Increased spinal monoamine concentrations after chronic thoracic dorsal rhizotomy in goats

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THORACIC SPINAL SENSORY PATHWAYS are important in ventilatory control during exercise. For example, during even mild exercise, goats exhibit ventilatory failure, characterized by progressive hypercapnia and erratic ventilatory patterns, with increased dead space after bilateral thoracic dorsal rhizotomy (TDR) (26, 40). In contrast, normal goats (no TDR) typically become slightly hypocapnic during exercise, either with or without increased dead space. During subsequent exercise trials, TDR goats exhibit progressive functional recovery, regaining the ability to regulate arterial blood gases in an experience- vs. time-dependent manner. In other words, with each exercise trial, TDR goats gradually increase their exercise ventilatory response until it approximates that of normal goats. The mechanism underlying functional recovery after TDR is unknown, but it could be due to dorsal root regrowth, collateral sprouting of intact primary afferent fibers, or alternate mechanisms of plasticity at the spinal or supraspinal levels. In this study, our objective was to investigate the effects of TDR on descending modulatory (monoaminergic) systems and (peptidergic) primary afferent neurons that may play a role in the functional deficits and/or recovery. Thus HPLC and immunocytochemistry were utilized to examine changes in thoracic and cervical monoamine concentrations, including serotonin (5-HT), norepinephrine (NE), and dopamine (DA). Immunohistochemical techniques were also used to investigate changes in the distribution of a neuropeptide associated with primary afferent neurons, calcitonin gene-related peptide (CGRP).

Immunoreactive CGRP in the spinal cord is restricted to primary afferent fibers and terminals in the dorsal horn and to cell bodies of the ventral horn (4, 24, 38). Although CGRP is not present in all primary afferent neurons, it serves as an effective marker for primary afferent fibers and, therefore, provides an index of intact spinal afferent innervation. Thus immunohistochemical examination of spinal CGRP serves as an indicator of the efficacy and persistence of thoracic sensory denervation (i.e., TDR).

The monoamines 5-HT, NE, and DA have many of the requisite characteristics to mediate (spinal) plasticity in respiratory motor control (1, 3, 22). For example, Kinkead et al. (17) found that cervical dorsal rhizotomy in adult rats increases 5-HT terminal density...
in the phrenic motor nucleus and augments 5-HT-dependent long-term facilitation of phrenic motor output after episodic hypoxia. Thus sensory deafferentation of the cervical spinal cord elicits both structural and functional changes in the descending serotoninergic pathways, apparently increasing the capacity for serotoninergic modulation of phrenic motor output.

Descending projections of NE- and DA-containing neurons mirror the spinal innervation patterns of 5-HT (14), exert similar actions on spinal motoneurons (36), and influence the activity of respiratory-related neurons (1, 3). Although less is known about the potential contributions of spinal NE and DA to plasticity in respiratory motor control, it is of interest to determine the effects of spinal sensory deafferentation on each of the descending modulatory systems. Thus our goal was to determine whether changes in spinal 5-HT, DA, and NE concentrations occur after TDR, thereby providing (correlative) evidence concerning their potential involvement in functional deficits and/or recovery of the exercise ventilatory response.

HPLC was used to detect changes in 5-HT, NE, and DA concentrations in thoracic and cervical spinal cord homogenates in goats that had undergone TDR at variable times before tissue collection. In addition, we utilized immunohistochemistry to detect changes in the anatomic distribution of 5-HT and CGRP. Collectively, these goats had experienced ventilatory failure during exercise followed by progressive functional recovery (26).

We tested three specific hypotheses: 1) thoracic spinal concentrations of 5-HT, NE, and DA are increased after TDR in goats; 2) CGRP immunoreactivity remains decreased in the superficial laminae of the thoracic dorsal horn, thus providing evidence that functional recovery did not result from regrowth of primary afferent neurons; and 3) changes in monoamine concentration are also observed in functionally associated regions of the spinal cord that were not directly affected by TDR. This third hypothesis was not stated a priori but arose from our somewhat surprising experimental results. Specifically, we noted unexpected changes in 5-HT concentration within the cervical spinal segments associated with the phrenic motor nucleus in ruminants (C5–C6) (20). These studies do not conclusively demonstrate causality among functional deficits, functional recovery, and observed neurochemical alterations. Nevertheless, they demonstrate the potential for spinal monoamines to be involved in the compensatory mechanisms that underlie functional recovery after TDR. These data have been presented previously in abstract form (27, 28).

**METHODS**

**Experimental Animals**

Nine intact female and six neutered male goats were used (20–55 kg). Seven animals underwent bilateral TDR from T2 to T12. Four animals underwent the same surgical procedure, but no roots were cut (sham-operated controls), and four goats served as unoperated controls. Spinal cords were harvested 4–15 mo after TDR or sham surgeries. All goats had similar histories in our laboratory (with the exception of variable times after TDR) and had undergone similar experiences (exercise training and so forth). All experimental procedures were approved by the University of Wisconsin School of Veterinary Medicine Animal Care and Use Committee.

**Surgical Preparation**

In goats anesthesia was induced with sodium thiamylal (iv) followed by anesthesia with halothane in oxygen (0.9–1.5% halothane). After laminectomy from T2 or T3 to T12 and durotomy, all visible dorsal rootlets were sectioned (i.e., TDR) or left intact (i.e., sham-operated controls). The dura was crosscut at intervals and left open to minimize damage from postoperative spinal compression resulting from edema. The thick dorsal muscle mass was sutured closed and served as a protective barrier for the exposed spinal cord.

One day before surgery, steroid therapy began with dexamethasone (0.2 mg/kg iv). During surgery, lactated Ringer solution was continuously infused (10 ml·kg⁻¹·h⁻¹ iv), and blood gases, temperature, blood pressure, and the electrocardiograph were monitored. After surgery, progressively diminishing steroid therapy continued for ~2 wk (prednisone iv; 1 mg/kg for 3 days, 0.5 mg/kg for 3 days, 0.25 mg/kg for 3 days, and 0.25 mg/kg as needed every other day). Both TDR and sham-operated goats were treated identically in their pre- and postoperative care. Approximately 4 wk after surgery, the goats were used in experiments to measure ventilation and blood-gas responses at rest, during treadmill exercise, and during hypercapnia at rest (26). During these experiments, many goats exhibited ventilatory failure during exercise (as indicated by an increase in arterial Pco₂ from rest to exercise), when wearing the respiratory mask alone, or with increased dead space. The ventilatory failure was of sufficient severity in some goats that they could not continue walking. In other goats, the ventilatory failure was mild and could only be detected by careful blood-gas analysis. All goats subsequently exhibited progressive functional recovery in an experience-dependent manner, restoring the ability to sustain treadmill exercise with essentially normal blood-gas regulation. Thus the goats generally experienced both thoracic sensory denervation and the variable experience of ventilatory failure followed by functional recovery during exercise before their spinal cords were harvested for analysis.

**Tissue Preparation**

Four to 15 mo after surgery, the goats were euthanized with an overdose of pentobarbital sodium, and their spinal cords were removed and divided into segments. Each segment was cut in half, and each half was either 1) fixed by immersion in 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1 M PBS at pH 7.4 for 1 wk (for immunohistochemistry) or 2) wrapped in foil, frozen immediately on dry ice, and then stored at −80°C (for HPLC analysis). Each goat with TDR was paired with a control animal (either unoperated or sham operated) and euthanized on the same day. Postfixed tissue was stored in PBS with 0.1% sodium azide. Immersion fixation might have increased background staining in tissue sections; however, we attempted to minimize this problem by examining at least five sections at each segmental level in each goat and by simultaneously processing tissues from matched TDR and sham-operated or unoperated control goats.
Immunohistochemistry

Immunohistochemical procedures were conducted by one of two different methods in two different laboratories (12, 35). Both methods yielded similar results, although they are difficult to compare quantitatively. Transverse, 40- to 50-μm tissue sections were cut from T4, T6, and T10 with a vibrating microtome and stained for 5-HT or CGRP immunoreactivity using the peroxidase-antiperoxidase (PAP) protocol of Sternberger (34) with rabbit as the primary host for the antibody. Briefly, the sections were first rinsed in 0.1 M PBS for 10 min and then placed in normal goat serum (diluted to 3.0% in 0.1 M PBS) for 30 min. The free-floating sections were subsequently incubated for 24 h in primary antisera for 5-HT (gift from M. S. Brownfield; 1:5,000 at room temperature) or CGRP (Peninsula Laboratories, 1:3,000 or 1:7,000 at room temperature). The sections were then incubated in peroxidase-conjugated goat anti-rabbit (GAR) IgG (1:50) followed by incubation in the PAP complex [1:80 (35) or 1:200 (12)]. Sections were rinsed in 0.1 M PBS twice for 10 min and once in 3.0% normal goat serum for 30 min and were then incubated for 7 to 10 min in 0.05% 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical) in 0.1 M PBS and 0.01 M H2O2. In one of the procedures, DAB incubations also included 2.5% nickel ammonium sulfate intensification (12). The primary antisera, link antibody (GAR), and PAP complex were diluted in 1% normal goat serum containing 0.75% Triton X-100 to ensure maximum antibody penetration throughout the 50-μm tissue section. The DAB-reacted sections were rinsed twice in 0.02 M PBS, mounted onto gelatin-coated slides, dehydrated in ethanol, cleared in xylene, and coverslipped with Permount for light-microscopic analysis.

To establish the specificity of the primary antibody and our technique, the following control experiments were performed: 1) the primary 5-HT antisera was either omitted or replaced with PBS, normal rabbit serum (1:5,000) or immunoabsorbed anti-5-HT (absorbed with 5-HT-BSA paraformaldehyde cross-linked conjugate (100 μg/ml of 1:5,000 anti-5-HT)) and 2) the primary CGRP antisera was preabsorbed with synthetic CGRP (100 μg/ml of dilute antiserum). We also documented staining specificity of the antibody for 5-HT, but not 5-hydroxyindoleacetic acid (5-HIAA) or 5-hydroxytryptophan (5-HTP), using the procedures of Shipper and Tilders (33). To do this, we stained spinal cord sections against cryostat sections of paraformaldehyde-conjugated indole-gel matrices at three different concentrations of 5-HT, 5-HIAA, and 5-HTP. No CGRP or 5-HT immunoreactivity was observed in any of the control procedures. Staining for endogenous peroxidase found in red blood cells was seen in all of these unperfused but fixed tissues, but it was easily distinguished from immunocytochemical staining on the basis of size, shape, and location.

HPLC Analysis

Frozen spinal segments (C5-C6, T3–T6, T7–T11, and L1–L2) were homogenized and analyzed via HPLC with electrochemical detection for 5-HT, NE, and DA. Two different HPLC systems and operators were used in this study, which was conducted over a period of 5–6 yr. Because it turned out that all females goats were analyzed with one HPLC system (12), whereas the neutered male goats were analyzed with another (29, 30), we did not believe that we were justified in making any statements concerning apparent gender differences in monoamine levels because other factors could confound our interpretation of the results. The following methods were adapted with modifications from Mefford (25) and are described more fully by Harkness and Brownfield (12) and by Olson et al. (29, 30) with modifications (9). In brief, neural tissue was homogenized in 0.4 M perchloric acid (10 vol/wt) containing dihydroxybutyric acid, a nonnatural monoamine that serves as an internal standard. After centrifugation, crude homogenates were injected directly onto a reverse-phase paired-ion HPLC system (21) with electrochemical detection. Mock neural tissue samples containing known monoamines at concentrations that spanned the range of tissue monoamine concentrations were extracted in parallel as an additional, daily control. For the catecholamine assays, crude tissue homogenates were extracted with alumina. Samples were mixed with internal standard (2.5 ng dihydroxybenzylamine or propranolol), 1.5 M Tris-EDETA, pH 8.6, and acid-washed alumina; shaken for 5 min, mixed on a rotator for 15 min; and then washed three times with 0.001 M sodium acetate. Adsorbed catecholamines were eluted from the alumina with 200 μl 0.05 M phosphoric acid and injected into the HPLC system in volumes of 50–100 μl. The chromatographic system consisted of a Waters M45 pump, auxiliary pulse dampener, Perkin-Elmer autosampler, a Bioanalytical Systems LC4B electrochemical detector, and a Spectra-Physics 4270 integrator. The column was a biphase 5-μm ODS column (4.6 × 250 mm). Two mobile-phase systems were used: 1) catecholamines were resolved by using 0.1 M sodium acetate, 0.02 M citric acid, 100 mg/l sodium octyl sulfate, 50 mg/l sodium EDTA, and 15% methanol; and 2) indoles were separated by using 0.1 M sodium acetate, 0.1 M citric acid, and 20% methanol. Peak areas were integrated, and results were reported as picograms per injection by the integrator (with corrections for recovery of internal standard).

Statistical Analysis

A two-way ANOVA with repeated-measures design was used to detect significant effects of treatment, segment level, and treatment-segment level interaction. Effects were considered significant if P < 0.05. Post hoc analysis of individual comparisons was conducted via the Bonferroni method. Because comparisons between sham-operated and unoperated control animals revealed no significant differences, these groups were combined in all data presentations. Clustered segmental analysis focused on discrete groups of spinal segments, including 1) C7–C8, chosen because these segments provide innervation of the phrenic motor nucleus in ruminants (20); 2) T7–T10, chosen because these rostral segments provide the dominant innervation of intercostal motoneurons (18); and 3) T11–T12, grouped because this region provides the dominant innervation of expiratory intercostal muscles (8, 18). In these analyses, monoamine concentrations for all segments and groups were averaged, and statistical inferences among treatment groups were made by using a one-way ANOVA with a Bonferroni post hoc test for individual comparisons.

To provide a semiquantitative assessment of 5-HT terminal density in the thoracic spinal cord (see Fig. 3), the immunoreactivity (terminal density) was scored on a scale of 0–5 by an experienced, blinded individual. These scores were assigned individually to the dorsal horn, ventral horn, and intermediolateral column of selected thoracic segments on at least five sections per segment. Scores per region and segment were averaged per goat, and the averages between goat groups were analyzed via Student’s t-test with the Bonferroni correction for multiple comparisons. To avoid variation due to the different immunohistochemical procedures used, and different individuals scoring the terminal densities, data represented in Fig. 3 are those collected exclusively in one
laboratory. Results from the other immunohistochemical methods were qualitatively similar.

RESULTS

Immunohistochemical Analysis

CGRP. Camera lucida reconstructions of spinal sections stained for CGRP from the thoracic spinal cord are shown in Fig. 1 for one sham and one TDR goat. Labeling of immunoreactive CGRP was nearly completely eliminated in the superficial laminae of the rostral thoracic dorsal horn in TDR goats. Although actual numbers of CGRP fibers were not determined, immunoreactive fibers in Lissauer’s tract and in the dorsal and dorsolateral funiculi appeared reduced in number. Laminae I and II also exhibited a pronounced loss of CGRP immunoreactivity. At more caudal thoracic levels, CGRP immunoreactivity still appeared reduced compared with control tissue, although the differences became progressively less pronounced in the caudal direction (Fig. 1). There was an apparent increase in CGRP labeling within the white matter and dorsal horn at T6 relative to T4 in TDR animals; increased numbers of immunoreactive fibers were suggested in the dorsolateral funiculus, Lissauer’s tract, and in laminae I and II (Fig. 1). At T10, CGRP labeling appeared greater than at T6, with the overall density of CGRP labeling in TDR sections approximating that in controls, particularly in laminae I and II and in the medial portion of the dorsal horn. All goats used for CGRP analysis showed qualitatively similar results.

5-HT. Camera lucida drawings of 5-HT-immunoreactive axons and boutons in the thoracic spinal cord of one sham-operated and one TDR goat are shown in Fig. 2. Immunocytochemical analysis of thoracic spinal 5-HT revealed a general increase in 5-HT immunoreactivity within the gray matter. 5-HT immunoreactivity was elevated in both the dorsal and ventral horns (with some fibers crossing the midline) in TDR goats relative to control goats. Although increased labeling...
HPLC Analysis

5-HT. HPLC analysis of 5-HT on homogenates of the thoracic spinal segments from these same goats yielded complementary results (Fig. 4). 5-HT concentrations appeared to be elevated in TDR (n = 7) relative to control goats (n = 8) at every thoracic segment, with the apparent difference becoming less pronounced in the rostral to caudal direction. Segments C5–C6, a region associated with the phrenic motor nucleus in ruminants (20), demonstrated an enhancement in 5-HT concentration, even though this area is not directly affected by TDR. ANOVA confirmed that 5-HT concentration was elevated in TDR goats relative to control (P < 0.004); however, the data were highly variable from one spinal segment to the next and did not attain overall significance in any individual comparison. The apparent increase at C5–C6 was highly significant (122%; P < 0.02) when data were clustered into segmental groups (Fig. 4B). Significance was not attained in either thoracic segment cluster despite a strong trend toward an elevation in the rostral segments.

NE. When spinal NE levels were examined by using HPLC, NE concentrations appeared to be elevated in TDR relative to control goats at every thoracic segment (Fig. 5A; P < 0.003 overall), although no individual comparisons were significant. Clustering the data into segmental groups (Fig. 5B) revealed a significant increase (53%; P < 0.04) in the caudal thoracic segments (T7–T11; Fig. 5B).

DA. One surprising result is that TDR elevates spinal DA in TDR relative to control goats (Fig. 6A; P < 0.001 overall). In thoracic segments, DA concentration was substantially elevated in both the rostral (234%; P < 0.03) and caudal (310%; P = 0.051) thoracic segments (Fig. 6B). In cervical segments, DA concentration was largely unchanged, with the exception that there was a strong trend to increase at C5–C6 (191%; P = 0.054; Fig. 6B).

DISCUSSION

These data provide strong evidence that TDR increases spinal concentrations of neurotransmitters as-

Fig. 3. Mean (± SE) semiquantitative (subjective) scores of 5-HT terminal density in the thoracic spinal cord. Open bars, control goats (n = 3); solid bars, TDR goats (n = 3). Immunoreactivity (terminal density) was scored on a scale of 0–5 by an experienced, blinded individual. These scores were assigned individually to the dorsal horn, ventral horn, and intermediolateral column of selected thoracic segments on at least 5 sections per segment. Scores per region and segment were scored only from the goats subjected to immunohistochemistry by using nickel intensification from a single laboratory; semiquantitative outcomes were the same for goats processed in a different laboratory without nickel intensification. Values were averaged per goat, and the averages between goat groups were analyzed via Student’s t-tests. *Significant difference, P < 0.05.

Fig. 4. A: mean 5-HT concentrations ([5-HT]) measured in control (●) and TDR goats (●) at spinal segments from C3 to L2. Dotted vertical lines, region of dorsal rhizotomy. Statistical analyses indicate that [5-HT] was elevated in TDR goats relative to control (P < 0.004). B: mean (± SE) [5-HT] within spinal regions of interest (C5–C6, T3–T6, and T7–T11) in TDR (solid bars) and control goats (open bars). Greatest increase in [5-HT] caused by TDR was observed in the cervical segments (122%; *significant difference, P < 0.02), a region not directly affected by the surgical procedure. Differences between TDR and control became progressively less in the rostrocaudal direction.

Fig. 3 (continued): A: mean 5-HT concentrations ([5-HT]) measured in control (●) and TDR goats (●) at spinal segments from C3 to L2. Dotted vertical lines, region of dorsal rhizotomy. Statistical analyses indicate that [5-HT] was elevated in TDR goats relative to control (P < 0.004). B: mean (± SE) [5-HT] within spinal regions of interest (C5–C6, T3–T6, and T7–T11) in TDR (solid bars) and control goats (open bars). Greatest increase in [5-HT] caused by TDR was observed in the cervical segments (122%; *significant difference, P < 0.02), a region not directly affected by the surgical procedure. Differences between TDR and control became progressively less in the rostrocaudal direction.

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sociated with descending modulation of spinal sensory-motor integration. Because the effects were observed in both the denervated thoracic region and in cervical regions not affected directly by surgical procedures (but associated with the phrenic motor nucleus), compensatory changes in monoaminergic brain stem-spinal cord pathways may play a role in the functional deficits and/or recovery of ventilatory control after TDR (27). Even 1 yr after TDR, there was no recovery of CGRP immunoreactivity in the rostral thoracic dorsal horn, thereby suggesting that regrowth of sensory afferent pathways is not a factor in functional recovery after TDR.

Critique of Methods

This study was conducted over a period of 5–6 yr and used female and castrated male goats. Tissue was collected from these animals at variable times after TDR surgery and analyzed by using two different HPLC systems and two different immunohistochemical methods with a number of different technical assistants. Undoubtedly, these issues introduced variability and uncertainty into portions of our study. Nevertheless, trends from all goats, regardless of sex or how or when the tissues were examined, are consistent with an increase in monoamine terminal density and concentration in specific regions of the spinal cord after TDR.

Quantitative analysis was conducted on spinal cross-sectional homogenates for HPLC. This technique precludes an analysis of ventrodorsal differences in monoaminergic innervation. Instead, our results reflect the total monoaminergic concentration in both sensory and motor regions of the spinal cord. Therefore, the complexity of descending monoaminergic systems and their potential role(s) in the recovery of an adequate exercise ventilatory response are not fully addressed in the present study. Nevertheless, on the basis of qualitative immunocytochemical results, it appears that changes in 5-HT levels were similar in both the dorsal and ventral horns after TDR, suggesting that TDR may cause changes in both sensory and motor integration in goats.

Effect of TDR on Immunoreactive CGRP

Bilateral TDR nearly eliminated CGRP immunoreactivity at rostral thoracic levels of the dorsal horn.

Fig. 5. A: mean norepinephrine concentrations ([norepinephrine]) measured in control (○) and TDR goats (●) at spinal segments from C₃ to L₂. Dotted vertical lines, region of dorsal rhizotomy. [Norepinephrine] was elevated in TDR goats relative to control (P < 0.002); there were no differential effects attributable to segmental level or a treatment-segment interaction. B: mean (± SE) [norepinephrine] within spinal regions of interest (C₅–C₆, T₃–T₆, and T₇–T₁₁) in TDR (solid bars) and control goats (open bars). Increase in [norepinephrine] caused by TDR was relatively small (39–57%) and was significant only from T₇ to T₁₁. *Significant difference, P < 0.05.

Fig. 6. A: Mean dopamine concentrations ([dopamine]) measured in control (○) and TDR goats (●) at spinal segments from C₃ to L₂. Dotted vertical lines, region of dorsal rhizotomy. [Dopamine] was substantially elevated in TDR goats relative to control (P < 0.001); there were no differential effects attributable to segmental level or a treatment-segment interaction. B: mean (± SE) [dopamine] within spinal regions of interest (C₅–C₆, T₃–T₆, and T₇–T₁₁) in TDR (solid bars) and control goats (open bars). [Dopamine] increase caused by TDR was relatively large (191–289%). There was a trend suggesting an increase in [dopamine] in the cervical spinal cord that was unique to the region of the phrenic nucleus (C₅–C₆; P = 0.054) and was notably similar to control at other cervical segments examined (see A). A similar trend was also seen in caudal thoracic segments (T₇–T₁₁; P = 0.051) *Significant difference, P < 0.03.
while leaving CGRP staining within the ventral horn unchanged. These data are consistent with other studies of CGRP immunoreactivity in the dorsal horn after rhizotomy in rats and cats (5, 39). Because CGRP, particularly in the superficial laminae of the dorsal horn, is largely present in primary afferent fibers, our data do not support the hypothesis that regrowth of dorsal rootlets occurs in rostral thoracic segments after dorsal rhizotomy. The relative preservation of CGRP labeling at more caudal sites (e.g., T₁₀) may suggest that some sprouting (11) is occurring at caudal thoracic levels, possibly in the zone of overlap between degenerating terminals and intact afferent fibers. In cats and rats, primary afferent fibers can ascend four to five segments before terminating in the dorsal horn (7, 39). Such ascending fibers may explain the progressive rostral-to-caudal increase in CGRP-labeled fibers.

**Effects of TDR on Spinal 5-HT**

5-HT-containing cell bodies are located mainly in the brain stem, in discrete midline clusters known as the raphe nuclei (16). The caudal groups of serotonergic neurons, consisting largely of the nucleus raphe magnus, raphe pallidus, and raphe obscurus, have divergent projections to many of the regions of the central nervous system that are important in respiratory control (1, 3, 22). With few exceptions, all serotonergic terminals in the spinal cord arise from these descending projections (16).

Both immunoreactive 5-HT terminal density and 5-HT concentration were elevated in the spinal cord after TDR. This led us to hypothesize that 5-HT contributed to the observed recovery of respiratory function during exercise in TDR goats (22). Recovery of an appropriate ventilatory response to exercise with increased dead space in TDR goats was extremely variable but seemed to depend more on the number of exercise trials presented to the animal rather than the passage of time (26). For example, some goats recovered within a given experimental day after four to eight exercise trials (5-min duration), whereas others recovered over a period of 1 mo with one to three exercise trials per week (26). Because the most consistent increase in spinal 5-HT concentration was in an area not directly affected by rhizotomy (C₅–C₆), we suspect that descending serotonergic projections provide a general, compensatory function, enhancing respiratory motor output in regions directly affected by the “injury” (i.e., thoracic), as well as in other, synergistic motor pools (e.g., phrenic motor nucleus).

5-HT plays an important role in plasticity of the central nervous system, particularly the spinal cord. After spinal cord injury, 5-HT or serotonergic neurons contribute to functional recovery of locomotor function (13, 32). For example, the timing of functional deficits and recovery of locomotor function correlate closely with 5-HT loss and recovery below a spinal hemisection in rats, and subsequent application of 5-HT-receptor antagonists at least partially reverses locomotor recovery (32). The mechanisms by which 5-HT or serotonergic neurons promote functional recovery after spinal cord injury are unclear. However, 5-HT acts as a trophic factor in some circumstances (19, 41) and elicits long-lasting enhancement of synaptic efficacy in the spinal dorsal horn (14). We postulate that plasticity of serotonergic neurons per se imparts a degree of functional plasticity in spinal motor control by enhancing the capacity for neuromodulation during motor behaviors such as respiration or locomotion. As yet, the hypothesis that increased capacity for serotonergic modulation contributes to functional recovery after TDR is untested.

**Effects of TDR on Spinal NE**

Descending noradrenergic projections arise from locus coeruleus (A₈) and area A₅ (another pontine noradrenergic cell group) (14). However, details of the descending innervation patterns are variable, even among rat substrains (6). Thus the precise origin of spinal NE detected in this study on goats is unknown.

Although TDR had marginal effects on spinal NE concentration, there was a rostrocaudal trend toward increasing NE concentration within the area of denervation. The functional significance of changes in NE after TDR remains unclear. However, this observation is consistent with the hypothesis that enhanced noradrenergic modulation of expiratory motor output contributes to functional recovery after TDR because caudal thoracic and lumbar segments predominantly innervate expiratory motor pools (8).

**Effects of TDR on Spinal DA**

Descending dopaminergic fibers arise from hypothalamic area A₁₁ (2) and project to the spinal cord, including the ventral horn (37). A link between the activity of descending dopaminergic pathways and motor activity has been established (10), suggesting a modulatory role for DA in the control of spinal motor function. Regardless, little is known about the release of DA from nerve terminals in regions of interest to respiratory motor control or its role in motor functions such as breathing.

Our demonstration that spinal cord DA concentration increases dramatically after TDR indicates that spinal dopaminergic pathways undergo plasticity after TDR. Although such changes may compensate for loss of sensory inputs from respiratory muscles, we do not yet have sufficient evidence to predict with clarity the functional significance of increased spinal dopaminergic modulation on respiratory motor output during exercise.

**Significance**

Immunocytochemistry and neurochemical analyses do not allow an assessment of causal relationships between changes in spinal neurotransmitters and recovery of function after TDR. Nevertheless, these studies provide compelling evidence that deafferentation of the spinal cord causes profound alterations of neurochemicals that are known to modulate respiratory mo-
tor activity. Furthermore, these changes can occur outside the spinal segments directly affected by TDR and, in fact (uniquely), in the cervical segments specifically associated with the phrenic motor nucleus in goats. Such coincidence raises the surprising and exciting prospect that increased phrenic neuromodulation is utilized as a form of compensation for functional deficits caused by loss of sensory feedback from thoracic respiratory muscles. To our knowledge, evidence for a similar compensatory mechanism is without precedent in the motor control literature.

Firm conclusions concerning the significance of our findings await a demonstration of a causal relationship between serotoninergic enhancement and recovery of function and/or a demonstration of the roles of NE and DA in plasticity after TDR.

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