Expired minute ventilation was calculated by multiplying Ve by respiratory rate. Both Ve and minute ventilation were indexed to body weight.

Animal groups were compared using repeated measures on the intact vagi vs. vagotomy. Multiple comparisons were made using the Kruskal-Wallis ANOVA and the Mann-Whitney U-test. Ve measurements before and after vagotomy were compared using the Wilcoxon matched-pairs test. All other means were compared using a two-factor ANOVA with repeated measures on the intact vagi vs. vagotomy. Multiple comparisons were made using t-tests with Bonferroni’s correction. Differences were considered significant if the overall significance level was P < 0.05.

RESULTS

Histological Assessment of Cervical SCI

The C2 spinal segments from all injured rats were examined histologically to confirm completeness of the hemisections for subsequent respiratory data inclusion. Figure 1 presents a representative example of a...
lesion that extended to the midline of the spinal cord, thereby removing the entire ipsilateral side without overt contralateral damage. No rats were excluded from data analysis on the basis of histological examination.

**Clinical Observations**

Rats became laterally recumbent immediately after injury and were unable to right themselves. Water and food were fed per os over the next 2–3 days until rats were able to eat by themselves. All rats were hemiparalyzed on the ipsilateral side and developed bilateral hind leg rigidity. Between 3 and 5 days postinjury, the rigidity in the hindlimbs subsided and rats began to compensate for the hemiparalysis by using the tail as a supporting appendage. By 7 days postinjury, rats had begun to use both hindlimbs to ambulate. The ipsilateral forelimb was maintained in a flexed position for the duration of the study and was not involved in grasping activities. Some rats required their bladders to be expressed of urine manually for the first 48 h postinjury. No abnormalities were noted with respect to defecation. No abnormalities were noted with respect to defecation.

**Effect of C₂ Hemisection on the Pattern of Spontaneous Ventilation in Vagally Intact Rats**

The pattern of spontaneous ventilation on room air was assessed in vagally intact normal, sham-operated, and C₂-hemisected rats at 1 and 2 mo postinjury. No differences in spirometric, cardiovascular, or arterial blood-gas measurements were detected between normal and sham-operated rats at either time point. These groups were subsequently combined as one control group for each time point after injury. All groups were of similar age during the study. No differences existed in body weight between controls (1 mo: 270 ± 14 g; 2 mo: 280 ± 30 g) and injured groups (1 mo: 250 ± 5 g; 2 mo: 278 ± 10 g). Rectal temperature and arterial blood pressure. Vₑ, minute ventilation. *P < 0.05 relative to controls. †P < 0.05 relative to the same group at 1 mo postinjury; ‡P < 0.001.

Minute ventilation was not different between the control and C₂-hemisected groups (Table 1) at either 1 or 2 mo postinjury and was reflected by similar arterial PCO₂ (PaCO₂) between the groups (Table 1). In addition, arterial blood pH, arterial PO₂ (PaO₂), and heart rate were not different between injury and control groups (Table 1). However, pH was significantly higher in injured rats at 2 mo than at 1 mo postinjury (P < 0.01; Table 1).

Although minute ventilation was not affected by SCI, the pattern of breathing was altered (Figs. 2 and 3). At 1 and 2 mo postinjury, C₂-hemisected rats maintained minute ventilation with a significantly lower Vₑ (P < 0.01; Fig. 2A) and higher respiratory rate (P < 0.01; Fig. 2B) than control rats. The higher respiratory rate in injured rats was due to a shorter Ti (P < 0.0001) and Te (P < 0.0001) at 1 mo postinjury and a shorter Te (P < 0.01) at 2 mo postinjury compared with controls (Table 2).

**Effect of C₃ Hemisection on the Pattern of Spontaneous Ventilation in Bilaterally Vagotomized rats**

Bilateral vagotomy did not alter heart rate (Table 1), which most likely reflects the treatment of all rats with atropine. In addition, vagotomy did not change mean arterial blood pressure or PaO₂ at either time point postinjury (Table 1). In contrast, PaCO₂ decreased in control (P < 0.001) and injury groups (P < 0.05) (Table 1). In addition, arterial pH increased in control (P < 0.01) and C₂-hemisected (P < 0.05) groups at 1 mo postinjury (Table 1).

Minute ventilation was again not different between the groups after bilateral vagotomy (Table 1). In addition, SCI did not affect the pattern of breathing postvagotomy (Figs. 2 and 3). Vagotomy increased Vₑ in both control (P < 0.0001) and C₂-hemisected (P < 0.0001) rats, but it was no longer different between the groups (Fig. 2A) at either 1 or 2 mo postinjury. In addition, respiratory rate decreased in both groups (P < 0.0001).

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<th>Table 1. Cardiovascular, arterial blood-gas, and temperature measurements from control and C₂-hemisected rats at 1 and 2 mo postinjury</th>
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Values are means ± SD. Measurements were made before (intact) and after (cut) bilateral vagotomy. HR, heart rate; MBP, mean arterial blood pressure. Vₑ, minute ventilation. *P < 0.05 relative to controls. †P < 0.05 relative to the same group at 1 mo postinjury; ‡P < 0.05 relative to the vagotomized measurement.
and also was no longer different between injured and control rats (Fig. 2B) at either time point. The decrease in respiratory rate was due to a prolongation of both Ti (P < 0.0001) and Te (P < 0.0001) in all four groups (Table 2).

Effects of C2 Hemisection on the Pattern of Augmented Breaths

Augmented breaths were identified before vagotomy in control and injured groups at both time points after injury. An augmented breath was characterized by a normal inspiratory effort followed by an additional stepwise inspiratory effort and an increased inspired and expired tidal volume (Fig. 4). The number of augmented breaths per minute (frequency) was significantly higher in C2-hemisected rats than controls (P < 0.01) (Fig. 5B) at 2 mo but not at 1 mo postinjury. The Ve for an augmented breath was greater than the tidal volume during eupnea in all groups (P < 0.0001). However, Ve (Fig. 5A), Ti, and Te (Table 3) of the augmented breaths were not different between groups at either time period after injury. After bilateral vagotomy, no augmented breaths were detected in any group.

DISCUSSION

We have demonstrated an effect of C2 hemisection on the pattern of breathing at 1 and 2 mo postinjury. Injured rats maintained minute ventilation with a higher respiratory rate and lower Ve than control rats. In addition, the frequency of augmented breaths was higher in injured rats at 2 mo postinjury. After bilateral vagotomy, the pattern of breathing in injured rats was no longer different from controls.

In our study, bilateral vagotomy decreased PaCO2 in all groups. A quantitatively similar effect of vagotomy
on \( P_aCO_2 \) in normal rats and rats with respiratory disease has been described by others (27). The mechanism by which vagotomy decreased \( P_aCO_2 \) in those studies was not addressed. The larger tidal volumes secondary to vagotomy may have improved ventilation-perfusion matching by decreasing physiological dead space ventilation and subsequently increasing alveolar ventilation. In our study, minute ventilation (Table 1) was not addressed. The larger tidal volumes postinjury, which may reflect the greater impact on respiratory motor output of a cervical lesion compared with a thoracic injury (39).

The rapid shallow breathing pattern after \( C_2 \) hemisection was mediated by vagal afferents. The stimulus by which vagal afferent feedback is altered after \( C_2 \) hemisection is unknown. A vagally mediated altered pattern of breathing after SCI may be due to changes in lung volume due to reduced respiratory motor output, muscle weakness secondary to disuse, and changes in the mechanical properties of the lungs and chest. In our study, \( V_E \) increased in injured rats after vagotomy and was not different from control rats. In addition, \( V_E \) was similar between groups during augmented breaths. This suggests that the pattern of breathing was not limited by neural motor output or muscle function and supports the hypothesis that the higher respiratory rate and lower \( V_E \) were due to changes in the mechanical properties of the lungs and/or chest wall.

A rapid shallow pattern of breathing is also present during pulmonary disease and has been attributed to vagal afferent feedback in a number of species (6). More specifically, this pattern of breathing has been described in the rat during experimentally induced pneumonitis (35, 47) and pulmonary fibrosis (27, 43). In these studies, the altered pattern of breathing was attributed to stimulation of pulmonary stretch receptors (27, 43) and C fibers (47). SCI in humans is frequently associated with atelectasis, pneumonia, bronchitis, bronchospasm, chest wall spasticity (18), decreased lung volumes (17, 20, 21, 39), and reduced lung and chest wall compliance (9, 11, 14, 41). All of these have the potential to increase vagal afferent feedback from the lungs (6) and may contribute to the rapid shallow breathing pattern described in some clinical studies (10, 25, 26).

Table 2. Inspiratory and expiratory times in spontaneously breathing anesthetized control and \( C_2 \)-hemisected rats at 1 and 2 mo after injury

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<tr>
<th>Vagus</th>
<th>1 Mo Postinjury</th>
<th>2 Mo Postinjury</th>
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<td></td>
<td>Control</td>
<td>( C_2 ) hemisected</td>
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<tr>
<td>( T_i )</td>
<td>( 0.22 \pm 0.03 )</td>
<td>( 0.15 \pm 0.03^a )</td>
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<td>Cut</td>
<td>( 0.34 \pm 0.04^† )</td>
<td>( 0.31 \pm 0.04^† )</td>
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<tr>
<td>( T_e )</td>
<td>( 0.29 \pm 0.06 )</td>
<td>( 0.19 \pm 0.03^† )</td>
</tr>
<tr>
<td>Cut</td>
<td>( 1.07 \pm 0.35^† )</td>
<td>( 1.34 \pm 0.33^† )</td>
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Values are means \( \pm \) SD given in s. Measurements were made before (intact) and after (cut) bilateral vagotomy. \( T_i \), inspiratory time; \( T_e \), expiratory time. \(^aP < 0.05\) relative to controls. \(^†P < 0.05\) relative to the prevagotomy measurement.

Fig. 4. Flow, \( V_E \), and \( V_I \) from an anesthetized control rat (left) and injured rat (right) at 2 mo postinjury. Rats were breathing spontaneously room air before vagotomy. An augmented breath (AB) was characterized by a biphasic inspiratory flow signal and increased \( V_I \) and \( V_E \). Scales for flow, volume, and time are identical for each panel. Note the similar \( V_E \) during the AB in the injured rat compared with the control rat before vagotomy.
The incidence of these complications after C2 hemisection in rats is unknown. In our study, the normal PaO₂ during room air breathing suggests that pneumonia was not present in our rats. In addition, all rats received atropine at the start of the study to reduce respiratory secretions and prevent occlusion of the tracheal cannula and lower airways. It is, therefore, unlikely that cholinergic-mediated bronchospasm contributed to the pattern of breathing in the injured rats before vagotomy. Whether the effects of C2 hemisection are due to an immediate mechanical change at the time of injury (i.e., decreased functional residual capacity) or a progressive effect of injury (i.e., spasticity of the chest wall or fibrosis) remains unknown. The pattern of breathing before 1 mo postinjury will need to be examined to help separate these alternate mechanisms.

In addition to the above peripheral effects, C2 hemisection may induce a rapid shallow breathing pattern by inducing plasticity of the central pathways mediating pulmonary vagal afferent reflexes.

The unilateral nature of a C2 hemisection introduces an asymmetric effect of injury on respiratory motor output to the diaphragm and chest wall muscles. As such, the effects of this lesion on the movements of the left and right lung are unlikely to be uniform. It is, therefore, plausible that the altered pattern of vagal afferent feedback is also asymmetric. An additional consideration is the possibility for paradoxical chest wall movement in injured rats. The superior chest wall has been demonstrated to collapse inward during inspiratory effort in people with cervical SCI and is attributed to paralysis of chest wall muscles during inspiration (7, 30, 31). The presence of paradoxical chest wall and lung motion, either unilaterally or bilaterally, was not measured in our rats but might have lead to out-of-phase patterns of vagal afferent traffic from the two lungs.

Augmented breaths are characterized by an enhanced inspiratory airflow that results in a larger V₁ than the eupneic tidal volume for a single breath (Fig. 4). Augmented breaths, or sighs, are an airway protective reflex and are a normal feature of spontaneous ventilation. Their physiological benefit has been attributed to expansion of atelectic areas of the lung, thereby restoring lung compliance (38). Augmented breaths occur periodically during eupnea but are present with increased frequency during conditions of lung deflation, hypoxia, pneumothorax, noxious inhaled irritants, and decreased lung compliance (1, 6, 13, 28).

In our study, the frequency of augmented breaths in C2-hemisected rats was higher than controls at 2 mo postinjury. This effect was unlikely to be related to chemoreceptor stimulation because hypercapnia does not stimulate augmented breaths in the rat (1) and blood gases were similar in all groups. Bilateral cervical vagotomy abolished augmented breaths in all groups, which is consistent with other studies in the rat, suggesting that this protective reflex is mediated by vagal afferents (1, 19, 28). In particular, evidence exists that these reflexes are mediated through pulmonary irritant receptors rather than stretch receptors (13, 28). The increased frequency of augmented breaths that we observed could be explained by a reduction in pulmonary compliance at 2 mo postinjury. Decreased pulmonary compliance could also explain
the vagally mediated alterations in respiratory pattern that we observed. In summary, we have demonstrated that rats with a high cervical SCI have an altered pattern of breathing that develops before 1 mo after injury. This most likely reflects altered pulmonary mechanics because the difference between injured and control rats was mediated by vagal afferents. The presence of an increased frequency of augmented breaths supports this hypothesis and indicates that airway protective reflexes also can be altered following SCI.

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