Serotonin$_2$ receptors mediate respiratory recovery after cervical spinal cord hemisection in adult rats

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Zhou, Shi-Yi, Gregory J. Basura, and Harry G. Goshgarian. Serotonin$_2$ receptors mediate respiratory recovery after cervical spinal cord hemisection in adult rats. J Appl Physiol 91: 2665–2673, 2001.—The aim of the present study was to specifically investigate the involvement of serotonin [5-hydroxytryptamine (5-HT$_2$)] receptors in 5-HT-mediated respiratory recovery after cervical hemisection. Experiments were conducted on C$_2$ spinal cord-hemisected, anesthetized (chloral hydrate, 400 mg/kg ip), vagotomized, pancuronium-paralyzed, and artificially ventilated female Sprague-Dawley rats in which CO$_2$ levels were monitored and maintained. Twenty-four hours after spinal hemisection, the ipsilateral phrenic nerve displayed no respiratory-related activity indicative of a functionally complete hemisection. Intravenous administration of the 5-HT$_2A$/2C-receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) induced respiratory-related activity in the phrenic nerve ipsilateral to hemisection under conditions in which CO$_2$ was maintained at constant levels and augmented the activity induced under conditions of hypercapnia. The effects of DOI were found to be dose dependent, and the recovery of activity could be augmented under conditions of hypercapnia. DOI-induced recovery was attenuated by the 5-HT$_2$-receptor antagonist ketanserin but not with the 5-HT$_2C$-receptor antagonist RS-102221, suggesting that 5-HT$_2A$ and not necessarily 5-HT$_2C$ receptors may be involved in the induction of respiratory recovery after cervical spinal cord injury.

CERVICAL SPINAL CORD HEMISECTION at the C$_2$ level disrupts brain stem bulbospinal impulses from the rostral ventral respiratory group to the phrenic motoneurons (PMNs) located in the ventral horns at midcervical levels (4, 10). This type of lesion interrupts normal respiratory drive to the PMNs, resulting in a quiescent ipsilateral phrenic nerve and paralysis of the ipsilateral hemidiaphragm. Anatomically, descending fibers from the rostral ventral respiratory group make ipsilateral, contralateral, and bilateral axonal connections with PMNs (8, 9), yet immediately after cervical hemisection, the spinal decussating axons cannot depolarize PMNs (9). Within hours after hemisection, however, plasticity converts these functionally ineffective synapses to functionally latent connections in that they do not restore reflex activity under normal conditions. The latent connections become functionally effective only after contralateral phrenicotomy or under conditions of asphyxia induced several hours after spinal hemisection. The asphyxia results in functional recovery of the hemidiaphragm paralyzed by spinal cord injury (9). The connections form the “crossed phrenic pathway” (CPP) and escape injury by descending in the spinal cord contralateral to the lesion before crossing the midline to innervate PMNs ipsilateral and caudal to the C$_2$ hemisection site (8, 9, 26). The recovered respiratory-related activity in the initially quiescent phrenic nerve under the above conditions has been referred to as “crossed phrenic nerve activity” (CPNA) (29).

Whereas the complete mechanisms driving CPNA are not currently known, evidence from our laboratory suggests that it may be mediated, in part, by serotonin [5-hydroxytryptamine (5-HT)] neurotransmission (11, 39, 40). Serotonin-containing fibers have been anatomically identified to project to, and distribute terminals near, PMNs (3, 16, 17, 31, 39). Interestingly, after C$_2$ hemisection, the 5-HT afferents demonstrate elements of plasticity evidenced by increases in axodendritic and axosomatic terminals within the ipsilateral phrenic nucleus (37). The plasticity may enhance CPNA and thus contribute to recovery of the paralyzed hemidiaphragm. Physiologically, reduction of 5-HT with the 5-HT synthesis inhibitor p-chlorophenylalanine before cervical hemisection attenuates the normal asphyxia-induced CPNA, suggesting that the recovery is dependent on sufficient levels of 5-HT (11). This hypothesis was recently supported in our laboratory with data demonstrating that administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) induced CPNA when CO$_2$ levels were maintained and augmented the asphyxia-induced CPNA in rats after cervical hemisection (40, 41). The increases were prevented with the general 5-HT-receptor antagonist methysergide, suggesting that 5-HT has the relevant characteristics to induce CPNA after cervical spinal cord hemisection. Given the broad-spectrum affinity of methysergide for serotonin$_2$ receptors and the endogenous distribution of serotonin$_2$ receptors mediating respiratory recovery after cervical spinal cord hemisection, future studies elucidating the role of serotonin$_2$ receptors in CPNA would provide important insights into the neural mechanisms responsible for respiratory recovery after spinal cord injury.
multiple 5-HT receptors, the present study was designed to specifically target 5-HT_2 receptors as a potential mechanism mediating hemidiaphragm recovery after cervical spinal cord hemisection. To investigate this, phrenic nerve activity in hemisectioned rats was monitored after the administration of the 5-HT(2A/2C)-receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), in the presence or absence of 5-HT-2a (ketanserin) and 5-HT-2c (RS-102221) receptor antagonists.

MATERIALS AND METHODS

Surgical procedures. Adult female Sprague-Dawley rats (n = 27; 250–350 g; Harlan) were used in these studies in accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and institutional approval by Wayne State University Animal Investigation Committee. Rats were randomly assigned to groups and injected with atropine sulfate (0.1 mg/kg im) 10 min before being anesthetized with 4% chloral hydrate (400 mg/kg ip). Animals were then prepared for cervical hemisection. After a dorsal midline incision in the neck, the paravertebral muscles were retracted, and a laminectomy of the second cervical vertebra was performed. The cervical spinal cord was exposed after a small incision of the dura mater and arachnoid. With the use of microscissors, the spinal cord was hemisectioned just caudal to the left dorsal root at C2. The muscle layers were sutured using interrupted silk, wound clips were utilized to close the skin incision, and the antibiotic Betadine was applied to the incision site. One day after surgery, animals were again pretreated with atropine and anesthetized as described above. Catheters were inserted into the left femoral artery and vein for recording arterial blood pressure (pressure monitor BP-1, World Precision Instruments) and intravenous drug administration, respectively. After insertion of a tracheal cannula, animals were paralyzed with pancuronium bromide (0.5 mg/kg iv) and then artificially ventilated with room air by a small-animal ventilator (Harvard Apparatus rodent ventilator). CO2 levels were recorded using a CO2 monitor (Datex, Normocap). A homeothermic blanket control unit (Harvard) was used to maintain the body temperature at 37 ± 1°C. Animals were bilaterally vagotomized to avoid locking respiratory drive to the cycle of the ventilator.

Neural recordings. Both left (ipsilateral to hemisection) and right (contralateral to hemisection) phrenic nerves were exposed in the neck via a ventral approach. The nerves were isolated, desheathed, and transected. A suture was passed through the cut end of the central stump of the phrenic nerve. Neural recordings were made monophasically with the suture tied to the caudal pole and the central nerve stump contacting the rostral pole of platinum bipolar electrodes. This procedure was adopted to minimize movement of the nerve during recording, especially as predrug and postdrug quantitative comparisons of nerve activity were conducted. Neural activity was filtered (bandwidth: 0.1–3 kHz), amplified (520 Tektronix, gain 5–20 K), and displayed on-line using a Cambridge Electronic Design (CED) 1401 data-acquisition system. Signals were also fed into a videotape recorder for off-line data analysis with Spike 2 (CED) software.

Experimental protocols. The functional completeness of each hemisection was verified first. After a left spinal cord hemisection at C2, the right phrenic nerve shows inspiratory-related activity. The left phrenic nerve, however, typically shows no respiratory-related activity because of the disruption of the bulbospinal respiratory pathways after cervical hemisection. Hemisections were considered functionally complete if the ipsilateral phrenic nerve showed a complete absence of respiratory-related activity. Only those animals showing a functionally complete hemisection were selected for the experiments. In the experiments designed to assess drug effects under conditions in which CO2 levels were monitored and maintained, CO2 levels were maintained by adjusting the rate (60–80 breaths/min) or stroke volume (3–5 ml) of the ventilator.

Other experiments were designed to assess drug effects during asphyxia. Briefly, the ventilation rate was lowered to increase CO2 levels >35 Torr for 60 s, and then asphyxia (hypoxia and hypercapnia) was induced by turning off the ventilator. During asphyxia, increasing respiratory-related activity in the right phrenic nerve reached a maximum just before the activity terminated. The ventilator was turned back on a few seconds after burst activity terminated to resuscitate the animal. In addition, CPNA was observed in the left phrenic nerve. Experiments were carried out for 5-HT-2-receptor effects only in those animals that had similar CPNA results from at least two respiratory stress tests, separated by a 15-min interval. During drug testing, asphyxia was induced 5 min after each drug administration, and the animal was then allowed to recover for at least 5 min before the next drug administration.

Drug treatments. All drug treatments were administered systemically (via the femoral vein) to rats that received left cervical (C2) spinal hemisections 24 h earlier. All compounds were obtained from Tocris Cookson and included the 5-HT-2A/2C-receptor agonist DOI, the 5-HT-2-receptor antagonist ketanserin, the 5-HT-2C-receptor antagonist RS-102221, the 5-HT-2C-receptor agonist MK-212 hydrochloride, and the general 5-HT-receptor antagonist methysergide. To test the effects of 5-HT-2A/2C-receptor stimulation on CPNA during asphyxia, DOI (0.2 mg/kg iv) was infused 5 min after asphyxia-induced CPNA. CPNA was assessed 5 min after DOI infusion, followed by administration of methysergide (4.0 mg/kg). The effects of methysergide on the DOI-induced CPNA in asphyxic rats were then recorded 5 min later.

In the group of animals designated to assess dose-dependent effects (CO2 is maintained), DOI was given in initial doses of 0.05 mg/kg and increased in successive dose increments at 15-min intervals, resulting in cumulative doses of 0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg. Phrenic nerve activity was recorded from both nerves at each dose. To assess the temporal maintenance of respiratory recovery after DOI administration, phrenic nerve activity was monitored 1, 3, 5, 15, and 25 min after a single dose of DOI (0.2 mg/kg). A final time point was recorded 2 h after DOI administration (data not shown). From the DOI dose-response data, the dose (0.2 mg/kg) was chosen based on its ability to induce phrenic motor recovery effectively.

To discriminate the effects of DOI from 5-HT-2A and/or 5-HT-2C receptors, the antagonists ketanserin (5-HT-2A) and RS-102221 (5-HT-2C) were utilized under conditions in which CO2 levels were monitored and maintained. CPNA was assessed 5 min after a single injection of DOI (1.0 mg/kg). Ketanserin (2.0 mg/kg dose; Ref. 19) or RS-102221 (2.0 mg/kg dose; Ref. 2) was subsequently infused, and the effects on DOI-mediated CPNA were recorded 5 min later. To counteract the adverse effects of ketanserin on blood pressure, epinephrine (1:5,000) was administered. In a separate group of animals, ketanserin was also administered to rats before DOI injection.

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Last, to assess the impact of 5-HT_{2A/C}-receptor stimulation on intact respiratory function, MK-212 (0.1 mg/kg) was administered, and phrenic nerve activity was recorded 3, 5, 10, and 50 min later. The dose utilized (0.1 mg/kg) was chosen through preliminary dose-response experiments in the laboratory before the study, which showed that any dose administered >0.1 mg/kg shut down respiratory activity. It should be noted that no animal in the study received postsurgical analgesia. One might argue that pain-induced stress after surgery may affect spinal synaptic pathways and is responsible for some of the observed results of the study. This is not likely because predrug phrenic nerve recordings, taken under general anesthesia in this study, showed no apparent pain-induced respiratory activity (see Results).

Statistical analysis. Filtered nerve activity was rectified and integrated (time constant, 100 ms) by a moving averager (MA-821, CWE) and then quantitatively analyzed using the Spike2 (CED) software. For evaluation of the effect of chemical injection on phrenic nerve activity, comparisons of percent changes in intensity of inspiratory-related activity before and after drug administration were made in each nerve. The intensity was estimated by determining the area under the integrated curve after subtracting background activity (noise plus spontaneous tonic activity). 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 areas of the last five consecutive inspiratory-related bursts (gasps) during asphyxia were then measured automatically by the computer software after the cursors was set between the onset of the first burst and the termination of the fifth burst. The last five bursts before the onset of apnea were usually the largest. From this value (total area between the cursors), background activity was subtracted. Measurements of nerve activity are expressed as a percent change from either predrug, intact right phrenic nerve activity (R-PNA) or as a percentage of the respective asphyxia-induced maximal nerve response.

Values in the text are expressed as means ± SE. For multiple-group comparisons involving dose response and 5-HT_{2A/C}-receptor antagonists, as well as analysis of respiratory frequency (bursts/min) and blood pressure changes, a repeated-measures ANOVA was conducted to assess the overall effects of drug treatments with F ratios reported (GB-STAT for MS Windows, version 5.4). Individual comparisons between groups were subsequently conducted using a Fisher least significant difference post hoc analysis (significance at *P* < 0.05). The present study utilized area under the curve (AUC) analysis as a measure of motor activity instead of conventional peak amplitudes of the phrenic nerve bursts, because AUC analysis is a more direct measure of the entire phrenic nerve motor output profile, whereas amplitude analysis alone only measures a single component of motor activity. In fact, previous work from our laboratory utilizing both measures found no significant differences in conclusions or outcome between amplitude alone or AUC analysis (11). Eldridge (6) showed that phrenic nerve measures using the “moving average” yielded values with the same form and meaning as the episodic true integration, concluding that the moving average can provide a good index of changes in respiratory and/or phrenic nerve output and/or function.

RESULTS

Effects of DOI on CPNA during asphyxia. Neurogram tracings in Fig. 1 are qualitative but are representative of similar results obtained from five animals. Specifically, Fig. 1 shows the influence of 5-HT_{2A/C}-receptor stimulation with DOI on CPNA induced by temporary asphyxia 24 h after C_2 spinal cord hemisection. Figure 1A shows that there was no obvious respiratory-related activity in the left (ipsilateral to hemisection) phrenic nerve before drug administration, indicative of a functionally complete cervical spinal hemisection. After cessation of mechanical ventilator support to induce asphyxia, and before drug administration, CPNA in the left phrenic nerve was induced (Fig. 1B). Five minutes after systemic administration of DOI (0.2 mg/kg), the asphyxia-induced CPNA in the left phrenic nerve is apparently augmented to levels
above that induced by temporary asphyxia alone (Fig. 1C). The DOI-enhanced CPNA under conditions of asphyxia was blocked 5 min after infusion of the general 5-HT-receptor antagonist methysergide (4.0 mg/kg; Fig. 1D). Whereas this paradigm utilizing repetitive bouts of asphyxia may be perceived to alter subsequent respiratory responses (e.g., tachyphylaxis), previously published data from our laboratory yielded reproducible results under a similar asphyxia-based paradigm (41).

Effects of DOI on CPNA without asphyxia. Figure 2 demonstrates the effects of systemic DOI administration on respiratory activity in a rat in which CO₂ levels were monitored and maintained at a constant level. Figure 2 exemplifies the dose-dependent effects of systemic DOI on phrenic nerve activity in rats after C₂ spinal cord hemisection. Predrug baseline demonstrates pronounced respiratory-related activity in the right phrenic nerve and an absence of respiratory-related activity in the left phrenic nerve, indicative of a functionally complete cervical spinal cord hemisection. DOI at cumulative doses of 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/kg induced and gradually increased CPNA in the left phrenic nerve under conditions of maintained CO₂ levels. At the higher doses (1.0–2.0 mg/kg), DOI did not significantly alter burst amplitude but increased burst rate activity (bursts/min) noticeably in the right, contralateral phrenic nerve compared with predrug controls (Figs. 2 and 3). Interestingly, DOI-induced respiratory recovery in the left nerve was sustained after a single injection (Fig. 3). Figure 3F demonstrates that, after a single administration of DOI (0.2 mg/kg), CPNA is maintained up to 25 min later. In fact, DOI-induced CPNA in the left phrenic nerve persisted for up to 2 h after a single administration (data not shown).

Effects of DOI and 5-HT₂A₂C-receptor antagonists on respiratory recovery. Figures 4 and 5 demonstrate the results of DOI-induced CPNA under conditions of maintained CO₂ in the presence or absence of antagonist compounds for both 5-HT₂ (ketanserin) and 5-HT₂C receptors (RS-102221). Figure 4 qualitatively demonstrates neurogram tracings for each group, whereas a quantitative summary of the results is shown in Fig. 5. Predrug analysis demonstrates a complete absence of respiratory-related activity in the left phrenic nerve, once again indicative of a functionally complete cervical spinal hemisection (Fig. 4A).
minutes after a single intravenous injection of DOI (1.0 mg/kg), CPNA is detected in the left phrenic nerve (Fig. 4B). Subsequent administration of the 5-HT$_{2c}$-receptor antagonist RS-102221 (2.0 mg/kg) appeared to decrease qualitatively respiratory burst rate induced by DOI but did not quantitatively alter the DOI-induced CPNA significantly (Fig. 4C and Table 1). Conversely, 5 min after a single dose of the 5-HT$_{2a}$-receptor antagonist ketanserin (2.0 mg/kg), the DOI-mediated CPNA was significantly attenuated but not completely abolished (Fig. 4D). These burst-amplitude data are consistent with the effects of DOI and antagonists on burst rate (bursts/min; Table 1). Specifically, DOI at all doses significantly increased respiratory rate (range: 63.3 ± 4.5 to 74.8 ± 6.2 bursts/min) compared with predrug controls (44.3 ± 4.8 bursts/min; Table 1). Interestingly, injection of RS-102221 did not significantly decrease the DOI-mediated increases in respiratory rate (64.1 ± 6.7 bursts/min); however, ketanserin reduced the DOI-evoked increases in respiratory rate to levels not significantly different from that of predrug controls (52.4 ± 4.4 bursts/min). It should also be noted that, whereas RS-102221 appeared to reduce blood pressure in Fig. 4C, Table 1 demonstrates no significant quantitative changes in blood pressure. Conversely, because ketanserin alone significantly lowers blood pressure (70.3 ± 4.6 mmHg; Table 1), epinephrine (1:5,000) was also given to rule out the possible effects of blood pressure on the attenuation of DOI-mediated CPNA. Figure 4E demonstrates that the addition of epinephrine, while normalizing blood pressure, did not significantly alter the ketanserin-mediated attenuation of the DOI-induced CPNA from that of ketanserin alone. For comparative purposes, Fig. 4F demonstrates the CPNA induced by asphyxia before any drug administration. Note that elevation of BP after epinephrine did not affect ketanserin-induced or ketanserin-induced attenuation of DOI-mediated respiratory recovery in the left phrenic nerve. For comparative purposes, phrenic nerve activity is shown during predrug asphyxia in which the ventilator is turned off and CO$_2$ levels are not maintained. Figure 5 quantitatively summarizes the dose-dependent effects of DOI as well as the influences of 5-HT$_{2a/2c}$-receptor antagonism on respiratory activity in both phrenic nerves. Figure 5A summarizes the data as a percentage of predrug R-PNA under conditions in which CO$_2$ levels were maintained. Data are expressed as a percentage of the intact contralateral (right) nerve, because predrug respiratory activity in the ipsilateral (left) phrenic nerve is zero. Asterisks show significantly different levels compared with predrug controls (52.4 ± 4.4 bursts/min). It should also be noted that, whereas RS-102221 appeared to reduce blood pressure in Fig. 4C, Table 1 demonstrates no significant quantitative changes in blood pressure. Conversely, because ketanserin alone significantly lowers blood pressure (70.3 ± 4.6 mmHg; Table 1), epinephrine (1:5,000) was also given to rule out the possible effects of blood pressure on the attenuation of DOI-mediated CPNA.
Fig. 5. Summary of quantitative data. A: phrenic nerve responses expressed as a percentage of predrug values from the right phrenic nerve under conditions in which CO2 levels were maintained. *Significant difference from predrug right phrenic nerve activity (R-PNA) (P < 0.05). Data are expressed as a percentage of the intact contralateral (right) nerve, because prepregnancy respiratory activity in the ipsilateral phrenic nerve is zero. DOI-induced significant increases in left phrenic nerve activity (L-PNA) at all doses. At the higher doses (0.2–2.0 mg/kg), DOI-induced recovery in the left phrenic nerve was not significantly different from asphyxia-induced recovery in the same nerve. DOI-induced recovery at 0.5–2.0 mg/kg was significantly attenuated by ketanserin (°significantly different compared with DOI + ketanserin) but not by RS-102221. Note that no drug treatment altered the R-PNA compared with predrug controls, with the exception of asphyxia-induced increases. B: phrenic nerve responses recorded under conditions in which CO2 levels were maintained expressed as a percentage of the respective maximal respiratory response in each nerve to assess what percentage of the maximal effect is elicited after 5-HT1A receptor stimulation. By setting the asphyxic response as maximum (100%) CPNA, activity in the left nerve predrug (1.0 ± 0.9%) or after DOI doses at 0.05 mg/kg (24.1 ± 2.8%) and at 0.1 mg/kg (31.9 ± 6.7%) was significantly less than asphyxia-induced activity in the respective left control nerve as indicated. However, L-PNA, after DOI at 0.2, 0.5, 1.0, and 2.0 mg/kg, was not significantly different from asphyxia-induced activity in the respective left control nerve. Subsequent application of RS-102221, although trending above DOI-induced levels, did not significantly alter DOI-induced L-PNA from maximal asphyxia control levels. However, after ketanserin, DOI-induced normalization (in 0.2–2.0 mg/kg DOI groups) was significantly different and/or attenuated (17.7 ± 8.1%; compared with DOI + ketanserin) but not completely abolished. R-PNA was significantly less than respective right asphyxia-induced controls. Values are means ± SE. Statistical differences from repeated-measures ANOVA were detected and F ratios derived from repeated-measures ANOVA were compared with predrug R-PNA controls; for R-PNA compared with respective left asphyxia controls (F9,45 = 8.9; P < 0.0001); and for L-PNA compared with respective right asphyxia controls (F9,48 = 10.1; P < 0.0001).

Table 1. Mean arterial BP and respiratory frequency after drug administration

<table>
<thead>
<tr>
<th>Dose, mg/kg</th>
<th>BP, mmHg</th>
<th>Respiratory Rate, bursts/min</th>
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<tbody>
<tr>
<td>Predrug</td>
<td></td>
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<tr>
<td>0.05</td>
<td>93.5 ± 5.6*</td>
<td>44.3 ± 4.8</td>
</tr>
<tr>
<td>0.1</td>
<td>87.2 ± 7.0†</td>
<td>63.3 ± 4.5*</td>
</tr>
<tr>
<td>0.2</td>
<td>84.4 ± 5.0</td>
<td>66.9 ± 4.8*</td>
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<tr>
<td>0.5</td>
<td>87.7 ± 3.7†</td>
<td>76.0 ± 4.0*</td>
</tr>
<tr>
<td>1.0</td>
<td>91.9 ± 5.7†</td>
<td>73.5 ± 6.2†</td>
</tr>
<tr>
<td>2.0</td>
<td>92.0 ± 4.7†</td>
<td>77.1 ± 6.8†</td>
</tr>
<tr>
<td>RS-102221</td>
<td>92.7 ± 7.4†</td>
<td>64.1 ± 6.7*</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>2.0</td>
<td>70.3 ± 4.6*</td>
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Values are means ± SE. BP, blood pressure; DOI, (-)-2,5-dimethoxy-4-iodosamphetamine hydrochloride. Values were taken 10 min after drug administration. Values were statistically analyzed by using a repeated-measures ANOVA, and individual group comparisons were made by using a Fisher least significant difference post hoc analysis (P < 0.05). °Significant difference compared with predrug controls, P < 0.05. †Significantly different compared with ketanserin, P < 0.05. F ratios derived from repeated-measures ANOVA for BP (F8,50 = 3.0; P < 0.009) and for frequency (F8,49 = 7.4; P < 0.0001).
significantly less in all experimental and predrug (range: 33.7 ± 9.7 to 61.2 ± 8.6%) groups compared with respective right phrenic asphyxia-response controls.

$5-HT_{2C}$-receptor stimulation temporarily depresses respiratory activity. Figure 6 demonstrates the qualitative effects of $5-HT_{2C}$-receptor stimulation on respiratory activity at various time points after a single injection of the $5-HT_{2C}$-receptor agonist MK-212 (0.1 mg/kg). Burst amplitude in the intact, right phrenic nerve is apparently depressed at 3, 5, and 10 min after a single injection yet appeared to return to predrug levels within 50 min. However, burst rate 50 min after a single injection of MK-212 was qualitatively increased (Fig. 6). The effects of MK-212 on the quiescent left phrenic nerve after cervical hemisection are not shown because no noticeable effects could be detected.

DISCUSSION

Serotonin $2A$, but not serotonin $2C$, receptors are likely involved in respiratory recovery after cervical spinal cord hemisection. The present study demonstrates that the $5-HT_{2A/2C}$-receptor agonist DOI elicits CPNA in the ipsilateral phrenic nerve rendered quiescent after a C2 spinal cord hemisection. DOI-mediated increases in burst amplitude and respiratory rate (bursts/min) were attenuated with ketanserin and not with RS-102221. Although not absolutely conclusive, these results strongly implicate $5-HT_{2A}$- and not $5-HT_{2C}$-receptor linkage to respiratory recovery in this model. Elevated amplitude in phrenic nerve burst activity is thought to be related to an increase in tidal volume (6) and is a normal response to asphyxia (36). The present study demonstrated an increase in integrated phrenic nerve waveforms recorded from both contralateral and ipsilateral nerves during asphyxia. Qualitatively, DOI augmented burst amplitudes in the left nerve above those of asphyxia-induced levels alone, replicating previous data that suggested that increases in $5-HT$ neurotransmission may also augment asphyxia-induced CPNA (40). Functionally, the results suggest that PMNs ipsilateral to hemisection likely do not reach maximum output capacity during asphyxia, and thus there is an opportunity for $5-HT_{2}$ receptors to enhance the CPNA.

Stimulation of $5-HT_{2}$ receptors in the present study also activated CPNA under conditions in which CO2 levels were maintained. This suggests that $5-HT_{2}$ receptors do not require asphyxia-induced cues to activate the CPP. In fact, under these conditions, DOI induced CPNA <1 min after injection and maintained burst activity for up to 2 h. The DOI-mediated increases in CPNA under the above conditions matched levels induced by asphyxia, indicating that $5-HT_{2}$ receptors alone are capable of completely activating the CPP. DOI-induced CPNA is dose dependent and leads to a gradual increase in burst amplitude and frequency, both of which are attenuated by ketanserin and not RS-102221. Interestingly, at higher doses, DOI evoked prolonged inspiratory burst duration. Because the medullary raphe nuclei have postinspiratory and expiratory-related bulbospinal projections (7) to the PMNs (18), it is possible that $5-HT_{2}$ receptors associated with these bulbospinal pathways were activated. Alternatively, it is possible that $5-HT_{2}$ receptors produced a long-lasting depolarization of the PMN membrane potential, because 5-HT has been shown to induce this excitatory effect at the spinal level (23). This is very likely because our laboratory recently demonstrated that PMNs colocalize $5-HT_{2A}$- and not $5-HT_{2C}$-receptor mRNA expression (1), suggesting that PMNs are capable of $5-HT_{2A}$-receptor biosynthesis, which may be focally linked with the postsynaptic regulation
of PMN activity. Therefore, it is possible that higher doses of DOI may lead to overstimulation of 5-HT_2 receptors and PMN excitation, leading to prolonged burst activity. The notion that 5-HT_2 receptors may be regulating CPNA at the level of the spinal cord is supported by studies showing that microapplication of 5-HT into the phrenic nucleus of the rabbit increased the peak integrated amplitude of phrenic nerve activity, an effect prevented by the 5-HT-receptor antagonist methysergide (33). Moreover, excitation of PMNs, after electrical stimulation of the raphe obscurus (13–15), is blocked with methysergide (14) and more specifically with the 5-HT_2-receptor antagonists ketanserin (23) or SR-46349B (12). Together, these observations, combined with the present results, point to the likely possibility that 5-HT_2A receptors may directly regulate the excitation of PMNs at the level of the spinal cord. Stimulation of these receptors leads to an enhancement of phrenic burst amplitude.

In the present study, DOI also mediated increases in respiratory rate (bursts/min), under conditions of both maintained CO_2 levels and asphyxia. An increase in respiratory frequency during asphyxia in rats is a normal response to hypercapnia (35, 36). DOI-mediated increases in respiratory rate under conditions of maintained CO_2 and asphyxia were attenuated with ketanserin but not RS-102221. This finding also points to the possibility that 5-HT_2A receptors may also regulate CPNA at medullary respiratory centers. The likelihood is supported by previous work in which application of 5-HT (27) or 5-HTP (28) to an in vitro bath containing rat brain stem-spinal cord explant preparations from newborn rats increased respiratory-related discharge frequencies recorded from the cervical ventral roots. The increases were prevented either by reduction in 5-HT with the 5-HT synthesis inhibitor p-chlorophenylalanine, or by general 5-HT-receptor antagonism with methysergide (28). Moreover, 5-HT microinjection into the rostral ventrolateral medulla also increased the frequency of respiratory-related activity in the cervical ventral roots (5). Electrical stimulation of the nucleus raphe obscuris increased the frequency of respiratory activity recorded from the ipsilateral phrenic nerve, implicating the influence of 5-HT afferents on medullary respiratory centers that regulate respiratory rhythm (13, 25). The above observations, combined with the present results, suggest that 5-HT_2 receptors, most likely 2A, may also mediate effects at the level of the medulla after C_2 spinal cord hemisection.

It should be noted that ketanserin produces hypotension after intravenous administration. To rule out the possibility that hypotension caused the attenuation of DOI-induced CPNA, epinephrine was coadministered with ketanserin in the present study. The administration of epinephrine normalized blood pressure to levels seen before ketanserin but did not alter the depression of the DOI-induced CPNA compared with ketanserin alone. It is generally accepted that ketanserin is more selective for 5-HT_2A receptors but is a less effective blocker of 5-HT_2C receptors (21, 22, 32, 38). Thus 5-HT_2A rather than 5-HT_2C-receptor activation is most likely involved in the induction of the CPNA after C_2 spinal cord hemisection. It is noteworthy to mention that, although ketanserin is a poor 5-HT_2C-receptor antagonist, it also shows only moderate binding affinity for histamine H_1 and α_1-adrenergic-receptor binding sites, binds very weakly to dopamine receptors, and is inactive in other known neurotransmitter-receptor binding assays (21). Therefore, given the binding characteristics of ketanserin, the results of the present study strongly implicate 5-HT_2A-receptor-mediated mechanisms yet do not fully rule out the involvement of 5-HT_2C receptors. However, with the growing availability of more specific 5-HT_2A-receptor antagonists (MDL-100907), future studies may be able to discriminate more selectively 5-HT_2A-receptor-mediated mechanisms in the spinal cord.

In the present study, RS-102221, a 5-HT_2C-receptor antagonist, did not significantly attenuate either burst amplitude or rate after DOI-induced CPNA. This result, combined with a lack of colocalization of 5-HT_2C-receptor mRNA expression with PMNs (1), suggests that 5-HT_2C receptors may not be functionally linked to PMNs at the level of the spinal cord. Interestingly, specific stimulation of 5-HT_2C receptors with MK-212 temporarily depressed phrenic nerve burst amplitudes in the intact right phrenic nerve. Similar observations have been reported in which 5-HT_2C receptor stimulation depressed respiratory rate in cats (20) and in vitro brain stem preparations (30). In addition, it has recently been shown that MK-212 inhibited neurons of the nucleus tractus solitarius, an effect blocked by RS-102221 (34). Together, these observations suggest that the influence of 5-HT_2C receptors on respiratory activity may be isolated to brain stem respiratory centers with little or no direct regulation over PMNs.

**Functional importance of serotonin_2A-receptor-mediated respiratory recovery.** The present study provides strong evidence that 5-HT_2, most likely 2A and not 2C, receptors may be functionally linked to the activation of respiratory pathways that become disrupted after cervical spinal cord injury. These findings are in direct agreement with previous work that implicated 5-HT in the activation of latent CPPs (24). Electrical stimulation of the lateral funiculus, contralateral to cervical hemisection, prompted short-latency responses in the ipsilateral phrenic nerve (24). Administration of 5-HTP induced tonic activity in the phrenic nerve, as well as two long latency responses. Similar findings were recently reported from our laboratory in which 5-HTP induced CPNA ipsilateral to cervical hemisection. This effect was prevented with methysergide (40, 41). Collectively, these data suggest that 5-HT may convert ineffective CPPs in the spinal cord to effective pathways capable of inducing CPNA ipsilateral to cervical hemisection. The present data, showing increases in burst rate and amplitude, implicate excitatory 5-HT_2-receptor mechanisms, likely mediated at both medullary and spinal cord levels. Whereas the pharmacological outcomes of the present study strongly point to a 5-HT_2A-receptor-mediated mechanism, additional...
studies will be required with more specific 5-HT2A-receptor antagonists to prove that the 5-HT2A receptor alone is responsible for these effects.

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REFERENCES


