Spinal Activation of Serotonin 1A Receptors Enhances Latent Respiratory Activity After Spinal Cord Injury

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ABSTRACT

Background/Objective: Hemisection of the cervical spinal cord results in paralysis of the ipsilateral hemidiaphragm. Removal of sensory feedback through cervical dorsal rhizotomy activates latent respiratory motor pathways and restores hemidiaphragm function. Because systemic administration of serotonin 1A receptor (5HT1A) agonists reversed the altered breathing patterns after spinal cord injury (SCI), we predicted that 5HT1A receptor activation after SCI (C2) would activate latent crossed motor pathways. Furthermore, because 5HT1A receptors are heavily localized to dorsal horn neurons, we predicted that spinal administration of 5HT1A agonists should also activate latent motor pathways.

Methods: Hemisection of the C2 spinal cord was performed 24 to 48 hours, 1 week, or 16 weeks before experimentation. Bilateral phrenic nerve activity was recorded in anesthetized, vagotomized, paralyzed Sprague-Dawley rats, and 8-OH-DPAT (5HT1A agonist) was applied to the dorsal aspect of the cervical spinal cord (C3–C7) or injected systemically.

Results: Systemic administration of 8-OH-DPAT led to a significant increase in phrenic frequency and amplitude, whereas direct application to the spinal cord increased phrenic amplitude alone. Both systemic and spinal administration of 8-OH-DPAT consistently activated latent crossed phrenic activity. 8-OH-DPAT induced a greater respiratory response in spinal injured rats compared with controls.

Conclusion: The increase in crossed phrenic output after application of 8-OH-DPAT to the spinal cord suggests that dorsal horn inputs, respiratory and/or nonrespiratory, may inhibit phrenic motor output, especially after SCI. These findings support the idea that the administration of 5HT1A agonists may be a beneficial therapy in enhancing respiratory neural output in patients with SCI.

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Key Words: Spinal cord injuries; Serotonin 1A receptor; Rats; Respiration; Crossed phrenic pathway; Sensory afferents; 5HT1A agonist.

INTRODUCTION

Spinal cord injury (SCI) affects approximately 11,000 new individuals annually, with more than one half affecting the upper cervical spinal cord (1). This level of injury results in a significant impact to the respiratory system. In fact, pneumonia, atelectasis, and respiratory failure are common problems in patients with tetraplegia and occur in 40% to 70% of patients (2,3). Indeed, problems

associated with the respiratory system are the leading cause of mortality of all patients with SCI (1). Although there have been significant improvements in patient care and morbidity, there still remains a significant lack in our basic understanding of the effect of SCI on the respiratory system.

Experimentation using animal models has considerably enhanced our understanding of basic respiratory function after SCI. Hemisection of the upper cervical spinal cord (C2) interrupts the primary descending input from respiratory premotor axons located in the lateral and ventral funiculi (4), effectively paralyzing the hemidiaphragm ipsilateral to the injury. Under conditions of elevated respiratory drive, a latent crossed respiratory motor pathway has been shown to become activated to restore function to a previously paralyzed hemidiaphragm (5–7). This crossed phrenic pathway consists of

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neurons located bilaterally in the medullary ventral respiratory group (VRG) whose axons descend the spinal cord contralateral to the hemisection and then cross below the site of injury to synapse with phrenic motor neurons (8). Excitation of this crossed pathway, through the excitation of descending inputs, can restore function to a previously paralyzed hemidiaphragm (9,10). This mode of functional motor restoration has been studied rather extensively. In addition, recovery of ipsilateral phrenic activity can occur spontaneously weeks after injury (11). Finally, the removal of sensory input by either acute (12) or chronic (13) cervical dorsal rhizotomy can activate or enhance crossed phrenic pathways to restore diaphragmatic function. Despite these observations, very little information is available regarding the roles and mechanisms underlying phrenic sensory afferent feedback after SCI.

Recently, Teng et al (14) and Choi et al (15) have shown that systemic activation of serotonin-1A (5HT1A) receptors can reverse the altered breathing patterns associated with SCI in awake rats, indicating the involvement of 5HT1A receptors in respiratory function after SCI. Therefore, we hypothesized that the systemic activation of 5HT1A receptors would enhance phrenic activity in spinally hemisected rats and activate crossed phrenic pathways. However, the specific location of the 5HT1A receptors that are involved in the alteration of breathing patterns after SCI remains unknown.

Within the spinal cord, 5HT1A receptors are heavily localized to the dorsal horn (16). After SCI, serotonin levels below the site of injury are dramatically reduced (17,18). This reduction leads to the hyperexcitability of dorsal horn sensory neurons (because activation of 5HT1A receptors results in cellular hyperpolarization) (19) and has been linked to mechanical allodynia and thermal hyperalgesia observed after SCI (20). In fact, animal studies have shown that 5HT1A agonists applied to the dorsal spinal cord reduce mechanical allodynia and thermal hyperalgesia back to control values (20).

Within the respiratory system, sensory afferent stimulation during inspiration results in the inhibition of phrenic motor output (21): the phrenic-to phrenic inhibitory reflex. This reflex is a segmental spinal reflex that is most likely mediated through nonrespiratory interneurons of the dorsal spinal cord (22,23). We hypothesized that, after SCI, these dorsal horn sensory neurons also become hyperexcitable and thus mediate a sustained inhibition of phrenic motor neurons. Activation of 5HT1A receptors in the dorsal spinal cord should reduce sensory neuron firing observed after SCI and thus disinhibit phrenic motor neurons, allowing for the expression of the crossed phrenic pathway. We therefore predicted that, in spinally hemisected rats, the application of 8-OH-DPAT, a 5HT1A agonist, to the dorsal spinal cord would increase phrenic motor output and activate crossed phrenic pathways.

METHODS

Animal Surgery Protocol

All procedures were approved before experimentation by the Animal Investigation Committee at Wayne State University acting under the guidelines provided by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Sprague-Dawley female rats ($n = 52$, Harlan, retired breeders) were housed in triplicate at the Wayne State University School of Medicine animal housing facility and provided with food and water ad libitum. Individual rats were removed from the facility and brought to the laboratory for sterile, spinal cord hemisection surgery. Animals were deeply anesthetized with ketamine and xylazine (70 and 20 mg/kg, respectively), and the surgical site was shaved and disinfected. An incision was made down the back of the neck, and the muscles were cut down the midline to expose the C2 vertebra. The dorsal aspect of the C2 vertebra was removed, and the dura was cut down the midline, exposing the spinal cord. The spinal cord was hemisected by cutting the left cord with fine spring scissors. To ensure that the hemisection was complete, a fine tip surgical probe was placed into the cord at the midline and moved laterally, scraping the floor and walls of the vertebral canal. The muscles were closed with absorbable suture (3-0 Vicryl suture, Ethicon Inc., Sommerville, NJ), and the skin was closed with wound clips. The wound was thoroughly cleaned and covered with a topical antibiotic ointment, and 10 mL of saline (containing 0.05 mg/kg buprenorphine) was injected subcutaneously. The rats were allowed to recover on a heating blanket until anesthesia began to wear off and then were placed individually in cages with rodent chow and water provided ad libitum. The food was supplemented with Nutrical (Evsco Pharmaceuticals, Buena, NJ), apples, and/or peanut butter. Sham surgeries were performed exactly as described above except that the spinal cord was not cut.

One group of animals (chronically injured) underwent a second surgery approximately 4 to 7 days. Because spontaneous recovery of hemidiaphragmatic activity was expected in this group of rats, we performed a second surgery to ensure that the hemisection surgery was complete and that the phrenic activity observed was caused by crossed phrenic pathways. Rats were anesthetized with ketamine and xylazine (70 and 20 mg/kg, respectively). An incision was made across the abdomen about 1 cm below the last rib. The diaphragm was visualized from the peritoneal cavity, and electromyogram recordings were taken from the left and right hemidiaphragms. The hemisection was considered to be functionally complete if there was no activity in the hemidiaphragm ipsilateral to hemisection. The abdominal muscles were sutured with absorbable suture, and the skin was stapled closed. Any rat that showed activity ipsilateral to hemisection was killed and not used in this

study. The rats with complete hemisections were allowed to recover for 16 weeks after hemisection.

Experimental Protocol

Animals were separated into 3 study groups: 1 group that recovered from surgery for 24 to 48 hours and received drug systemically, 1 group that recovered from surgery for 16 weeks and received drug systemically, and 1 group that recovered from surgery for 1 week and had drug applied directly to the dorsal spinal cord. On the day of experimentation, animals were anesthetized with chloral hydrate (400 mg/kg, IP), a tracheotomy was performed, and the animal was placed on a rodent ventilator (Harvard Apparatus, Holliston, MA) supplied with oxygen mixed with air ($\text{FIO}_2 \sim 0.50$). The femoral artery and vein were cannulated to record blood pressure and administer drugs, respectively. Blood gases were taken only from the group of animals in which 8-OH-DPAT was directly applied to the spinal cord, because a blood-gas analyzer was not available at the time of the other experiments. The rats were vagotomized in the midcervical region and paralyzed with pancuronium bromide (0.5 mg/kg, IV), and both phrenic nerves (PNs) were isolated and transected. In the 2 groups that received 8-OH-DPAT systemically, both right and left PNs were placed on bipolar electrodes from a ventral approach, and mineral oil was applied to prevent desiccation. In the group of rats in which 8-OH-DPAT was applied to the spinal cord, rats were placed into a stereotaxic frame and the dorsal aspect of the cervical vertebrae, and dura was removed to expose the dorsal spinal cord from C2 to C7. Both right and left PNs were recorded from a dorsal approach. Body temperature was maintained at 37°C by a rectal thermometer attached to a Harvard Rodent temperature controlled heating pad, and end-tidal CO_2 (Novamatrix, Respiromics, Wallingford, CT) was monitored continuously. The apneic threshold was determined by slowly increasing the ventilator rate and/or volume until PN discharge disappeared. The ventilator was adjusted to achieve an end-tidal CO_2 of approximately 4 mmHg above apnea (24–48 hours after injury: control, 39.33 ± 0.577 mmHg; hemisected, 39.7 ± 3.56 mmHg; 1 week after injury: sham 37.2 ± 2.86 mmHg; hemisected, 36.14 ± 1.57 mmHg; 16 weeks after injury: control, 43.25 ± 3.49 mmHg; hemisected, 45.13 ± 3.91 mmHg). The animals were allowed to stabilize for approximately 20 to 30 minutes before drug was applied. Systemic injections of 8-OH-DPAT (17 $\mu\text{g/kg}$) were given intravenously.

In one group, 8-OH-DPAT (100 μg dissolved in 4 μL of saline) or saline (4 μL) was applied to a Kim wipe pledget ($\sim 12 \times 2$ mm), which was placed carefully on the midline of the dorsal surface of the spinal cord (20). PN activity was amplified and filtered (3,000 Hz, band pass, Tectronix Model TM502A, Beaverton, OR) and recorded continuously on computer using data acquisition software (CED, Cambridge, UK).

To ensure that the respiratory responses that were generated after 8-OH-DPAT administration were caused by the specific activation of the 5HT1A receptor, specific 5HT1A-receptor antagonists were used just before 8-OH-DPAT administration. Systemically, pMMP1 hydrochloride [4-iodo-*N*-(2-[*r*-(methoxyphenyl)-1-piperazinyl] ethyl)-*N*-2-pridinybenzamide; Sigma, St. Louis, MO] followed by 17 $\mu\text{g/kg}$ 8-OH-DPAT was administered to normal rats ($n = 3$). On the dorsal spinal cord, another antagonist, WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt; Sigma) was applied followed by 8-OH-DPAT (100 μg dissolved in 4 μL of saline) in both hemisected ($n = 3$) and sham control rats ($n = 2$).

Data Analysis

Raw signals from PN recordings were full-wave rectified, integrated, and analyzed using CED data analysis software. The phrenic recordings were analyzed for frequency, burst peak height, burst duration, and burst area. Approximately a 1-minute segment was analyzed before drug application, and a 1-minute segment was analyzed after drug was applied (once phrenic recordings remained stable, approximately 5–15 minutes after 8-OHDPAT was applied).

Statistics

Statistics were performed using Systat software v.10.2 (Richmond, CA). A multivariate repeated measures ANOVA was applied to determine the effect of drug on phrenic motor output, and paired and unpaired *t* tests were used to determine if SCI had any effect on any other parameters (body weight, blood gases, etc.) All values are presented as means $\pm$ SE, and $P < 0.05$ was considered significant.

RESULTS

Hemisection surgery caused a significant reduction in body weight that occurred during the first 24 hours after surgery and continued for at least the first week after surgery (Table 1). Animals eventually gained weight; there was no difference in body weight 16 weeks after injury compared with presurgical values (Table 1). At 1 week after hemisection, blood pH, Po_2 and Pco_2 , and apneic thresholds did not differ between hemisected and control rats (Table 1). Blood gases were not taken at 24 hours and 16 weeks after hemisection; however, end-tidal CO_2 was monitored. From these data, there was no calculated difference in the apneic thresholds between hemisected rat and controls. The absolute apneic threshold values were significantly different among the 3 groups (1 week vs acute vs chronic; $P = 2.6 \times 10^{-6}$). Because blood gases were not taken, and the 3 experimental groups were not run at the same time, we cannot be certain that Paco_2 values changed over time. However, because the apneic thresholds for the hemisected and control rats within each study group did not

Table 1. Body Weight at the Time of Hemisection Surgery or at the Time of Experimentation (Times After Injury), Blood Pressure, Apneic Threshold, and Blood Gas Parameters in the 3 Experimental Groups of Rats

	Body Weight Before	Body Weight After	Blood Pressure	Apneic Threshold	pH	Po ₂	Pco ₂
<i>Female Sprague-Dawley rats, 1 week after hemisection</i>							
Hemisectioned (n = 10)	321.0 ± 7.72	290.8 ± 9.4*	86.9 ± 7.05	32.6 ± 0.896	7.31 ± 0.444	158.3 ± 10.2	37.0 ± 1.39
Sham (n = 5)	306.4 ± 14.5	305.6 ± 14.9	88.2 ± 8.94	33.0 ± 1.414	7.31 ± 0.078	146.7 ± 12.3	37.1 ± 2.33
Antagonist (n = 5)	318.2 ± 9.66	300.4 ± 8.5	70.5 ± 10.13	30.2 ± 1.03	6.96 ± 0.08	140.0 ± 13.8	40.4 ± 2.62
<i>Female Sprague-Dawley rats, 24 to 48 hours after hemisection</i>							
Hemisectioned (n = 10)	302.1 ± 5.05	276.1 ± 5.4*	83.5 ± 8.14	35.9 ± 1.1	—	—	—
Control (n = 8)	NA	291.5 ± 6.82	86.7 ± 5.88	35.5 ± 0.5	—	—	—
<i>Female Sprague-Dawley rats, 16 weeks after hemisection</i>							
Hemisectioned (n = 8)	302.8 ± 7.0	305.9 ± 10.3	92.06 ± 4.91	41.1 ± 1.38	—	—	—
Sham (n = 6)	309.7 ± 8.5	322.7 ± 1.42	99.0 ± 4.97	39.3 ± 1.42	—	—	—

All values are presented as means ± SE, and $P < 0.05$ is considered significant.

*Significant effect of surgery on body weight. No other significant changes were noted.

NA, not applicable.

differ, it is likely that the Paco₂ values did not change over time.

Left spinal cord hemisection caused paralysis of the left hemidiaphragm; thus, no activity was observed in the left PN after hemisection in acutely injured rats and 1 week after injury (Figure 1). 8-OH-DPAT consistently caused a significant increase in the peak amplitude of the phrenic motor output (Figures 1 and 2; Table 2) and resulted in the expression of crossed phrenic activity ipsilateral to the hemisection. The increase in phrenic peak amplitude was observed in all treatment groups, both hemisectioned and control rats, at various times after injury, whether it was applied directly to the spinal cord or injected systemically. Predrug respiratory-related activity in the nerve ipsilateral to hemisection (left PN) in the 16 weeks after hemisection group was caused by spontaneous activation of the crossed phrenic pathway.

Application of 8-OH-DPAT directly to the dorsal spinal cord resulted in (a) a significantly increased peak phrenic amplitude ($P = 2.46 \times 10^{-8}$), and (b) the expression of crossed phrenic activity in the nerve ipsilateral to the hemisection in all rats (10 of 10; Figure 2; Table 2). The hemisection surgery caused a significant reduction in peak phrenic amplitude ($P = 0.027$; ie, there was no respiratory-related activity in the left nerve after hemisection). There was also a significant interaction between the surgery and the nerves ($P = 0.023$), which was evident both before ($P = 0.023$) and after drug was applied ($P = 0.048$). In other words, before the drug was applied, only the hemisectioned group had significantly reduced left phrenic amplitude. After the drug was applied, phrenic amplitude increased, but the increase was significantly greater in the right nerve of hemisectioned rats. It seemed that the hemisection group showed a greater increase in peak amplitude after 8-OH-DPAT in the right nerve compared with the left nerve, whereas the sham controls showed a greater response to 8-OH-DPAT in the left nerve relative to the right. The area of the integrated burst was also significantly reduced by hemisection ($P = 0.009$) and showed a greater response to drug in hemisectioned rats compared with controls. Before application of drug, the burst area was smaller in the hemisectioned rats compared with controls ($P = 0.02$), but after drug application, the burst area was greater in the hemisectioned rats ($P = 0.006$). Finally, when 8-OH-DPAT was applied directly to the spinal cord, the frequency of the neural output was not affected.

The respiratory response to systemic administration of 8-OH-DPAT resulted in increases in frequency, burst peak amplitude, and burst area; however, hemisectioned rats and control rats showed a slight difference in their response (Figure 1; Table 2). Twenty-four to 48 hours after hemisection surgery, systemic injection of 8-OH-DPAT (intravenously) significantly increased the phrenic burst amplitude ($P = 7 \times 10^{-6}$) and resulted in a significant expression of crossed phrenic activity in the nerve ipsilateral to the hemisection (5 of 10). This was

A. 8-OH-DPAT applied to the dorsal horn of the spinal cord 1 week post hemisection

B. 8-OH-DPAT injected systemically (iv) 24-48 hrs post hemisection

C. 8-OH-DPAT injected systemically (iv) 16 weeks post hemisection

Figure 1. Right and left integrated PN activity in response to 8-OH-DPAT. (A) Representative tracings of the response to direct application of drug to the spinal cord in rats hemisected 1 week before experimentation. (B) Tracings of the response to systemic administration (intravenous) in acute injured rats (24–48 hours after hemisection). (C) Tracings of the response to systemic administration in chronic injured (16 weeks) rats. All tracings are 30-second segments, both before and after 8-OH-DPAT.

accompanied by a significant increase in the area of the integrated phrenic burst ($P = 0.02$). Systemic injection of 8-OH-DPAT also caused a significant increase in the respiratory frequency in control rats but not hemisected rats.

Spontaneous recovery of crossed latent pathways was observed in all hemisected rats 16 weeks after hemisection (Figure 1; Table 2). The peak amplitude of the phrenic bursts (hemisected, sham, both left and right) were significantly enhanced with a systemic injection of 8-OH-DPAT (intravenously; $P = 10^{-4}$), and again, there was a greater response to 8-OH-DPAT in the hemisected rats than in the sham control rats ($P = 0.07$; Figure 2). Like application directly to the cord, 8-OH-DPAT in chronically hemisected rats caused a significantly different response in the right and left nerves. There was also a significant reduction in the peak amplitude of both the right ($P = 0.01$) and left nerve ($P = 0.012$) in the hemisected rats compared with controls, indicating a reduced motor drive in chronic injured rats (16 weeks after injury) compared with controls. In addition, the burst area ipsilateral to hemisection increased significantly in response to 8-OH-DPAT.

To verify that the respiratory responses observed with 8-OH-DPAT were caused by the specific activation of 5HT_{1A} receptors, we systemically blocked 5HT_{1A} receptors using the selective 1A receptor antagonists, p-MPPI (which crosses the blood brain barrier) in 3 control rats and WAY 100635 on the dorsal cord in both control

Figure 2. Graph of the peak neural response of 8-OH-DPAT in the right and left PNs relative to control values. Application of the drug was either applied directly to the dorsal spinal cord 1 week after hemisection or systemically in acute injured rats (24–48 hours after injury) or chronic injured rats (16 weeks after injury). *Difference between the right or left hemisected and control values. The bar denotes a significant difference between the hemisection group and the control group.

and hemisected rats. We found that both highly selective 5HT_{1A} receptor antagonists blocked the respiratory responses observed after 8-OH-DPAT was applied. In fact, we observed a significant reduction in motor output after the 5HT_{1A} receptor antagonist, WAY 100635, was applied (Table 3).

DISCUSSION

This study showed that application of 8-OH-DPAT, a 5HT_{1A} agonist, to the dorsal horn of the spinal cord caused a significant increase in bilateral phrenic motor output and elicited latent crossed phrenic activity in rats with SCI. Systemic administration of 8-OH-DPAT had a similar effect in both acutely injured (24–48 hours) and chronically injured (16 weeks) rats, supporting the idea that 5HT_{1A} agonists may be a useful therapy in enhancing respiratory neural output in patients with SCI (14,15), even years after injury.

The National Spinal Cord Injury Statistical Center (Birmingham, AL) reported that more than 50% of all SCIs occur in the cervical spinal cord and result in significant impairments to the respiratory system (1). Animal studies have shown that hemisection of the cervical spinal cord rostral to the phrenic nucleus results in paralysis of the hemidiaphragm ipsilateral to the site of injury (5–13). Furthermore, studies show that both increasing respiratory drive (5–10) and removal of sensory input through dorsal root rhizotomy (12,13) activate latent motor pathways to restore motor function to the paralyzed hemidiaphragm. Understanding multi-

Table 2. Respiratory Parameters in Response to the Application of 8-OH-DPAT for 3 Experimental Groups of Rats

		Peak Amplitude		Frequency	Burst Duration	Burst Area
		Right PN	Left PN			
<i>5HT1A agonist applied directly to dorsal spinal cord 1 week after hemisection</i>						
Hemi (n = 10)	Predrug	0.144 ± 0.03	0.00†	41.28 ± 3.6	0.55 ± 0.04	0.056 ± 0.01‡
	8-OH-DPAT	0.24 ± 0.08*‡	0.09 ± 0.05*†	37.17 ± 2.8	0.41 ± 0.02	0.067 ± 0.02‡
Sham (n = 5)	Predrug	0.142 ± 0.02	0.24 ± 0.078	42.48 ± 3.6	0.514 ± 0.06	0.058 ± 0.01
	8-OH-DPAT	0.19 ± 0.04*	0.34 ± 0.10*	44.97 ± 5.2	0.504 ± 0.05	0.063 ± 0.02
<i>5HT1A agonist injected systemically (intravenous) 24 to 48 hours after hemisection</i>						
Hemi (n = 10)	Predrug	0.107 ± 0.02‡	0.00†	35.48 ± 3.1	0.459 ± 0.02	0.021 ± 0.006
	8-OH-DPAT	0.14 ± 0.02*‡	0.042±.003*†	33.95 ± 2.5	0.423 ± 0.02	0.03 ± 0.005*
Control (n = 8)	Predrug	0.21 ± 0.05	0.12 ± 0.05	35.65 ± 5.3	0.359 ± 0.08	0.026 ± 0.003
	8-OH-DPAT	0.26 ± 0.08*	0.16 ± 0.05*	45.7 ± 5.5*	0.391 ± 0.03	0.034 ± 0.01*
<i>5HT1A agonist injected systemically (intravenous) 16 weeks after hemisection</i>						
Hemi (n = 8)	Predrug	0.11 ± 0.017	0.036 ± 0.01†	40.7 ± 5.46	0.418 ± 0.03	0.031 ± 0.01
	8-OH-DPAT	0.14 ± 0.02*	0.05 ± 0.01*†	39.1 ± 4.53	0.39 ± 0.02	0.04 ± 0.008
Sham (n = 6)	Predrug	0.17 ± 0.054	0.084 ± 0.015	39.4 ± 6.14	0.44 ± 0.02	0.05 ± 0.014
	8-OH-DPAT	0.21 ± 0.07*	0.108 ± 0.02*	33.4 ± 3.37	0.41 ± 0.03	0.06 ± 0.02

All values are presented as means ± SE, and $P < 0.05$ is considered significant.

*Significant effect of 8-OH-DPAT compared with predrug values on a given variable.

†Significant difference between left and right phrenic nerve (PN).

‡Significant difference between the hemisection (hemi) value and the control (sham) value.

ple mechanisms underlying the activation of latent respiratory motor pathways and enhancing respiratory motor output, in general, may ultimately help to wean patients from ventilator dependency and/or aid in basic respiratory function after SCI.

Recently, systemic administration of 5HT1A agonists has been shown to improve respiratory dysfunction in a number of different conditions: Rett Syndrome (24), obstructive sleep apnea (25), and even respiratory disturbances caused by SCI (14,15). The mechanism(s) by which 5HT1A agonists improve ventilation are not completely known. Activation of 5HT1A receptors (using 8-OH-DPAT and buspirone) in spinal contusion-injured rats (T8) reversed the altered breathing patterns associated with the injury: tidal volume increased, breathing frequency decreased, and the respiratory responses to hypercapnia were normalized (14). Similarly, in C5 hemicontused rats, Choi et al (15) showed that 8-OH-DPAT administration restored ventilatory function to near normal levels and normalized the ventilatory responses to hypercapnia. Both papers suggested a possible role of 5HT1A receptor activation affecting sensory afferent pathways, but the exact mechanism is unknown. The results from this paper show that activation of 5HT1A receptors, most likely located in the dorsal horn of the spinal cord, are directly involved with an increase in phrenic motor output and the activation of crossed phrenic activity, strongly indicating 5HT1A receptor involvement at the level of local spinal circuitry.

Although the exact neurons in the spinal cord that are affected by 8-OH-DPAT are unknown, the increases in respiratory motor output observed after application of 8-OH-DPAT could be caused by the following: (a) phrenic afferents inhibit phrenic motor neurons through ascending sensory-supraspinal pathways, (b) phrenic afferents inhibit phrenic motor neurons through local cervical respiratory interneurons, (c) phrenic afferents inhibit descending bulbospinal axons presynaptically, and/or (d) neurons in the dorsal horn that are nonrespiratory modulated (ie, do not fire in phase with the respiratory cycle) may be excited by phrenic afferents and/or other sympathetic sensory inputs to inhibit phrenic motor neurons. These possibilities are briefly discussed below.

Dorsal Horn Afferent-Supraspinal-Mediated Respiratory Reflexes

The results of this study could be caused by phrenic afferents normally inhibiting phrenic motor neurons through ascending sensory-supraspinal pathways. In fact, Teng et al (14) and Choi et al (15) suggested that the respiratory effects that they observed in response to 5HT1A agonists may have been mediated through ascending sensory-supraspinal pathways, specifically the intercostal and abdominal group II afferent pathways. Indeed, stimulation of chest wall afferents by changing lung volume leads to an excitation of diaphragmatic output (26), and this may be mediated by serotonin (27). However, in this study, we examined the effect of 8-OH-DPAT at the level of the cervical spinal cord, and the

Table 3. Respiratory Parameters in Response to the Application of Way 100535 (5HT1A antagonist) Followed by 8-OH-DPAT (5HT1A Agonist)

		Peak Amplitude	Frequency	Burst Duration	Burst Area
On spinal cord Way 100635 + 8-OH-DPAT	Predrug	0.174 ± 0.029	34.49 ± 3.06	0.56 ± 0.0279	0.071 ± 0.014
	After drugs	0.15 ± 0.027*	30.14 ± 4.23	0.578 ± 0.031	0.065 ± 0.012

All values are presented as means ± SE, and $P < 0.05$ is considered significant.

*Significant effect of the combined treatment of 5HT1A antagonist and agonist.

diaphragm is essentially devoid of all group II sensory afferents. In addition, most phrenic afferent-supraspinal respiratory reflexes result in excitation of respiratory output (28,29), whereas inhibitory mechanisms are mediated at a spinal level (21,22,30). This suggests that the role of 5HT1A agonists in increasing phrenic activity that was observed in this study must be mediated by something other than group II afferents.

Cervical Respiratory Interneurons

The results of this study could also be caused by phrenic afferents inhibiting phrenic motor neurons through local cervical respiratory neurons. First, no evidence of any functional connections between cervical respiratory interneurons and phrenic motor neurons using cross-correlogram techniques were found in the phrenic-phrenic inhibitory reflex (30). Second, cervical respiratory interneurons, inspiratory and expiratory, responded to phrenic afferent stimulation with diverse cellular activities; excitation, inhibition, or nothing at all, indicating that respiratory cervical interneurons are not involved in the prolonged inhibition of phrenic motor neurons (22,23). Therefore, it seems unlikely that the 5HT1A receptor activation on a cervical interneuron would result in the disinhibition of phrenic motor neurons as we observed in this study.

Presynaptic Inhibition of Descending Respiratory Premotor Axons

While phrenic primary afferents may be involved in the inhibition of descending respiratory premotor axons, the literature suggests that this is not mediated through the 5HT1A receptor and thus could not be the source of increased phrenic motor output after 8-OH-DPAT application. There are anatomical reports that place phrenic afferents in close proximity to phrenic motor neurons (31) as well as the presence of axo-axonic synapses on phrenic motor neurons (32), indicating a possible monosynaptic inhibition of phrenic motor neurons by phrenic afferents. Physiological studies also support that phrenic motor neurons are presynaptically inhibited. In fact, serotonin decreases the inspiratory current in phrenic motor neurons (33); however, this response was blocked through the 5HT1B receptor and not the 5HT1A receptor (34).

Nonrespiratory Modulated Dorsal Horn Sensory Neurons

Phrenic afferents also stimulate nonrespiratory modulated interneurons in the dorsal spinal cord (22,23). The role of cervical spinal interneurons in the control of respiration was examined, and 2 types of neurons found: respiratory-modulated and “phrenic-driven” neurons (22). Phrenic-driven neurons were localized to the dorsal horn and did not fire in phase with respiration, but did respond to phrenic afferent stimulation and seem responsible for mediating the phrenic-to-phrenic inhibitory reflex (22). The data indicate that nonrespiratory related cervical interneurons are stimulated by phrenic afferent input.

After spinal hemisection, multireceptive dorsal horn neurons in the lumbar spinal cord become spontaneously active and more responsive to stimuli on the side ipsilateral to hemisection (19). The reason for this hyperexcitability is most likely caused by the lack of serotonergic innervation after SCI and the removal of descending central command. Under normal conditions, the dorsal horn of the spinal cord is heavily innervated by serotonin and modulates dorsal horn sensory neurons by acting on 5HT1A receptors to inhibit dorsal horn neurons (16). After SCI, serotonin levels are dramatically reduced (17,18), which leads to the hyperexcitability and spontaneous firing of dorsal horn sensory interneurons (19,20). Studies showed that mechanical allodynia and thermal hyperalgesia associated with SCI are ameliorated with the administration of 5HT1A agonists to the dorsal horn of the spinal cord (20).

A similar effect of SCI on dorsal horn sensory neurons may occur in cervical regions below the hemisection injury and affect respiratory related circuitry. Consistent with this hypothesis, this study showed that activation of 5HT1A receptors in the dorsal horn increased phrenic output significantly more in hemisected rats compared with controls, suggesting that an inhibitory input might arise from phrenic afferents after SCI.

Systemic vs Dorsal Horn 8-OH-DPAT Administration

The data from this study show that all treatments, direct application and systemic administration of 8-OH-DPAT, resulted in an increase in amplitude of phrenic motor output. Systemic administration, however, also resulted in an increased frequency in 1 group of control rats.

Systemic administration of drug would surely have activated 5HT1A receptors globally, including respiratory centers in the brainstem. The reason why only one group of rats showed a positive frequency response is unknown. 5HT1A receptors are located in brainstem regions and affect ventilation in a complex manner. Activation of these receptors has resulted in both excitatory (35,36) and inhibitory (36,37) effects on ventilation.

The data from this study also show that the 2 PNs showed a relative difference in their response to drug (ie, in control rats, 8-OH-DPAT caused a slightly larger amplitude response in the left nerve compared with the right nerve, whereas in hemisectioned rats, 8-OH-DPAT caused a slightly larger response on the left nerve). The difference might simply reflect a lack of descending ipsilateral respiratory input on the left side, such that, after hemisection, the right nerve tended to show a larger response.

The effects that were observed in this study after 8-OH-DPAT administration were mediated through the activation of the 5HT1A receptor, because antagonists specific to the 5HT1A receptor blocked its effect. The results also clearly indicate that activation of spinal 5HT1A receptors results in the activation/enhancement of latent crossed phrenic pathways. Choi et al (15) discounted any potential role of crossed phrenic pathways in their C5 hemicontusion model, because the number of surviving phrenic motor neurons was extremely low. However, similar mechanisms might also be in place in the thoracic spinal cord (ie, 5HT1A activation and hyperpolarization of thoracic dorsal horn neurons may disinhibit intercostal motor neurons and thus contribute to the activation of putative “crossed intercostal pathways”).

CONCLUSION

Application of 8-OH-DPAT to the dorsal spinal cord or systemic administration of 8-OH-DPAT resulted in increased phrenic motor output: primarily an increase in the peak amplitude of the burst and the activation of crossed phrenic pathways. The results from this study suggest that inhibitory inputs arising from within the cervical dorsal spinal cord depress phrenic motor output in a local spinal reflex under both normal and spinal cord-injured conditions but that this inhibition is greater after SCI. Moreover, systemic 8-OH-DPAT enhanced ventilatory output in both acute and chronic injured rats, which indicates its effectiveness over a wide range of times after injury. Finally, the results from this study, along with other studies (14,15), strongly support the idea that 5HT1A agonists may be a beneficial therapy in treating patients with post-SCI respiratory dysfunction.

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