5-Hydroxytryptophan-induced respiratory recovery after cervical spinal cord hemisection in rats

SHI-YI ZHOU AND HARRY G. GOSHGARIAN

Department of Anatomy and Cell Biology, Wayne State University,
School of Medicine, Detroit, Michigan 48201

Received 10 April 2000; accepted in final form 8 June 2000

Zhou, Shi-Yi, and Harry G. Goshgarian. 5-Hydroxytryptophan-induced respiratory recovery after cervical spinal cord hemisection in rats. J Appl Physiol 89: 1528–1536, 2000.—The present study investigates the role of serotonin in respiratory recovery after spinal cord injury. Experiments were conducted on C2 spinal cord hemisedected, anesthetized, vagotomized, paralyzed, and artificially ventilated rats in which end-tidal CO2 was monitored and maintained. Before drug administration, the phrenic nerve ipsilateral to hemisection showed no respiratory-related activity due to the disruption of the descending bulbospinal respiratory pathways by spinal cord hemisection. 5-Hydroxytryptophan (5-HTP), a serotonin precursor, was administered intravenously. 5-HTP-induced time- and dose-dependent increases in respiratory recovery in the phrenic nerve ipsilateral to hemisection. Although the 5-HTP-induced recovery was initially accompanied by an increase in activity in the contralateral phrenic nerve, suggesting an increase in descending respiratory drive, the recovery persisted well after activity in the contralateral nerve returned to predrug levels. 5-HTP-induced effects were reversed by a serotonin receptor antagonist, methysergide. Because experiments were conducted on animals subjected to C2 spinal cord hemisection, the recovery was most likely mediated by the activation of a latent respiratory pathway spared by the spinal cord injury. The results suggest that serotonin is an important neuromodulator in the unmasking of the latent respiratory pathway after spinal cord injury. In addition, the results also suggest that the maintenance of 5-HTP-induced respiratory recovery may not require a continuous enhancement of central respiratory drive.

serotonin; spinal cord injury; crossed phrenic phenomenon; respiration

HIGH CERVICAL SPINAL CORD hemisection causes ipsilateral hemidiaphragm paralysis due to a disruption of the bulbospinal respiratory pathways. Previous studies have shown that there is a latent crossed phrenic pathway that escapes spinal cord hemisection by descending into the cord contralaterally and then crossing the spinal midline before terminating on phrenic motoneurons ipsilateral to the hemisection (10, 23). This latent bulbospinal respiratory pathway can be activated by increasing central respiratory drive. Drive is increased in a spontaneously breathing animal by performing a contralateral phrenicotomy after C2 hemisection. In pancuronium-paralyzed, C2 hemisected rats, drive is increased by turning off the ventilator and inducing asphyxia. In previous studies, these conditions, under which drive is increased, have been defined as “respiratory stress” (30, 41). The activation of the latent pathway under these conditions restores lost respiratory activity in the phrenic nerve ipsilateral to the spinal cord hemisection (19, 29). Recent studies have demonstrated that theophylline, a methylxanthine adenosine receptor antagonist, also has an excitatory effect on respiratory drive and induces recovery in the phrenic nerve and hemidiaphragm ipsilateral to spinal cord hemisection (25, 26, 27).

It is well established that serotonin is an important modulator of respiration in the central nervous system (1, 2). A broad effect of serotonin is depression of central respiration. Respiratory-related activity recorded from phrenic nerves are diminished after systemic administration of certain serotoninergic agents (16, 20, 21). Nevertheless, serotonin could also have excitatory effects on phrenic motoneurones as systemic administration of 5-hydroxytryptophan (5-HTP), a serotonin precursor, significantly increases spontaneous tonic activity in the phrenic nerves of either cervical spinalized or hemisected animals (18, 22).

Several lines of evidence have suggested that serotonin could contribute to the unmasking of the latent crossed phrenic pathway after spinal cord injury. Ultrastructural studies have demonstrated a significant increase in the number of serotonergic axodendritic and axosomatic terminals in the ipsilateral phrenic nucleus after C2 spinal cord hemisection (36). Furthermore, electrical stimulation of the lateral funiculus of the spinal cord contralateral to hemisection evoked responses in the phrenic nerve ipsilateral to hemisection that were absent before 5-HTP administration (18, 22).

A recent study from this laboratory showed that 5-HTP increases the amplitude of asphyxia-induced recovery in the phrenic nerve ipsilateral to hemisection in C2 spinal cord-hemisected rats (41). However, as-
phyxia may result in uncontrolled alterations in central respiratory drive and changes in systemic blood pressure. To further investigate the role of serotonin in the unmasking of the latent crossed phrenic pathway, the present study was designed to test the hypothesis that serotonin induces respiratory recovery in the phrenic nerve ipsilateral to C2 hemisection in rats by mechanisms that do not require enhanced respiratory drive. That is, the animals were not subjected to respiratory stress, but, rather, end-tidal CO₂ was maintained at a constant level. The results demonstrate that manipulation of serotonin levels can induce and maintain recovery in the phrenic nerve after spinal cord injury without subjecting the animal to respiratory stress.

MATERIALS AND METHODS

General procedures. Experiments were performed on 27 adult female Sprague-Dawley rats (9-mo-old, retired breeders, 250–350 g; Harlan). Animal care and handling were conducted in accordance with the guidelines of the Division of Laboratory Animal Research at Wayne State University. Atropine sulfate (0.04 mg/kg body wt im) was given before anesthesia to reduce mucus secretions. The animals were anesthetized with chloral hydrate (375 mg/kg ip). A left spinal cord hemisection was made just caudal to the C2 dorsal roots, as previously described (29). Wounds were sutured, and the animals were then returned to their cages for recovery. One day after spinal hemisection, animals were once again anesthetized with chloral hydrate. The depth of anesthesia was assessed by blood pressure changes or reflex response to toe pinches. Additional doses of the anesthetic were administered throughout the physiological studies when necessary. Catheters were inserted into the left femoral artery and vein for recording arterial blood pressure and injection of drugs, respectively. Animals were paralyzed with pancuronium bromide (0.5 mg/kg, iv) and artificially ventilated. End-tidal CO₂ was monitored by a CO₂ monitor (Nor-mocap, Datex) and maintained between 25 and 32 Torr by adjusting ventilation rate (60–80 breaths/min) and tidal volume (3–5 ml). A homeothermic blanket was used to maintain the body temperature at 37 ± 1°C. Animals were bilaterally vagotomized in the midcervical region to avoid entrainment of respiratory drive to the cycle of the ventilator. Dextrose (5%) was occasionally administered intravenously to maintain blood pressure during the general surgical preparation of the animals. There was no attempt to regulate blood pressure after the 5-HTP studies were initiated.

Neural recordings. Both left (ipsilateral to spinal cord hemisection) and right (contralateral to spinal cord hemisection) phrenic nerves were exposed in the neck via a ventral approach and were transected. The central cut ends of the phrenic nerves were mounted on conventional bipolar platinum electrodes and immersed in mineral oil pools. As previously described (41), neural recordings were made monophasically. Phrenic nerve activity was band-pass filtered (bandwidth 0.1–3 kHz), amplified, and displayed on-line using a Cambridge Electronic Design 1401 data acquisition system. Signals were also fed into a videotape recorder for off-line data analysis.

Functional completeness of the hemisection. The functional completeness of the C₂ hemisection was verified in all animals. When end-tidal CO₂ ranged from 25 to 32 Torr, the right phrenic nerve showed pronounced respiratory activity. However, the left phrenic nerve typically had no discernible respiratory-related activity due to the disruption of the bulbospinal respiratory pathways after spinal hemisection. Only those animals with a complete absence of respiratory-related activity in the left phrenic nerve were selected for the experiments.

Experimental protocols. Animals were allowed at least 20 min before drug testing to obtain stable blood pressure, end-tidal CO₂, and phrenic nerve activities. Animals were treated with pargyline (25 mg/kg iv), a monoamine oxidase inhibitor to prevent the degradation of serotonin by monoamine oxidase.

Pargyline and 5-HTP were dissolved in saline solution. With all drugs, animals received 0.1 ml drug/100 g body wt, followed by a 0.3-ml saline slow flush injection over at least 30 s. In 24 rats, injection of pargyline induced no major response in the phrenic nerves. However, in three animals, pargyline enhanced respiratory-related activity in the right phrenic nerve and induced respiratory recovery in the left phrenic nerve. Because the subsequent administration of 5-HTP would have evoked responses that were in addition to those observed after pargyline, the resulting data would not be attributable to 5-HTP alone, and the experiments in these three cases were terminated. After administration of pargyline, the first group of rats (group 1, n = 15) received a single injection of 5-HTP (4.0, 2.0, 1.0, 0.5, or 0.2 mg/kg). The effects of 5-HTP on phrenic nerve activity were monitored while the animal’s physiological status was maintained in a stable condition, usually up to 120 min. Control experiments for pargyline were conducted on three additional rats that were administered pargyline only to ensure that 5-HTP-induced changes in the phrenic nerve were not related to pargyline- or time-dependent factors. Rats in the second group (group 2, n = 6) were designated to assess cumulative dose-dependent responses. 5-HTP was administered in an initial dose of 0.5 mg/kg and was increased in successive dose increments at 10-min intervals. Methysergide (4.0 mg/kg iv), an antagonist of serotonin receptors, was given in some experiments in group 1 and in all experiments in group 2.

Data analysis. For quantitative data analysis, phrenic nerve activity was rectified and integrated (time constant = 100 ms) by a moving averager (MA-821, CWE). Respiratory-related activity in each nerve was quantitatively analyzed using Spike2 software (Cambridge Electronic Design). Comparisons of changes in intensity of inspiratory-related activity before and after drug administration were made in each nerve, as described previously (41). Briefly, the intensity was estimated by determining the area under the integrated curve after subtracting background activity (noise + spontaneous tonic activity). The background activity level was first determined by measuring activity during the expiratory phases. Background activity during the inspiratory phase was estimated by extrapolating a line between the adjacent background activity level in the expiratory phases. The total area of five consecutive inspiratory-related bursts was then measured by the computer after setting the cursors between the onset of the first burst and the end of the fifth burst. From this value, background activity was subtracted. Phrenic nerve activity and respiratory recovery were expressed as a percentage of the predrug value of respiratory activity in the right phrenic nerve. Values in the text are expressed as mean ± SE. Wilcoxon and Mann-Whitney’s rank sum tests were performed to assess significant differences, and the significance level was set at P < 0.05.
RESULTS

Time-dependent responses of phrenic nerve activity to 5-HTP. Before drug administration, the right phrenic nerve showed pronounced respiratory-related activity, but the left phrenic nerve had no respiratory-related activity due to the disruption of the descending bulbo-spinal respiratory pathways by spinal cord hemisection (Fig. 1A).

To find an effective dose at which recovery would persist in the left phrenic nerve during the experimental period (2 h), single doses of 5-HTP (0.2–4.0 mg/kg) were administered to 15 pargyline-treated animals (Table 1). After 5-HTP administration, respiratory activity in the right phrenic nerve was initially enhanced as respiratory recovery in the left phrenic nerve occurred. Maximal respiratory recovery (RR_max), onset (time) of respiratory recovery, and time of RR_max in the left phrenic nerve occurred in a time- and dose-dependent manner. A clear example of the typical time-dependent changes in phrenic nerve activity at a dose of 4.0 mg/kg is shown in Fig. 1. 5-HTP initially increased respiratory activity in the right phrenic nerve and produced respiratory recovery in the left phrenic nerve. 5-HTP-induced respiratory recovery increased in both burst amplitude and duration (Fig. 1, B–E). This recovery, most likely, was not due to a drug-induced fluctuation of either respiration or blood pressure because end-tidal CO_2 was nearly constant throughout the experiment and mean arterial blood pressure varied minimally after the first few minutes. Note that the amplitude of respiratory activity in the right phrenic nerve started to decline 5–8 min after 5-HTP injection (Fig. 1, D and E), but respiratory recovery in the left phrenic nerve was sustained. In this case, 5-HTP resulted in a respiratory depression 10 min after injection (Fig. 1F).

In the two cases studied at the 2.0 mg/kg dose of 5-HTP, RR_max in the left phrenic nerve was reached at 15 and 30 min, respectively (see Table 1). At doses of 2.0–4.0 mg/kg, 5-HTP usually resulted in a depression of respiratory-related activity and the appearance of spontaneous tonic activity in both phrenic nerves in 10–40 min (Table 1). Prolonged inspiratory duration (Fig. 1E), i.e., an apneustic respiratory pattern, was usually observed immediately before respiratory depression. In cases of 5-HTP-induced respiratory depression, the left phrenic nerve usually developed more spontaneous tonic activity than the right phrenic nerve (Fig. 1F).

In the two cases of a single 1.0 mg/kg injection, 5-HTP induced respiratory recovery in the left phrenic nerve within 1 min, and recovery reached its maximum at 35 and 40 min, respectively (Table 1). Both phrenic nerves converted to spontaneous tonic activity by 90 min.

The onset of 5-HTP-induced respiratory recovery in the left phrenic nerve was temporally related to dose. At 0.5 mg/kg, 5-HTP-induced recovery was apparent within 1 min in the majority of animals tested. However, at 0.2 mg/kg, the recovery was observed from 30 to 50 min after 5-HTP administration (Table 1). More importantly, at both doses, the drug-induced changes lasted to the end of the experiment, i.e., up to 120 min. An example of the typical time-related changes in phrenic nerve activity after a single 5-HTP injection (0.5 mg/kg dose) is shown in Fig. 2. 5-HTP initially increased respiratory activity in the right phrenic nerve during the first hour after injection while pro-

Fig. 1. Effects of a single injection of 5-hydroxytryptophan (5-HTP), at a dose of 4.0 mg/kg body wt, on phrenic nerve activity in a rat subjected to a left C_3 spinal cord hemisection. A: before drug administration (predrug). Note that there was no discernable phasic activity before drug administration [end-tidal CO_2 (T_{CO_2}) = 30 Torr] in the left phrenic nerve (L-PNA), but the right phrenic nerve (R-PNA) showed pronounced respiratory activity. The animal was given pargyline (25 mg/kg iv), and a single injection of 5-HTP (4.0 mg/kg) was given 10 min after the pargyline. B–F: phrenic nerve activities at 1, 2, 5, 8, and 10 min after the 5-HTP injection, respectively. In this case, respiratory recovery in L-PNA was observed 1 min after 5-HTP administration and increased in intensity until 5 min (D). At 8 min (E), note that respiratory recovery in L-PNA was enhanced, whereas the amplitude of respiratory activity in R-PNA was decreased compared with B–D. The respiratory-related phasic activity converted to spontaneous tonic activity ~10 min after 5-HTP administration in both phrenic nerves (F). G: methysergide reversed the effects of 5-HTP on phrenic nerve activity within 5 min. BP, blood pressure; ‘, min.
5-HTP-induced respiratory recovery in hemisected rats

Table 1. 5-HTP-induced respiratory recovery in the left phrenic nerve in 15 left C2 spinal cord-hemisected rats

<table>
<thead>
<tr>
<th>Animal No</th>
<th>5-HTP mg/kg</th>
<th>T_RR,min</th>
<th>T_RRmax,min</th>
<th>T_Tonic,min</th>
<th>RR_max, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0</td>
<td>&lt;1</td>
<td>5</td>
<td>10</td>
<td>90.7</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>&lt;1</td>
<td>10</td>
<td>12</td>
<td>123.9</td>
</tr>
<tr>
<td>3</td>
<td>2.0</td>
<td>&lt;1</td>
<td>15</td>
<td>40</td>
<td>40.5</td>
</tr>
<tr>
<td>4</td>
<td>2.0</td>
<td>5</td>
<td>30</td>
<td>35</td>
<td>53.5</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>&lt;1</td>
<td>35</td>
<td>90</td>
<td>46.6</td>
</tr>
<tr>
<td>6</td>
<td>1.0</td>
<td>&lt;1</td>
<td>40</td>
<td>90</td>
<td>17.5</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>&lt;1</td>
<td>60</td>
<td></td>
<td>120.2</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>&lt;1</td>
<td>70</td>
<td></td>
<td>24.2</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>&lt;1</td>
<td>80</td>
<td></td>
<td>126.3</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>&lt;1</td>
<td>90</td>
<td></td>
<td>62.0</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>8</td>
<td>90</td>
<td></td>
<td>33.8</td>
</tr>
<tr>
<td>12</td>
<td>0.5</td>
<td>&lt;1</td>
<td>100</td>
<td></td>
<td>88.2</td>
</tr>
<tr>
<td>13</td>
<td>0.2</td>
<td>30</td>
<td>(120)</td>
<td></td>
<td>57.9</td>
</tr>
<tr>
<td>14</td>
<td>0.2</td>
<td>40</td>
<td>(120)</td>
<td></td>
<td>50.2</td>
</tr>
<tr>
<td>15</td>
<td>0.2</td>
<td>50</td>
<td>(120)</td>
<td></td>
<td>55.8</td>
</tr>
</tbody>
</table>

5-Hydroxytryptophan (5-HTP) was given as a single intravenous injection in each rat. T_RR, time when 5-HTP-induced respiratory recovery in the left phrenic nerve was observed and when it was ≥5% of predrug value for respiratory activity in the right phrenic nerve; T_RRmax, time when 5-HTP-induced respiratory recovery reached maximum value; T_Tonic, time when respiratory-related activity in both phrenic nerves converted to tonic activity; RR_max, maximal 5-HTP-induced respiratory recovery, expressed as a percentage of predrug value for respiratory activity in the right phrenic nerve. Numbers in parentheses indicate that 5-HTP-induced respiratory recovery in the left phrenic nerve was still increasing at the end of the 120-min observation period. Note that there was no respiratory depression observed for the 120-min time point in rats 7–15.

...progressively increasing respiratory recovery in the left phrenic nerve. Interestingly, the respiratory activity in the right phrenic nerve returned to predrug levels in the second hour of the study, whereas the respiratory recovery in the left phrenic nerve persisted.

RR_max after a single injection of 5-HTP, at doses of 0.2–4.0 mg/kg, ranged from 17.5 to 126.3% of the predrug values for respiratory activity in the right phrenic nerve (Table 1).

Time-dependent changes in phrenic nerve respiratory activity after a single 0.5 mg/kg injection of 5-HTP (n = 6; Table 1) are summarized in Fig. 3A. The data show the dissociation of changes of respiratory activity in the right phrenic nerve and respiratory recovery in the left phrenic nerve. During the first 60 min, 5-HTP initially increased respiratory activity in the right phrenic nerve and induced respiratory recovery in the left phrenic nerve, simultaneously. However, during the second 60 min, 5-HTP-enhanced respiratory activity in the right phrenic nerve diminished, whereas 5-HTP-induced respiratory recovery in the left phrenic nerve persisted. Blood pressure was 108 ± 8.4 mmHg before drug administration and was 87.2 ± 5.1 mmHg by 120 min after 5-HTP administration (not significant; Fig. 3B). Pargyline did not induce significant effects on respiratory recovery in the left phrenic nerve in this group of animals (Fig. 3A).

A time control for pargyline was conducted on three animals. The drug did not produce appreciable respiratory-related activity in the left phrenic nerve (<5% of predrug values for respiratory activity in the right phrenic nerve) for up to 120 min.

Dose-dependent responses of phrenic nerve activity to 5-HTP. Quantitative analysis of dose-dependent responses of the phrenic nerves to 5-HTP was conducted on six animals that received cumulative doses of 5-HTP at 10-min intervals. A qualitative example of the effects of cumulative doses of 5-HTP on phrenic nerve activity is shown in Fig. 4. Increasing doses of 5-HTP resulted in inducement of and then increases in respiratory recovery in the left phrenic nerve (Fig. 4, B–D). After a cumulative dose of 4.0 mg/kg, 5-HTP produced a depression of respiratory-related activity in the right phrenic nerve. Activity in both phrenic nerves was converted to tonic firing at this dose. Figure 5 summarizes the quantitative results. 5-HTP at 0.5, 1.0, and 2.0 mg/kg induced and enhanced respiratory recovery in the left phrenic nerve by 13.9 ± 3.3, 29.1 ± 4.7, and 62.0 ± 18.6% of the predrug value for respiratory activity in the right phrenic nerve, respectively. However, respiratory activity in the right phrenic nerve was not significantly different at 0.5 and 1.0 mg/kg 5-HTP (121.0 ± 12.6 and 113.6 ± 16.8%, respectively). Moreover, respiratory activity in the right phrenic nerve was significantly reduced (79.6 ± 16.4%) at 2.0 mg/kg 5-HTP, whereas respiratory recovery in the left phrenic nerve (62.0 ± 18.6%) remained significantly...
enhanced. Pargyline had no significant effects on activity in both phrenic nerves in this particular series of experiments (104.4 ± 4.4%).

Methysergide reversed 5-HTP effects on phrenic nerve activity. The effects of a single dose of 5-HTP or of cumulative doses of 5-HTP were reversed by systemic administration of methysergide (4.0 mg/kg), a broad-spectrum 5-HTP receptor antagonist. Examples of the reversal are shown in Figs. 1G and 4F. A summary of the quantitative analysis \( (n = 6) \) of the methysergide/5-HTP interaction is shown in Fig. 5. After methysergide, respiratory activity in the right phrenic nerve was restored to 85.9 ± 12.6% of predrug values and respiratory recovery in the left phrenic nerve was 8.7 ± 2.6% of the predrug values for respiratory activity in the right phrenic nerve. The action of methysergide on attenuation of 5-HTP-induced activity was eliminated by an additional 2.0 mg/kg of 5-HTP.

DISCUSSION

The present study tested the putative role of 5-HTP, a serotonin precursor, in the restoration of respiratory-related phrenic nerve activity in rats subjected to C₂ spinal cord hemisection. Our data demonstrate that 5-HTP can induce the recovery of respiratory-related activity in the phrenic nerve ipsilateral to hemisection. Quantitative analysis shows that 5-HTP-induced recovery is time and dose dependent and is blocked by methysergide, a serotonin receptor antagonist. These data strongly suggest the involvement of serotonin receptors in the unmasking of the latent crossed phrenic pathway.

Methodological considerations. The intensity of respiratory-related activity in the phrenic nerve was estimated by determining the area under the integrated wave and was normalized by subtracting background activity that contained spontaneous tonic activity, white noise, and potential artifacts. Because there was no respiratory-related discharge in the left phrenic nerve, except background activity, after hemisection and the absolute values provided no meaningful information, recovery data were expressed as a percentage of the predrug value of respiratory activity in the functionally active right phrenic nerve in the same animal.

To assess the temporal responses of phrenic nerve activity to 5-HTP, single doses of 5-HTP ranging from 0.2 to 4.0 mg/kg were administered to pargyline-treated animals. As a result, only 5-HTP at a dose of 0.5 mg/kg allowed a stable observation of respiratory recovery in the phrenic nerve. In six animals that received 5-HTP at doses >0.5 mg/kg, the time to initial and maximal recovery was decreased, but both phrenic nerves converted to spontaneous tonic activity within 90 min. In the three animals that received 5-HTP at a dose of 0.2 mg/kg, the time to initial recovery was longer, and 5-HTP-induced respiratory recovery in the left phrenic nerve was still increasing at the end of the 120-min observation period.

In the study involving single doses of 5-HTP, right phrenic nerve activity reached a plateau in 10 min after drug administration, as seen in Fig. 1A. These results are consistent with those of Mitchell et al. (22), who suggested that the maximal effect of 5-HTP on phrenic nerve activity occurs in ~10 min. In the study involving dose-dependent responses of the phrenic nerves, 5-HTP was administered at 10-min intervals, which allow the maximal effect of 5-HTP on the central respiratory drive.

Because pargyline is a monoamine oxidase inhibitor, the interaction of pargyline with endogenous serotonin may have also contributed to changes in respiratory activity. Although pargyline did not produce major effects on phrenic nerve activity in most animals, pargyline enhanced respiratory-related activity in the right phrenic nerve and induced respiratory recovery in the left phrenic nerve in a few cases \( (n = 3) \). It is possible that pargyline interaction may also alter phrenic nerve activity in response to successive 5-HTP doses.

Effects of 5-HTP on the central respiratory network. The latent crossed phrenic pathway can be activated by enhancing central respiratory drive during respiratory stress (19, 23) or by local stimulation of the medullary...
chemoreceptor center (40). Systemic administration of theophylline, an adenosine receptor antagonist, produces respiratory recovery in a similar animal model of spinal cord injury (25–27). Because the excitatory effect of theophylline on respiration is mediated at the brain stem level (6), respiratory recovery after theophylline administration in hemisected rats is most likely due to its central effect of increasing descending respiratory drive (25). Thus the above studies confirm that the latent crossed phrenic pathway is activated under conditions that enhance descending respiratory drive.

Previous studies have provided evidence for the involvement of serotonin receptors in the excitation of respiratory neurons at the medullary level to produce a prolonged increase of respiratory activity (5, 21, 24). The results in the present study are consistent with these studies, because respiratory activity was initially increased after 5-HTP administration. Consequently, 5-HTP-induced respiratory recovery is likely due to enhanced descending respiratory drive, mediated by the modulation of serotonin receptors that excite the central respiratory network.

There were biphasic effects on the central respiratory network when high doses of 5-HTP were given systemically in pargyline-treated rats in the present study. For example, 5-HTP administration, at a dose of 4.0 mg/kg, initially stimulated respiration and then depressed respiratory activity within 10 min (see Fig. 1). The respiratory-related phasic activity converted to spontaneous tonic activity in both phrenic nerves when high doses of 5-HTP were injected. Our results are consistent with other studies, which report that intravenous administration of 5-HTP at higher doses induces a prolonged respiratory inhibition in the cat (20, 21). 5-HTP-converted tonic activity could be maintained as long as 4 h before it returned to normal respiratory phasic activity (20).

A study by Richmonds and Hudgel (31) demonstrated a 5-HTP-induced respiratory depression rather than depression in urethane-anesthetized rats. In that study, a dose-dependent increase of phrenic nerve activity reached its peak at a cumulative dose of 4.0 mg/kg. This is clearly different from the results of the present study, and many factors may account for the differences. First, the use of pargyline in the present study may induce a hypersensitive response of the phrenic nerve to 5-HTP. Pargyline was not used in the study by Richmonds and Hudgel. In addition, Richmonds and Hudgel observed a dramatic depression in blood pressure in their study, an observation that has also been reported by other investigators (18, 21). It is likely that the results reported by Richmonds and Hudgel may be directly related to hypotension; Kinkead and Mitchell (15) have suggested that hypo-

![Fig. 4. Dose-dependent effects of 5-HTP on phrenic nerve activity in a rat subjected to left C2 spinal cord hemisection. Upper traces, activity in R-PNA; lower traces, activity in L-PNA. A: phrenic nerve activity before 5-HTP administration. B–E: changes in phrenic nerve activity after systemic administration of cumulative doses of 5-HTP (0.5, 1.0, 2.0, and 4.0 mg/kg, respectively). Phrenic nerve activities were recorded 10 min after each 5-HTP administration. Note that in D, 5-HTP-induced respiratory recovery was enhanced in L-PNA, whereas the amplitude of respiratory activity in R-PNA decreased. Respiratory phasic activity was converted to tonic activity at a cumulative dose of 4.0 mg/kg in E and was restored after administration of methysergide (4.0 mg/kg body wt) in F (see Methysergide reversed 5-HTP effects on phrenic nerve activity for details).

![Fig. 5. Bar chart showing the effects of cumulative doses of 5-HTP on both phrenic nerves in C2 hemisected animals (n = 6). Pargyline was administered 10 min before 5-HTP. Cumulative doses of 5-HTP were administrated at 10-min intervals. Note that there are dose-dependent changes of 5-HTP-induced respiratory recovery in L-PNA. At doses of 4.0 mg/kg, 5-HTP disrupted respiratory-related activity. The effects of 5-HTP were reversed by methysergide. Pargyline had no significant effect on activity in either nerve. *Significantly different from predrug values in each nerve (P < 0.05).}
tension may lead to the facilitation of phrenic nerve responses. It is noteworthy that, in the present study, a small dose of 5-HTP did not significantly change blood pressure. Finally, it must be pointed out that the type of anesthetic and surgical preparation may have also contributed to the differences seen in the 5-HTP-induced responses in phrenic nerve activity of our study and that of Richards and Hudgel (31).

Interestingly, results from the present study demonstrate that the intensity of respiratory activity in the right phrenic nerve, which was used as an indicator of central respiratory drive, was decreased in the second hour after a single injection of 5-HTP (0.5 mg/kg; Fig. 3A). At this time, 5-HTP-induced respiratory recovery in the left phrenic nerve was not attenuated. Moreover, in cases of administration of a cumulative dose of 2.0 mg/kg, 5-HTP-induced respiratory recovery in the left phrenic nerve either persisted or increased when activity in the right phrenic nerve decreased (Fig. 5). The present data suggest that the initial activation of the crossed phrenic pathway by 5-HTP may be related to enhanced central respiratory drive. However, the present data also show that there is subsequent dissociation in the drug-induced activity of the right phrenic nerve (an indicator of central respiratory drive) and the left phrenic nerve (an index of activation of the latent respiratory pathway). It can, therefore, be inferred that maintenance of 5-HTP-induced recovery may be mediated by factors other than enhanced central respiratory drive.

Effects of 5-HTP at the spinal level. Results from the present study are consistent with other observations of spontaneous tonic activity developing in both phrenic nerves after a large dose of 5-HTP (18, 22), suggesting excitatory effects on respiratory motoneurons by activation of serotonin receptors at the spinal level. Because the results of the present study suggest that maintaining 5-HTP-induced respiratory recovery does not have to rely on the enhancement of central respiratory drive, we further hypothesize that excitatory modulation of serotonin at the spinal cord level may play an important role in maintaining 5-HTP-induced respiratory recovery. This hypothesis is supported by several lines of evidence. It is well known that serotonin receptor agonists increase excitability of spinal motoneurons (38) as well as phrenic motoneurons (17). Local application of 5-HTP to phrenic nuclei increases the peak amplitude of respiratory activity in the phrenic nerves (35). One of our previous studies demonstrated different effects of 5-HTP on phrenic nerve activity during respiratory stress (41). Specifically, 5-HTP enhanced activity in the phrenic nerve ipsilateral to hemisection but not in the phrenic nerve contralateral to hemisection. Because excitation of the central respiratory network should result in an enhancement of respiratory activity in both phrenic nerves (for further discussion, see Ref. 41), it is possible that excitatory effects induced by 5-HTP at the spinal cord level may explain this previous result.

Both the present study and the study by Ling et al. (18) show that, after systemic administration of 5-HTP, there are differences in the degree of facilitation of tonic activity in the phrenic nerves ipsilateral and contralateral to hemisection. The phrenic nerve ipsilateral to hemisection converted to more spontaneous tonic activity than did the phrenic nerve contralateral to hemisection (e.g., Figs. 1F and 4E). Hemisection-induced facilitation of spontaneous tonic activity in the ipsilateral phrenic nerve after 5-HTP administration has been explained by the interruption of a descending inhibitory pathway after spinal cord hemisection (18, 22). That is, phrenic motoneurons ipsilateral and caudal to spinal cord injury have less descending inhibitory inputs and become more excitable in response to serotonin than phrenic motoneurons contralateral to spinal cord injury. This may also explain why 5-HTP-induced respiratory recovery is facilitated in the left phrenic nerve, whereas the amplitude of respiratory activity in the right phrenic nerve is decreased. It appears that 5-HTP-induced facilitation may override the depression of central respiratory drive on phrenic motoneurons ipsilateral to hemisection.

Effects of 5-HTP on different types of serotonin receptors. 5-HTP is a serotonin precursor that has a broad, nonselective effect on serotonin receptors. The results of the present study may be due to the drug’s influence on multiple serotonin receptor subtypes (1, 9). Several investigations have suggested that different types of serotonin receptors, at different levels of the respiratory pathway, are responsible for the diverse effects of serotonin. In in vitro preparations, it has been shown that serotonin-induced excitatory effects of phrenic motoneurons are likely to be mediated by 5-HT2 (17, 24), perhaps by 5-HT2A receptors (13). Other investigations suggest that 5-HT1A receptors may play a role in the excitatory modulation of respiration but at the supraspinal level (5, 13). However, 5-HT1A receptors may also contribute to a central depression, because respiratory depression was observed after local application of the 5-HT1A receptor agonist 8-hydroxy-dipropylaminotetralin into the pre-Bötzing complex, a region that is essential for respiratory rhythm (32). 5-HT1B receptors may have no effects on tonic activity (24) but may depress respiratory-related activity at the spinal level (4, 5). In addition, a recent study suggested that 5-HT2C receptors are probably responsible for central depression of respiration, because a 5-HT2C agonist induced a decrease in the respiratory rate in the newborn rat (30).

In addition, 5-HT1A or 5-HT2A receptors are involved in either inhibitory or excitatory modulation of spinal axons (33). Several lines of evidence suggest that there are serotonin autoreceptors in peripheral and central axon terminals (12, 37). Selective activation of 5-HT1A or 5-HT2A receptor subtypes on spinal dorsal column axons results in either inhibitory or excitatory effects, respectively (33). Whether there are serotonin receptors on the axons of the crossed phrenic pathway is not yet known. However, the demonstration of the existence of such receptors on crossed phrenic axons in future experiments could be useful in explaining the 5-HTP-induced unmasking of the latent crossed.
phrenic pathway that leads to respiratory recovery in the C2 spinal hemisected rat.

Systemic administration of 5-HTP will only provide information on the total effects of the drug on the respiratory system. Additional studies, using more selective agonists and antagonists injected into either spinal or medullary centers, must be conducted before specific mechanisms can be proposed. Also, the present results do not exclude a possible role for interaction of serotonin with other neuromodulators; it has been found that serotonergic agents cause the release of other neuromodulators, such as dopamine (28), and block the excitatory effects of substance P (14).

Clinical implications. Serotonin is an important neuromodulator that is involved in the recovery of locomotor function after spinal cord injury (3, 7, 11, 34). The present study is the first to report respiratory recovery after administration of 5-HTP to rats with spinal cord injury. One of the major, life-threatening consequences of high cervical spinal cord injury in humans is interruption of the brain stem bulborespiratory pathways, which leads to paresis of the diaphragm and respiratory stress. Previous studies have demonstrated that the latent crossed respiratory pathway can be activated by increasing central respiratory drive in the rat (19, 26, 27, 29) and probably in humans as well (8). The present study suggests that modulation of serotonin levels can produce respiratory recovery without increasing central respiratory drive. Thus recovery may be achieved therapeutically, without the risk of respiratory motoneuron fatigue or stress. This approach could potentially lead to pharmacological treatments that may improve respiratory function in cervical spinal cord injury patients.

We thank Dr. K. D. Nantwi for reading and helpful discussion of this manuscript. This work was supported by National Institute of Child Health and Human Development Grant HD-31550 (to H. G. Goshgarian).

REFERENCES


