Serotonin 1A Receptor Agonists Reverse Respiratory Abnormalities in Spinal Cord-Injured Rats

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Contusion spinal cord injury (SCI) at T8 produces respiratory abnormalities in conscious rats breathing room air and challenged with CO_2. In seeking ways to improve respiration after SCI, we tested drugs that stimulate serotonin 1A (5-HT_{1A}) receptors, based on our previous findings that these agents can counteract respiratory depression produced by morphine overdose. Respiratory function was measured with a head-out plethysmograph system in conscious rats. T8 SCI rats ($n = 5$) showed decreased tidal volume (Vt; 0.90 ± 0.02–0.66 ± 0.03 ml; $p < 0.05$) and increased respiratory rate (f; 91 ± 3.7–132 ± 5.7 breaths/min; $p < 0.05$) with room air ventilation at 24 hr after injury. They also exhibited a diminished response to the respiratory stimulating effect of 7% CO_2; minute ventilation increased to 250 ± 17 ml/min before, but only to 162 ± 15 ml/min at 24 hr after SCI ($p < 0.05$). Respiratory deficits during room air ventilation were also observed at 7 d after injury ($n = 3$). Treatment with the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylmino)tetralin (8-OH-DPAT; 250 μg/kg, i.p.) at 24 hr ($n = 5$) or 7 d ($n = 3$) after injury normalized Vt, f, and the respiratory response to 7% CO_2. Identical results were obtained with another 5-HT_{1A} receptor agonist, buspirone (1.5 mg/kg, i.p.; $n = 3$). In contrast, intraperitoneal saline vehicle administration ($n = 5$) showed no beneficial effects on SCI-impaired respiration. Finally, pretreatment with a specific antagonist of 5-HT_{1A} receptors, 4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-benzamide (3 mg/kg, i.p.; $n = 3$) given 20 min before 8-OH-DPAT, prevented 8-OH-DPAT from restoring respiration to normal. Our results demonstrate that drugs that stimulate 5-HT_{1A} receptors counteract respiratory abnormalities in conscious rats after SCI.

Key words: **rat; 5-HT1A; 8-OH-DPAT; buspirone; *p*-MPPI; tidal volume; respiratory rate; minute ventilation; plethysmograph; spinal cord injury**

Introduction

We have reported that incomplete contusion at T8 results in consistent and significant abnormalities in respiratory function (Teng et al., 1999). These abnormalities were documented in conscious rats using a head-out plethysmograph system to evaluate respiratory activity. At 24 hr after spinal cord injury (SCI), there was an abnormal pattern of respiration during room air breathing and a reduction in the ability of rats to respond appropriately to breathing higher than normal levels of CO_2. The abnormal respiratory pattern of SCI rats consisted of a decreased tidal volume (Vt) and an increased respiratory rate (f), a pattern that is also found in patients with lower thoracic SCI (Prakash, 1989). The respiratory abnormalities seen in rats after SCI appear related to loss of motoneurons innervating muscles of respiration, because acute treatment with basic fibroblast growth factor increases survival of these motoneurons and prevents the respiratory deficits after contusion injury (Teng et al., 1999).

Loss of motoneurons occurs rapidly after SCI. In our injury model, approximately half of the ventral horn motoneurons that are lost chronically are already gone by 4 hr after injury, and the remainder are gone by 24 hr (Teng et al., 1998; Grossman et al., 2001). Thus, treatments to increase their preservation have a relatively limited therapeutic window. An alternative or additional strategy would be enhancing the function of surviving respiratory motoneurons. The goal of the present study was to evaluate whether a newly recognized group of respiratory stimulant drugs, 5-HT_{1A} receptor agonists (Sahibzada et al., 2000), would exert a beneficial effect on SCI-induced respiratory abnormalities.

The prototype drug of the group is 8-hydroxy-2-(di-*n*-propylmino)tetralin (8-OH-DPAT), and this and a related agent, buspirone, have been shown to counteract respiratory disturbances (i.e., apneustic breathing) produced by hypoxia, pentobarbital, and antagonists of the NMDA receptor complex (Lalley et al., 1994; Wilken et al., 1997). These agents given systemically will reverse apnea produced by morphine and dizocilpine overdose in anesthetized rats (Sahibzada et al., 1999, 2000). Respiratory stimulant effects of 5-HT_{1A} receptor agonists are also observed when these agents are administered under conditions where breathing is normal. For example, administration of buspirone intravenously to anesthetized cats increased f, tidal phrenic activity, and minute phrenic activity (Garner et al., 1989). Additionally, buspirone decreased the apneic threshold and shifted the CO_2 response curve to the left of the control CO_2 response curve. Mendelson et al. (1990) administered buspirone intraperitoneally to conscious rats and reported an increase in f, Vt, and minute ventilation (Ve). This group of investigators went

Received Nov. 18, 2002; revised Feb. 28, 2003; accepted March 5, 2003.

This work was supported by National Institutes of Health Grants P01-NS-28130 and R01-NS-35647.

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 0270-6474/03/234182-08$15.00/0

on to study buspirone in five patients with obstructive sleep apnea (Mendelson et al., 1991) and found that buspirone decreased the number of apneas by one-third. Based on the above positive findings of 5-HT_{1A} receptor agonists in stimulating respiratory function, we set out to determine whether these agents can restore breathing to normal in the rat model of SCI.

Materials and Methods

Spinal cord injury

Female Sprague Dawley rats (250–280 and 360–390 gm; Taconic, Germantown, NY) were anesthetized with 4% chloral hydrate (360 mg/kg, i.p.). An incomplete spinal cord contusion injury was produced at the T8 vertebral level with a weight drop device (10 gm × 2.5 cm) as previously described (Wrathall et al., 1985). After SCI, manual expression of bladders was performed twice daily until a reflex bladder was established. Animal care also included housing the rats in pairs to reduce isolation-induced stress, maintaining ambient temperature at 22–25°C, and using highly absorbent bedding. No prophylactic antibiotics were given.

Monitoring of respiratory parameters by plethysmograph

Experiments were conducted in unanesthetized, awake, spontaneously breathing rats at 24 hr before SCI, and at 24 hr and 1 week after injury.

Acclimation of the animals. We found that correct plethysmograph recording of respiratory parameters of conscious rats required animal training for acclimatization. Animals were placed in the body cylinder of the plethysmograph (Fig. 1*A*) for 60 min/d for at least 5 d. This procedure led them to become used to the environment. After acclimatization, rats remained quietly in the cylinder, allowing for the acquisition of data without physical signs of stress (i.e., defecation, urination, and bloody secretions in the eyes and nose) and motion artifacts.

Noninvasive measurements of f, Vt, and Ve. Noninvasive measurements of respiratory function in conscious rats were performed with a restrained head-out plethysmograph specially designed for rodents (BUXCO Electronics Inc., Sharon, CT) (Fig. 1*A*). The plethysmograph apparatus has a neck seal that prevents leakage of air from between the animal's neck and the plethysmograph opening. Displacement of the thoracic wall produced by the animal's respiratory movements causes changes in the cylinder pressure, which results in air flowing across a pneumotachograph located on the wall of the cylinder. The pressure drop across the pneumotachograph is measured with a pressure transducer and is proportional to the flow. This signal is amplified and integrated into volume. From measurements of volume and flow a computer and appropriate software provides respiratory parameters, such as *f*, Vt, and Ve. An additional opening on the wall of the box allows volume calibration by injecting and removing air from the box with a calibrated syringe.

The noise level in the laboratory was kept to a minimum to avoid startling the animals. Furthermore, the animals were visually isolated from the investigators by means of a chamber made of an opaque material that surrounded and covered the front end of the body plethysmograph (Fig. 1*B*). This arrangement, although blocking the vision of the animal, allowed the experimental observer to continuously monitor the movements of the rat's body, i.e., observe the rat's body from the neck down, inside the transparent cylinder. In this way, the experimental observer could exclude any recorded indices of respiratory function caused by unexpected noise and by body movements unrelated to respiratory movements. As an additional safeguard against registering signals unrelated to respiratory movements, the plethysmograph software used rejects all recorded signals that are generated by air flow dynamics different from those of regular breathing triggered by thoracic and abdominal changes.

Measurement of ventilatory response to carbon dioxide. For measurement of the ventilatory response to CO_2, animals were exposed to air containing 7% CO_2 (mixed with 60% O_2 and 33% N_2) for 7 min with recording of respiratory activity during the last 2 min. Hyperoxia hypercapnia was used with the expectation that any changes in respiratory activity would be caused by the increased CO_2 and not by any significant change in peripheral chemoreceptor activity caused by changes in oxygen.

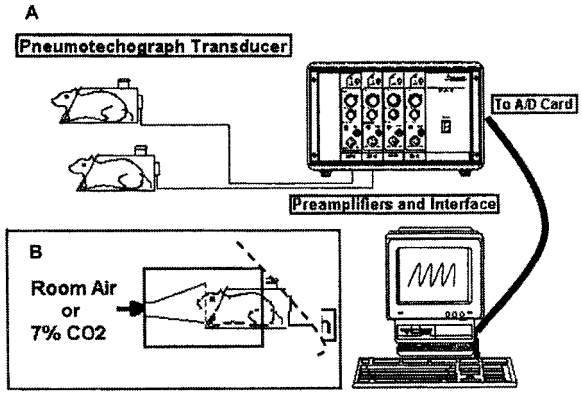

Figure 1. Noninvasive measurements of respiratory function in conscious rats. *A*, Schematic presentation of the restrained head-out plethysmograph system for rodents. *B*, The animals breathe from a funnel fixed in the front wall of a box made of an opaque material. The box surrounds the front two-thirds of the body cylinder of the plethysmograph, and the rear outlet of the box is covered with a piece of bath towel (illustrated by a dashed line). The animals were exposed to room air for baseline recordings and then to a gas mixture containing 7% CO_2 mixed with 60% O_2 and 33% N_2) for 5 min, and recording of respiratory activity was continued for another 2 min (a total recording duration of 7 min). After a new baseline was obtained by allowing the animals to breathe room air for 20 min, the rats were injected with drugs that affect 5-HT_{1A} receptors, and the recordings were repeated as specified in each experiment (figure modified from Teng et al., 1999).

Drug administration

The 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone (both purchased from Research Biochemicals, Natick, MA) were dissolved in 0.9% saline (pH adjusted to 7.4). Both agonists were administered intraperitoneally in 0.5 ml of final injection volume per rat and in doses of 250 μg/kg for 8-OH-DPAT and 1.5 mg/kg for buspirone. The 5-HT_{1A} receptor antagonist *p*-MPPI (Research Biochemicals) was also dissolved in 0.9% saline and given intraperitoneally at a dose of 3 mg/kg (pH 7.4; final volume, 0.5 ml). The doses used of the above drugs were based on data from an earlier study that demonstrated that 5-HT_{1A} agonists could reverse morphine-induced respiratory depression (Sahibzada et al., 2000). Vehicle solution was 0.9% saline and was also injected intraperitoneally (pH 7.4; volume, 0.5 ml/rat).

Experimental protocol

SCI surgical procedures were performed only after animals finished at least 5 d of plethysmograph acclimatization (see above) and at 24 hr after plethysmograph data acquisition for preinjury respiratory parameters. Tests of functional deficits (Gale et al., 1985; Basso et al., 1995) were performed at 24 hr before SCI, and at 24 hr and 1 week afterwards to confirm that a proper degree of SCI was achieved.

Baseline respiratory function was measured with room air ventilation and after each animal was stabilized inside a body cylinder (Fig. 1*A*,*B*) for 30 min at each time point analyzed before SCI and after injury. Immediately after the evaluation of baseline respiration, the animals were exposed to a gas mixture containing 7% CO_2 for 7 min to monitor their ventilatory response to CO_2 stimulus (Teng et al., 1999). For vehicle and 8-OH-DPAT studies, at 24 hr after injury, respiratory function of a SCI rat was first evaluated by plethysmograph for baseline performance as well as respiratory response to 7% CO_2 challenge. Twenty-four minutes after the end of CO_2 breathing and after a new baseline was recorded for 4 min starting at the 20 min time point, the rat was removed from the body cylinder (Fig. 1*A*). The animal was then injected with saline (0.5 ml, i.p.) and immediately put back into the cylinder in a smooth manner for continuing respiratory monitoring (the injection procedure took ~1.2 min on average). Baseline respiration (i.e., with room air ventilation) was examined for another 10 min, and at the end of this 10 min, ventilatory responses were evaluated when the animal was challenged by 7% CO_2 for 7 min. Twenty-four minutes after the end of the CO_2 stimulus (including a recording of a new baseline for 4 min), the rat was again taken out from the body cylinder. The animal was injected with 8-OH-DPAT (250 μg/kg

in 0.5 ml, i.p., with the injection procedure requiring an average of 1.2 min) and immediately put back into the cylinder for continuing respiratory monitoring. After the drug administration, baseline respiration (i.e., with room ventilation) was examined continuously for another 23 min. At the end of the twenty-third minute, ventilatory response was evaluated once more when the rat was challenged with 7% CO_2 for 7 min. A similar 8-OH-DPAT study was repeated at 7 d after SCI, except that no saline treatment was given.

In the time course study of the respiratory effect of 8-OH-DPAT, recordings of baseline respiratory function (for 4 min) and ventilatory response to 7% CO_2 (for 7 min) were repeated hourly for up to 5 hr after the administration of 8-OH-DPAT. In experiments of *p*-MPPI antagonism of 8-OH-DPAT effects, *p*-MPPI (3 mg/kg in 0.5 ml/rat, i.p.) was given at 20 min before the administration of 8-OH-DPAT. Baseline respiratory function was examined beginning at 4 and 18 min after *p*-MPPI injection (each lasted for 2 min). Baseline recording was performed again at 4 and 8 min after intraperitoneal 8-OH-DPAT (each lasted for 2 min), and at the end of the tenth minute after 8-OH-DPAT, ventilatory response to breathing 7% CO_2 was measured. For the study of buspirone effects, similar sequential procedures as those in the 8-OH-DPAT experiments were followed. However, the 7% CO_2 challenge was given at 10 min after intraperitoneal injection of buspirone (1.5 mg/kg in 0.5 ml), because buspirone has a shorter duration of action than 8-OH-DPAT (Sahibzada et al., 2000). Neither a time course nor an antagonism study was performed for buspirone.

All experimental procedures were performed in strict accordance with the Laboratory Animal Welfare Act, Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD; DHEW Publication No. 78–23, Revised 1978) and after review and approval of our protocol by the Animal Care and Use Committee of Georgetown University. All animals survived the entire study.

Statistical analyses

Experimental data are expressed as mean ± SEM. Statistical significance was defined at the $p < 0.05$ level. Respiratory data were analyzed statistically using repeated measures ANOVA, followed by Tukey's or Dunn's test for multiple comparisons between groups as used in previous studies (Teng et al., 1999). The same statistical tests were used for analyzing respiratory data from drug treatment studies.

Results

Effects of 8-OH-DPAT on T8 SCI-induced respiratory dysfunction

Respiratory function was evaluated both with the animals breathing room air and breathing a gas mixture containing 7% CO_2, as described in Materials and Methods. Before SCI ($n = 5$), Vt, *f,* and Ve were 0.90 ± 0.02 ml, 91 ± 3.7 breaths/min and 82 ± 3.9 ml/min, respectively (Fig. 2). Exposure to 7% CO_2 resulted in a statistically significant increase in all three indices of respiratory function (Fig. 2). As described in our previous study (Teng et al., 1999), 24 hr after injury there was a statistically significant decrease in Vt and a significant increase in *f*, resulting in a shallow and rapid breathing pattern (Fig. 2). There was also a reduced response to 7% CO_2. After SCI the tidal volume increased from 0.66 ± 0.03 to 1.10 ± 0.03 ml (66%), as compared to an increase from 0.90 ± 0.02 to 1.6 ± 0.09 ml (82%) before SCI (Fig. 2). The changes in Ve elicited by 7% CO_2 were also reduced. Before SCI, CO_2 increased Ve from 82 ± 3.9 ml/min to 250 ± 17 ml/min (205%), whereas after SCI, Ve increased only from 86 ± 6.4 ml/min to 162 ± 15 ml/min (88%) (Fig. 2). Each of the five SCI animals then received vehicle for 8-OH-DPAT (i.e., intraperitoneal saline) followed by 8-OH-DPAT (250 μg/kg, i.p.). Figure 2 shows that vehicle treatment did not alter respiratory activity in animals breathing room air and had no effect on their response to CO_2. In contrast, ~24 min after treatment with 8-OH-DPAT, *f* and Vt values were restored to values not significantly different from the corresponding values before SCI. Most importantly, exposure to 7% CO_2 now produced a ventilatory response that was identical to the normal response seen before SCI (Fig. 2).

In another set of animals ($n = 3$), we studied the time course of the effect of 8-OH-DPAT. The stimulatory effect of 8-OH-DPAT on the ventilatory response to CO_2 peaked at ~20 min and remained near the normal range for up to 4 hr after the administration of the drug, as shown by the effect of 8-OH-DPAT on Ve (Fig. 3). After 5 hr the ventilatory response to CO_2 was similar to the response observed 24 hr after SCI and before administering 8-OH-DPAT. There was also a transient stimulatory effect of 8-OH-DPAT on spontaneous respiration after SCI that was seen at 3 min after 8-OH-DPAT, and had, for the most part, disappeared by 20 min after administering the drug (Fig. 3).

We also examined the respiratory function and the response to 8-OH-DPAT of these same SCI animals at 7 d after injury. These results are summarized in Figure 4. SCI-induced respiratory dysfunction was still present; that is, during room air breathing, Vt was significantly reduced ($p < 0.05$) from values before SCI, and *f* was significantly increased ($p < 0.05$) as compared to preinjury values. These animals still exhibited a breathing pattern that was more shallow and rapid than before injury. However, the ventilatory response to 7% CO_2 of these animals was not significantly different from that observed before SCI (96 ± 5.1 to 254 ± 41 ml/min vs 82 ± 3.9 to 250 ± 17 ml/min; $p < 0.05$). The effect of treatment with 8-OH-DPAT on respiration 7 d after SCI is also shown in Figure 4. The most striking effect of 8-OH-DPAT was on CO_2-induced increases in Ve (Fig. 4*C*). Seven percent CO_2 was a powerful stimulant of respiration in these 8-OH-DPAT-treated rats. The increase in Ve was even greater than that before SCI. A similar enhancement in *f* by 8-OH-DPAT can also be noted (Fig. 4*B*). In contrast, injection of the saline vehicle had no effect on respiration.

Effects of 8-OH-DPAT on respiratory function of normal rats

Three uninjured animals were studied for the purpose of determining the effect of 8-OH-DPAT (250 μg/kg, i.p.) on their respiratory function. The time points chosen for analyzing an effect of 8-OH-DPAT were the same, i.e., at 4 and ~20–30 min after administering the drug. Data are presented in Figure 5 and indicate that at 4 min after administering 8-OH-DPAT, there were statistically significant increases in Vt, *f,* and Ve. The significant increases in *f* and Ve, but not the increase in Vt, persisted until 22 min after administering the drug. However, unlike the effect on SCI animals, the ventilatory response to 7% CO_2 was not significantly changed by 8-OH-DPAT administration in normal rats.

Effects of buspirone on T8 SCI-induced respiratory dysfunction

The effects of a second 5-HT_{1A} agonist, buspirone, were studied in a similar manner to that described for 8-OH-DPAT. The data are presented in Figure 6. In this group of animals ($n = 3$), at 24 hr after SCI, respiration also became more shallow and rapid, and a striking decrease in the ventilatory response to CO_2 was observed. Eight to 15 min after administration of buspirone (1.5 mg/kg, i.p.), there was a nonsignificant tendency toward increased Ve, and both *f* and Vt values were restored to values not significantly different from corresponding values present before SCI (Fig. 6). Most importantly, the ventilatory response to 7% CO_2 became normal.

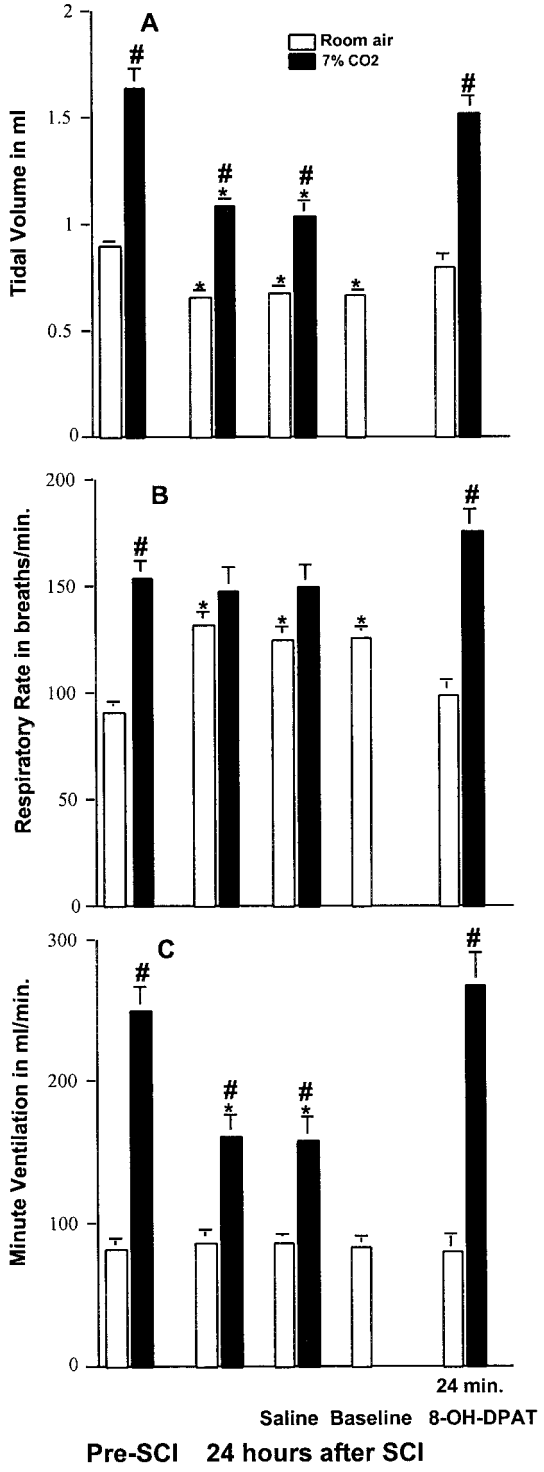

Figure 2. Effects of 8-OH-DPAT on SCI-induced respiratory dysfunction at 24 hr after injury. *A*, Vt is significantly decreased after SCI as compared with preinjury data for the same rats breathing room air or a gas mixture containing 7% CO_2. In addition, the increase in Vt normally elicited by 7% CO_2 is diminished. Administration of saline (vehicle for 8-OH-DPAT) had no effect on SCI-induced alterations in Vt. A baseline was re-established, and then 8-OH-DPAT (250 μg/kg, i.p.) was administered. Twenty-four minutes later, the Vt with room air and with 7% CO_2 was normalized. *B*, The measurement of *f* under room air conditions was significantly increased at 24 hr after injury. There was no further increase in *f* in response to 7% CO_2. Administration of saline had no effect. At 24 min after injection with 8-OH-DPAT, *f* with room air was reduced to preinjury levels and demonstrated a normal increase when respiration was stimulated with 7% CO_2. *C*, Ve while breathing room air was not affected by SCI, but there was a decreased response to 7% CO_2. Saline administration had no effect. The response to 7% CO_2 returned to preinjury levels at 24 min after injection with 8-OH-DPAT. Data reported are means ± SEM for five rats and were analyzed by one-way ANOVA followed by Tukey's and Dunn's tests. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with value obtained breathing room air.

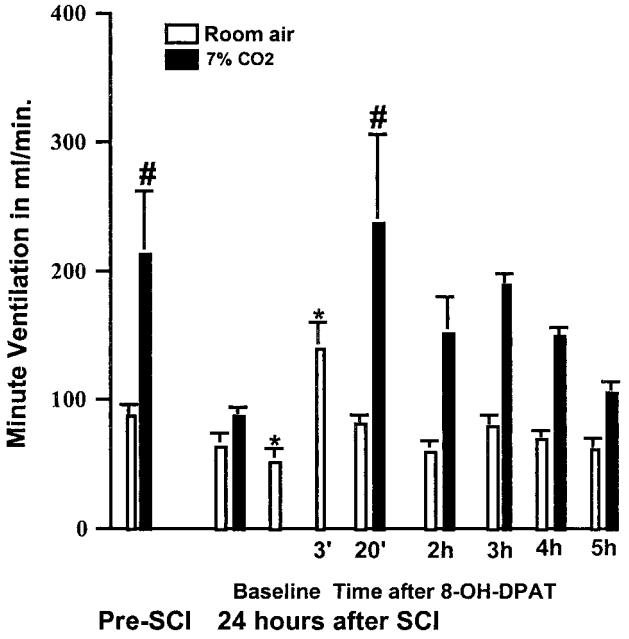

Figure 3. The time course of the effect of 8-OH-DPAT on Ve in rats at 24 hr after SCI. Bars represent the average Ve ± SEM for rats ($n = 3$) before and after SCI, the baseline measure before 8-OH-DPAT administration, and at specified times after the drug injection (250 μg/kg, i.p.) at 24 hr after injury. In this group of rats, SCI resulted in a small drop in baseline Ve when breathing room air, and greatly diminished the increase in Ve in response to 7% CO_2 challenge. 8-OH-DPAT treatment produced a transient increase in Ve with room air conditions at the 3 min point. At 20 min after treatment, Ve returned to a preinjury level, and the increase in Ve in response to 7% CO_2 was similar to that seen before SCI. Data reported are means ± SEM and were analyzed by repeated measures ANOVA followed by Tukey's test. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with value obtained breathing room air.

Effects of treatment with 8-OH-DPAT on T8 SCI-induced respiratory dysfunction occurring at 24 hr in animals pretreated with an antagonist (*p*-MPPI) of the 5-HT_{1A} receptor

Twenty-four hours after SCI, animals ($n = 3$) exhibited the shallow, rapid breathing pattern (Fig. 7) previously described. At this time, they were given *p*-MPPI (3 mg/kg, i.p.), a specific antagonist of the 5-HT_{1A} receptor (Kung et al., 1994). Treatment with *p*-MPPI did not significantly ($p > 0.05$) alter respiratory function of animals breathing room air (data not shown). The animals were given 8-OH-DPAT (250 μg/kg, i.p.) ~9–18 min after administration of *p*-MPPI. The antagonist, MPPI, blocked the transient early stimulatory effects of 8-OH-DPAT respiration (data not shown). Furthermore, 8-OH-DPAT failed to restore the ventilatory response to CO_2 to normal, in contrast to the powerful effect observed in SCI animals not pretreated with *p*-MPPI (compare data in Fig. 7 with data in Fig. 2). Indeed, in animals not pretreated with *p*-MPPI, Ve increased from 81 ± 11 to 268 ± 22 ml/min, an increase of more than threefold (Fig. 2). However, in animals that were treated with *p*-MPPI before receiving 8-OH-DPAT, Ve increased only from 88 ± 13 ml/min to 143 ± 28 ml/min (Fig. 7), an increase similar to that seen at 24 hr after SCI in animals receiving no treatment (87 ± 6.3 to 162 ± 15 ml/min) (Fig. 2).

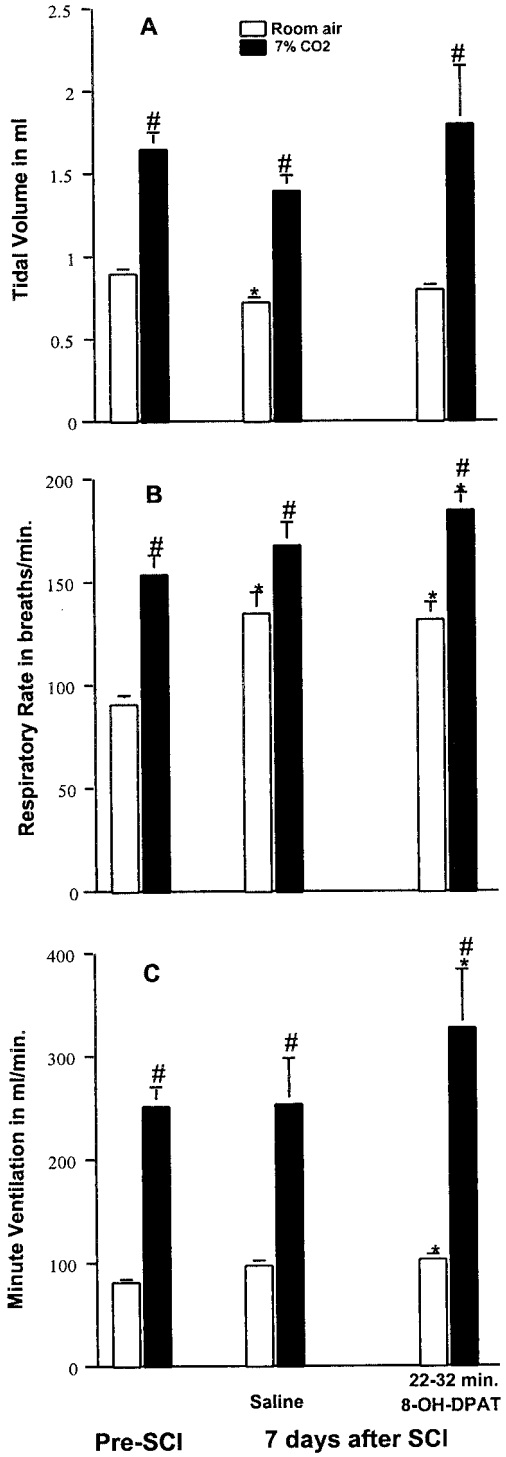

Figure 4. Respiratory response to 8-OH-DPAT of SCI animals at 7 d after injury. Tidal volume (*A*) was significantly decreased, and respiratory rate (*B*) significantly increased at 7 d after SCI as compared with preinjury data for the same rats, whereas Ve (*C*) was similar to that before injury. Stimulation with a gas mixture containing 7% CO_2 increased Vt, *f*, and Ve, similar to that seen before injury. Saline injection has no effect. Administration of 8-OH-DPAT (250 μg/kg, i.p.) normalized the Vt with room air conditions, but *f* and Ve were significantly greater than that before injury with both room air and 7% CO_2 conditions. Data reported were means ± SEM for three rats and were analyzed by one-way ANOVA followed by Tukey's and Dunn's tests. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with values obtained breathing room air.

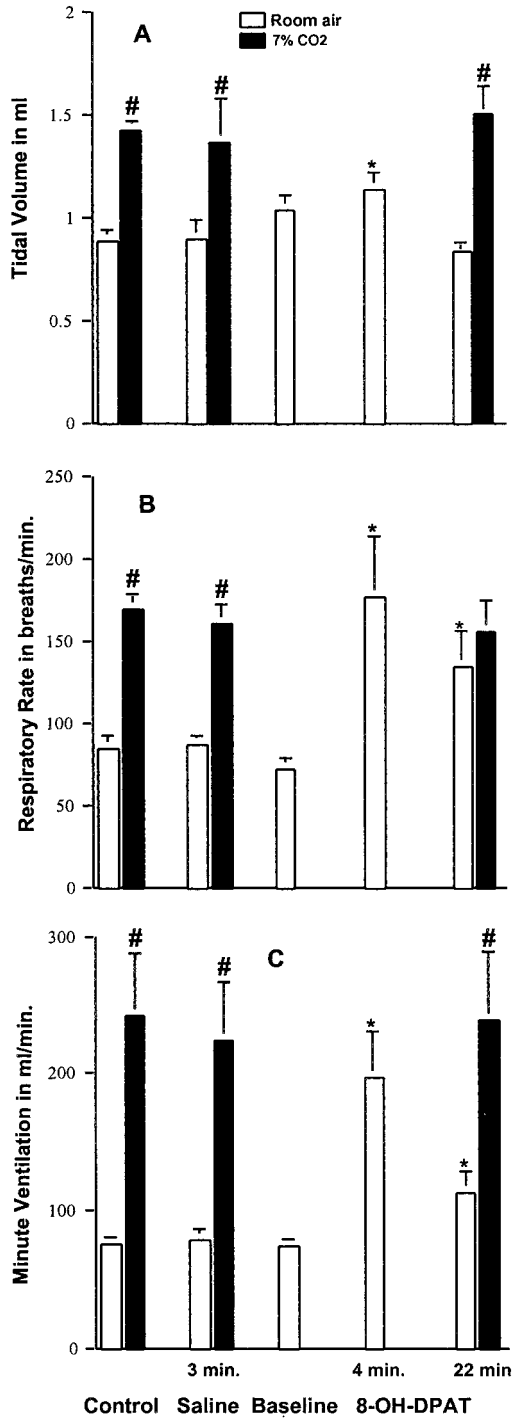

Figure 5. Effects of 8-OH-DPAT on respiratory function of normal rats. Normal rats breathing room air exhibited a transitory increase in Vt (*A*) at 4 min and a longer lasting increase in *f* (*B*) and Ve (*C*) at 4 and at 22 min after administration of 8-OH-DPAT (250 μg/kg, i.p.). The values for Vt, *f*, and Ve obtained with 7% CO_2 conditions were not affected by administration of 8-OH-DPAT. Data reported are means ± SEM for three rats and were analyzed by one-way ANOVA followed by Dunn's tests. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with values obtained breathing room air. Administration of saline at the 3 min time point had no effect.

Discussion

Our data confirm and extend previous results (Teng et al., 1999) demonstrating that incomplete spinal cord contusion injury at T8 in rats results in significant disturbances in respiratory function at 24 hr and 7 d after injury. The disturbances in respiratory

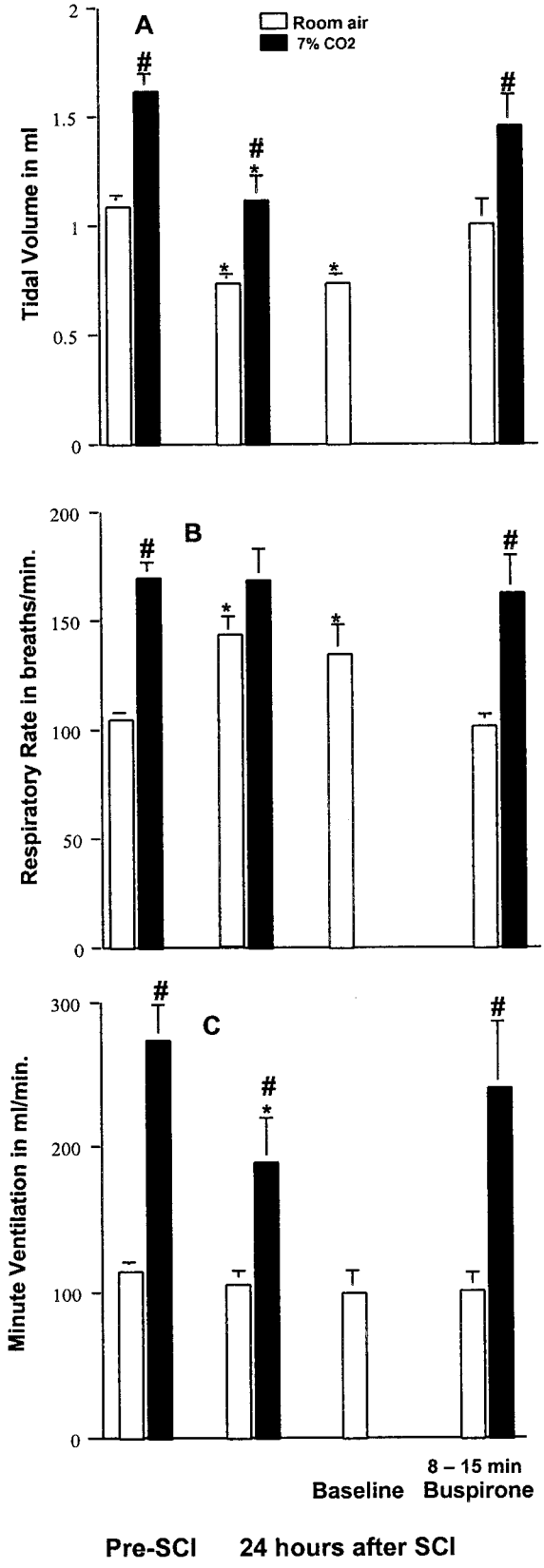

Figure 6. Effects of buspirone on SCI-induced respiratory dysfunction. At 24 hr after SCI, rats demonstrated reduced Vt (*A*) and increased *f* (*B*) with room air breathing conditions, although Ve (*C*) was similar to that obtained before injury. Stimulation with 7% CO_2 increased Ve to a lesser extent than before injury. A baseline was re-established, and then buspirone (1.5 mg/kg, i.p.) was administered. Buspirone normalized Vt and *f* with room air breathing conditions and increased the response of Ve to 7% CO_2 to equal that seen before SCI. Data reported are mean ± SEM for three rats and were analyzed by one-way ANOVA followed by Tukey's and Dunn's tests. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with values obtained breathing room air.

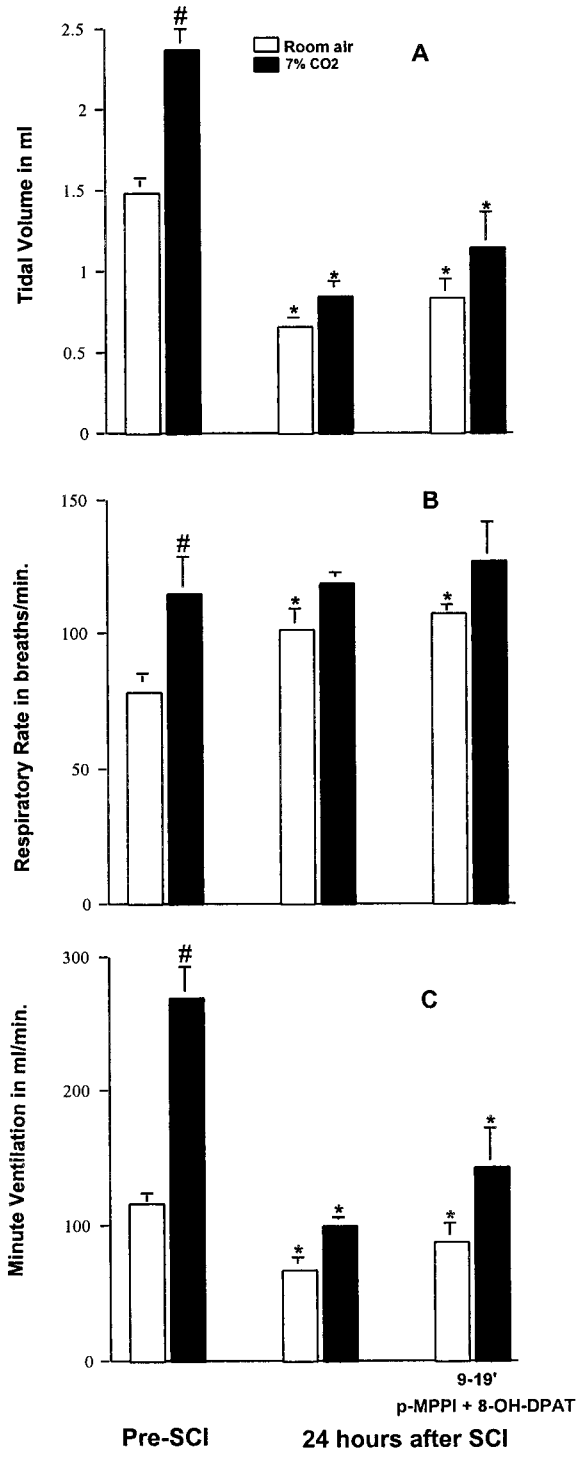

Figure 7. Effects of 8-OH-DPAT on SCI-induced respiratory dysfunction at 24 hr in animals treated with the 5-HT_{1A} receptor antagonist *p*-MPPI. Twenty-four hours after SCI, animals exhibited decreased Vt (*A*), increased *f* (*B*), and decreased Ve (*C*) with room air breathing conditions as well as a reduced response to 7% CO_2. Treatment with *p*-MPPI (3 mg/kg, i.p.) followed by 8-OH-DPAT (250 μg/kg, i.p.) blocked the normalization of respiration previously seen with 8-OH-DPAT (Fig. 2). Data reported are means ± SEM for three rats and were analyzed by one-way ANOVA followed by Tukey's and Dunn's tests. *Significant difference from preinjury value obtained with the same breathing condition (room air or 7% CO_2); #significant difference compared with values obtained breathing room air.
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function consisted of a decrease in Vt and an increase in *f* during room air breathing, as well as a reduction in the ventilatory response to breathing 7% CO_2. Although Ve was normal in the SCI animals during room air breathing, the combination of depressed Vt and increased *f* may impair gas exchange and cause respiratory failure (Rochester, 1993) and is found in patients with lower thoracic SCI (Prakash, 1989), with respiratory muscle weakness (Gibson et al., 1977) and with muscular dystrophy (Bégin et al., 1980). Administering the 5-HT_{1A} receptor agonist drug 8-OH-DPAT intraperitoneally to these rats at 24 hr after injury fully counteracted the respiratory disturbances created by SCI while rats were breathing room air. At both time points, 8-OH-DPAT was found to restore Vt and *f* values to the normal range. Furthermore, administering 8-OH-DPAT at 24 hr after injury fully restored the ventilatory response to CO_2.

Two lines of evidence suggest that the beneficial effects of 8-OH-DPAT on respiratory function after SCI were mediated through 5-HT_{1A} receptors. First, although the bulk of our data were obtained with 8-OH-DPAT, we observed that buspirone, a partial agonist at 5-HT_{1A} receptors (Taylor, 1988), also reversed the respiratory disturbances. Second, pretreatment of animals with *p*-MPPI, a selective antagonist of the 5-HT_{1A} receptor (Kung et al., 1994), prevented 8-OH-DPAT from counteracting SCI-induced impairment of respiratory function. Based on the totality of the data obtained with 8-OH-DPAT, buspirone, and *p*-MPPI, we conclude that activation of 5-HT_{1A} receptors is an effective way of reversing disturbed respiratory function after incomplete contusion at T8. Additional studies will be needed to determine whether serotonin agonists will be effective with the more severe respiratory deficits expected from an upper thoracic or cervical SCI.

The reason that 5-HT_{1A} receptor stimulation with drugs such as 8-OH-DPAT and buspirone benefits our respiratory impaired SCI rats is unclear. In seeking clues regarding the possible site of action of these drugs, it is important to consider what causes respiratory dysfunction after contusive SCI at T8. Data on cell loss over the first 24 hr in this model (Grossman et al., 2001) show that loss of ventral motoneurons at specified distances rostral and caudal to the injury epicenter progressed symmetrically with time. At 24 hr, tissue destruction was so severe that ventral motoneurons were completely absent at the epicenter and 2 mm in either direction, thus, a 4 mm length of cord was devoid. Further, at 4 mm rostral and caudal only ~44% of ventral motoneurons survived. In addition, glia were significantly reduced at the lesion epicenter and at distances of up to ±4 mm distal from it. With such tissue destruction, functional innervation of both intercostal and abdominal muscles (motoneurons at T5–L3) (Holstege, 1991) would be significantly impaired causing the respiratory dysfunction seen at 24 hr after injury in this and our earlier study (Teng et al., 1999). Drugs that activate the 5-HT_{1A} receptor eliminated the respiratory defects caused by SCI in the present study. We suggest that 5-HT_{1A} receptor activation might increase the excitability of those ventral motoneurons that have survived injury. The basis for this suggested mechanism is that recent findings indicate that 5-HT_{1A} receptors do exist on these neurons (Kheck et al., 1995), and when activated, amplify their excitatory output (Takahashi and Berger, 1990).

Although we speculate that 5-HT_{1A} receptor activation could restore SCI-induced respiratory dysfunction to normal by affecting motoneurons, 5-HT_{1A} receptors on other neurons could also be involved. The spinal cord regions most adversely affected by the contusion injury at T8 are the dorsal horns (Noble and Wrathall, 1985, 1989) that are involved in processing sensory input. Most importantly, intercostal and abdominal muscle afferents (including group II afferent fibers) influence supraspinal respiratory group neurons in the brainstem and motor output to skeletal muscles responsible for breathing (Shannon, 1980; Shannon and Lindsey, 1983). Indeed, removing some of this afferent input by performing a thoracic dorsal rhizotomy has been shown to decrease Vt, increase *f,* and reduce the ability of CO_2 to stimulate breathing (Gautier, 1973). This profile of respiratory effects produced by dorsal rhizotomy mimics the profile of respiratory changes that we observed in our SCI rats. The densest population of 5-HT_{1A} receptors in the spinal cord is in the dorsal horn (Thor et al., 1993) in laminae I-IV, and particularly in lamina II (Thor et al., 1993), suggesting a role in processing sensory inputs. A significant proportion of 5-HT_{1A} receptor sites are located on the terminals of primary afferent neurons (Daval et al., 1987), whereas others are postsynaptic (Wikberg and Hajos, 1987) and located on neurons intrinsic to the dorsal horn (Pompeiano et al., 1992). In addition, confocal and electron microscopic study of contacts between serotonin-immunoreactive fibers and interneurons in the dorsal horn reveals that axodendritic synaptic contacts exist between 5-HT fibers and interneurons in pathways from muscle afferents with dorsal horn group II interneurons (Jankowska et al., 1997). Furthermore, 5-HT axons contact spinal interneurons that project to motor nuclei and are activated by muscle afferents (Maxwell et al., 2000). Locally applied 5-HT tested on extracellularly recorded responses of spinal interneurons evoked by group II muscle spindle afferents exerts a modulatory action (Jankowska et al., 1997). Jankowska et al. (1997) suggest that transfer of information from group II muscle afferents to supraspinal centers may be gated by descending serotonergic pathways to adjust to the requirements of a specific behavioral situation. Because the neural pathways in the spinal cord responsible for transferring afferent information from intercostal muscles to supraspinal centers are influenced by serotonin, presumably via 5-HT_{1A} receptors, we speculate that drugs such as 8-OH-DPAT and buspirone might act in the dorsal horn of SCI rats to restore respiratory function to normal by acting on these pathways. Consistent with this speculation, Remmers (1970) found that sustained stimulation of chest wall mechanoreceptors provokes a slowing of the breathing rate, a response that we also observed with 5-HT_{1A} receptor activation in rats after SCI.

The possibility of a supraspinal site of action of 5-HT1A receptor agonists to counteract respiratory dysfunction created by SCI seems less likely. 8-OH-DPAT has shown little or no effect on output from key brainstem respiratory centers (Johnson et al., 1996, 2001). Further studies will be needed to examine various possible sites of action of 5-HT1A receptor agonists in reducing the respiratory deficit after SCI.

Our data obtained with CO_2 challenge 7 d after SCI injury indicated that animals no longer exhibited a depressed respiratory response while they continued to exhibit respiratory dysfunction when breathing room air. Furthermore, when animals at 7 d after SCI received 8-OH-DPAT, and their room air breathing was restored to normal, CO_2 challenge now evoked a significantly greater response on Ve than was noted before injuring the spinal cord. This is evidence of plasticity by 7 d after injury in circuits involved in controlling respiratory function, specifically those involved in eliciting the respiratory changes evoked by CO_2.

5-HT_{1A} receptors may be involved in the functional plasticity of these respiratory pathways. Serotonin appears to activate a latent pathway used for recovery of ipsilateral phrenic nerve activity in a C2 hemisection model (Zhou and Goshgarian, 2000). Giroux et al. (1999) demonstrated that 5-HT_{1A} receptors labeled

with radioactive 8-OH-DPAT significantly increased in laminae II, III, and X of lumbar segments at 15 d after spinal cord transection. Upregulation of 5-HT_{1A} receptors was suggested to be attributable to denervation supersensitivity, specifically postsynaptic hypersensitivity in response to loss of descending input. Kinkead et al. (1998) showed that cervical dorsal rhizotomy enhanced serotonin innervation of phrenic motor neurons. Baker-Herman and Mitchell (2002) reported that respiratory long-term facilitation of phrenic amplitude (i.e., long-lasting increase in respiratory amplitude after repeated hypoxic episodes) requires spinal serotonin receptor activation. Similar studies at 7 d after contusion SCI will be needed to determine whether upregulation of 5-HT_{1A} receptors also occurs in our model and serves to explain the difference in effect of serotonin agonists administered at 1 and 7 d after SCI. However, others have reported proliferation of 5-HT-containing terminals in lamina II following chronic SCI in rats (Zhang et al., 1993), consistent with our finding full recovery of respiratory function by 35 d after SCI (Teng et al., 1999).

In summary, our data suggest that drugs that stimulate 5-HT_{1A} receptors such as 8-OH-DPAT and buspirone are effective in restoring disturbances in respiratory function after SCI, specifically, incomplete spinal cord contusion injury produced at T8. Additional data of ours also show that these drugs will reverse morphine-induced apnea and dizocilpine-induced apnea (Sahibzada et al., 1999, 2000). Others have reported that these drugs will also reverse apneustic type breathing (Lalley et al., 1994; Wilken et al., 1997). Thus, the positive effect of 5-HT_{1A} receptor agonists on disturbed respiratory function may be a general phenomenon and not limited to SCI.

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