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Effects of corticospinal tract stimulation on renal sympathetic nerve activity in rats with intact and chronically lesioned spinal cords

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Pan B, Zahner MR, Kulikowicz E, Schramm LP. Effects of corticospinal tract stimulation on renal sympathetic nerve activity in rats with intact and chronically lesioned spinal cords. *Am J Physiol Regul Integr Comp Physiol* 293: R178–R184, 2007. First published April 22, 2007; doi:10.1152/ajpregu.00044.2007.—Sympathetic preganglionic neurons and interneurons are closely apposed (presumably synapsed upon) by corticospinal tract (CST) axons. Sprouting of the thoracic CST rostral to lumbar spinal cord injuries (SCI) substantially increases the incidence of these appositions. To test our hypothesis that these additional synapses would increase CST control of sympathetic activity after SCI, we measured the effects of electrical stimulation of the CST on renal sympathetic nerve activity (RSNA) and arterial pressure (AP) in α-chloralose-anesthetized rats with either chronically intact or chronically lesioned spinal cords. Stimuli were delivered to the CST at intensities between 25–150 μA and frequencies between 25 and 75 Hz. Stimulation of the CST at the midcervical level decreased RSNA and AP. These decreases were not mediated by direct projections of the CST to the thoracic spinal cord because we could still elicit them by midcervical stimulation after acute lesions of the CST at caudal cervical levels. In contrast, caudal thoracic CST stimulation increased RSNA and AP. Neither the responses to cervical nor thoracic stimulation were affected by chronic lumbar SCI. These data show that the CST mediates decreases in RSNA via a cervical spinal system but excites spinal sympathetic neurons at caudal thoracic levels. Because chronic lumber spinal cord injury affected responses evoked from neither the cervical nor thoracic CST, we conclude that lesion-induced or regeneration-induced formation of new synapses between the CST and sympathetic neurons may not affect cardiovascular regulation.

sympathetic preganglionic neurons; sympathetic interneurons; spinal cord injury; cardiovascular regulation

PROGRESS IS BEING MADE IN the regenerative repair of injured spinal cords. Although this progress is gradual, clinical trials are already under way (22, 26). Indeed, spinal cord regeneration has captured the imagination of both the public and the pharmaceutical industry (25). However, many fundamental questions remain to be answered (17). One of the most important and one of the least discussed among these is whether regenerating or sprouting axons will form functionally appropriate synapses and avoid forming aberrant synapses. In particular, we have suggested that aberrant synapses between spinal systems normally controlling movement and systems normally controlling the output of the sympathetic nervous system could lead to regulatory dysfunction (30). The formation of such aberrant synapses is plausible in light of previously reported sprouting and functional plasticity of corticospinal tract synapses rostral to spinal cord lesions (16).

In a previous study, we used the sprouting of the corticospinal tract (CST) rostral to a chronic spinal cord lesion to mimic the axonal sprouting and formation of new synapses that would occur in a regenerating spinal pathway (30). This sprouting is well described and very robust (16, 20). The motivation for the present experiments arose from our observation that sprouting of the thoracic CST after a lumbar spinal lesion caused a significant increase in close appositions (putative synapses) between axons of the CST and spinal sympathetic interneurons (IN) and between axons of the CST and sympathetic preganglionic neurons (SPN) at caudal thoracic levels. Similar increases in close appositions between CST collaterals and spinal neurons previously have been observed rostral to lesions of the CST (4). We interpreted the increase in close appositions with IN and SPN as an example of aberrant somatic-autonomic synapses and hypothesized that, given the increase in appositions, the CST would have an abnormally large effect on sympathetic activity in rats that had undergone chronic lumbar spinal cord lesions.

To test this hypothesis, we first determined the effects of electrical stimulation of the CST on renal sympathetic nerve activity (RSNA) and mean arterial pressure (AP) in rats with no previous lesion to their spinal cords. These rats were designated chronically intact. Then, we compared those effects with responses evoked in rats whose spinal cords had been lesioned at rostral lumbar levels 4–6 wk earlier, a period of time sufficient for the formation of many new close appositions between CST collaterals and spinal sympathetic neurons (30). These rats were designated chronically lesioned. We discovered that stimulation of the CST at cervical and caudal thoracic levels had opposite effects: cervical stimulation decreasing and thoracic stimulation increasing, RSNA. However, chronic lumbar lesions did not affect RSNA responses to stimulation of the CST at either cervical or thoracic levels.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were used in these experiments (Charles River, Raleigh, NC). All surgical procedures and postoperative care were provided in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and approved by the Johns Hopkins University Committee on Animal Care and Use. We used 175-g male rats for the chronically lesioned group. These rats survived for 4–6 wk after spinal cord lesions before we studied the effect of CST stimulation on RSNA and AP in acute experiments. By this time, rats weighed 300–400 g. Our chronically

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intact control group consisted of unoperated, weight-matched male rats. Forty rats met the criteria for inclusion in our data: *1*) stimulation sites could be unambiguously identified histologically, *2*) histological examination showed that stimulation sites were completely within the CST, and *3*) recordings of RSNA and AP were stable and free from artifact during stimulation periods. Forty-two rats did not meet at least one of these criteria. All stimuli were delivered to the left dorsal CST. Unless indicated, all spinal cords were acutely transected several segments rostral to stimulation sites to prevent antidromic activation of more rostral systems, brain stem nuclei in the case of cervical stimulation, and cervical spinal cord and brain stem systems in the case of thoracic spinal cord stimulation.

We conducted four experiments in chronically intact rats. First, we stimulated the CST between C3 and C5 after making an acute spinal transection at C2 ($n = 9$). Second, to gauge the necessity for the acute rostral transections, we stimulated the CST between C3 and C5 without previous acute C2 spinal transection ($n = 6$). Third, we stimulated the CST between C3 and C5 after both a complete acute transection of the spinal cord at C2 and a surgical lesion that destroyed the dorsal CST between C7 and C8 ($n = 7$). The purpose the latter experiments was to determine whether responses elicited from the cervical CST were mediated by direct projections to the thoracic spinal cord or, indirectly, by cervical systems activated by cervical CST stimulation. Fourth, in the thoracic spinal cord, we stimulated the CST between T10 and T11 after a T8-spinal transection ($n = 5$).

We conducted two kinds of experiments in chronically lesioned rats (lesions in the rostral lumbar spinal cord, as described below). First, we stimulated the CST between C3 and C5 after complete acute transection of the spinal cord at C2 ($n = 7$), and, second, we stimulated the CST between T10 and T11 in rats with complete acute lesions of the spinal cord at T8 ($n = 6$).

Chronic spinal cord injury. Under halothane anesthesia, T12 to L1 vertebrae were removed to expose the rostral lumbar segments. We incised the dura and hemisected the dorsal spinal cord at the L2-3 level with a microsurgical blade as described previously (30). The lesion extended slightly ventral to the central canal. Therefore, the area destroyed included the dorsal columns, containing both left and right dorsal CST, the left dorsolateral funiculus, the left dorsal horn, and the left intermediate zone (see Fig. 1*C* in Ref. 30). The locations and transverse extent of all lesions were determined histologically as described below. Muscle and skin incisions were closed separately. Rats were treated with an antibiotic (20,000 units im; Pfizerpen, Pfizer, NY), and an analgesic (1.1 mg/kg sc Banamine; Shering-Plough, Union, NJ) before cessation of anesthesia. We manually expressed the rats' bladders for 3–5 days after surgery by which time spontaneous micturition recovered.

Preparation for recording RSNA and AP. Anesthesia was induced by halothane and continued by administration of 100 mg/kg a-chloralose (7.5%; Sigma) via the right femoral vein. Rats were placed under a lamp and on a heating pad to maintain body temperature between 35 and 37°C, monitored with a rectal probe. The trachea was intubated for artificial respiration with 100% oxygen. Once secured in a stereotaxic frame, rats were paralyzed with gallamine triethiodide (40 mg/kg iv). The depths of anesthesia and paralysis, as indicated by the level and variability of RSNA and AP or the presence of corneal reflexes, were maintained with supplements of a-chloralose and gallamine triethiodide.

In rats in which we stimulated the cervical spinal cord, we made a laminectomy between the C1 and C6 vertebrae. This laminectomy was extended to the C8 vertebra when the CST of rats was to be lesioned at the C8 spinal segment. In rats in which we stimulated the caudal thoracic spinal cord, we made a laminectomy between the T6 and T10 vertebrae. The spinal cord was transected at the T8 spinal segment. In these rats, a pneumothorax and mechanical stabilization with a vertebral clamp were necessary to reduce respiratory movement of the spinal cord. An injection of 0.5–1 ml of human serum albumin (Baxter Healthcare, 25% solution, 12.5 g/50 ml iv) was administered if needed to stabilize AP. In all preparations, the exposed spinal cord was kept moist with warm mineral oil.

RSNA recording, signal processing, and quantification. Preparation for RSNA recording has been described elsewhere in detail (Chau et al., 1997). The left kidney was approached via a left flank laparotomy, reflected ventrally, and retracted. The renal nerve, which typically was found at the junction of the aorta and the renal artery or traversing the aorta and extending toward the kidney, was dissected from the surrounding tissues and epineurium and mounted on a bipolar hook electrode. RSNA was amplified by a differential amplifier at a bandpass of 300–3,000 Hz. Total amplification was between ×5,000 and ×10,000. Sympathetic activity was further processed by rectification and low-pass filtering at a time constant of 0.1 s. Unfiltered RSNA, processed RSNA, AP, and heart rate were recorded simultaneously with a Cambridge Electronic Design Micro1401 using Spike 2 software. At the end of each experiment, the renal nerve was cut proximal to the recording electrode. The electrical activity that remained was recorded and processed as described above for 3–5 min. Once it had stabilized, the average amplitude of this activity over a 4-s period was recorded and used to compute actual zero RSNA. Before analysis, recordings of RSNA were corrected for differences in amplifier gain.

We delivered all stimuli for 5 s. Because responses often had transient components that lasted for 0.1 to 0.5 s, we measured the magnitude of responses as the average of the RSNA during the last 4 s of stimulation, minus the zero activity measured as described above. To compensate for differing resting levels of RSNA between rats, we normalized all responses by the average of RSNA during the last 4 s before each stimulation, again, minus zero activity. Therefore, all responses in RSNA were expressed as %changes.

CST stimulation. We inserted microelectrodes (0.5 megaOhm tungsten, World Precision Instruments) into the cervical CST to a depth of 1.2–1.4 mm between segments C3 and C5 and into the left thoracic CST to a depth of 0.8–1.0 mm between T10 and T11. Electrodes were precoated with 1% 1,1′ dioctadecyl-3,3,3′3′ tetramethyl-indocarbocyanine perchlorate (DiI) in 100% ETOH to ensure precise localization of electrode tracks (15). We used a constant-current stimulator to deliver square wave stimuli at all combinations of 25, 75, 125, and 150 μA and 25, 50, and 75 Hz. Pulse duration was set at 200 μs. Upon completion of each 12-stimulation paradigm, the stimulation site was marked with a small direct current lesion.

Histology. At the end of experiments, rats were perfused transcardially with buffered saline (pH 7.4), followed by 4% buffered paraformaldehyde (pH 7.4). The spinal cord was removed and postfixed in fixative overnight. After cryoprotection in 30% sucrose for 48 h, 40 μm horizontal sections were cut on a sliding microtome, mounted on gelatin-coated glass slides, and air dried. The exact sites of electrode tracks were identified by both DiI labeling and electrolytic lesions using fluorescence and conventional microscopy. In rats lesioned acutely at C7-8, complete destruction of the left and right dorsal CSTs was confirmed histologically in 40-μm transverse sections. Lumbar spinal cords of chronically lesioned rats were similarly sectioned, and the extent of each chronic lesion was reconstructed.

Data presentation and statistical analysis. Data are expressed as means ± SE. Statistical analysis employed one-way ANOVA with repeated measures or paired Student's *t*-test for comparison between responses to stimuli within groups, and two-way ANOVA for comparison between groups, as appropriate. Values of $P < 0.05$ were considered significant.

RESULTS

Cervical CST stimulation decreased RSNA. Electrical stimulation of the CST in segments C3-C5 evoked decreases in RSNA in rats previously transected at C2 (Fig. 1*A*). In most rats, larger stimulus intensities and frequencies tended to evoke larger decreases (Fig. 1*B*, white bars). Moreover, at each

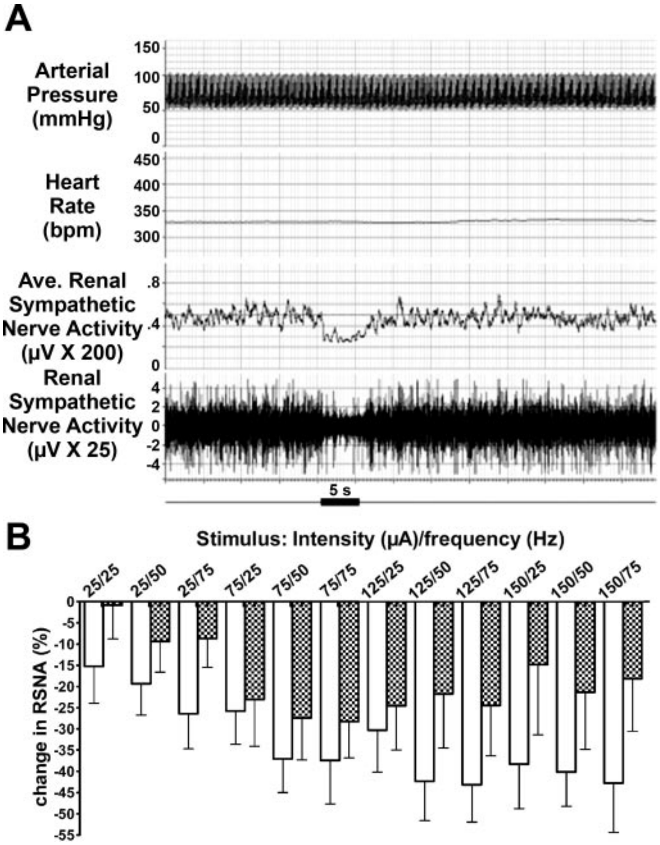

Fig. 1. *A*: representative response of renal sympathetic nerve activity (RSNA), heart rate, and arterial pressure (AP) to stimulation of the cervical corticospinal tract (CST). Note rapid onset of inhibition of RSNA with stimulation. RSNA returned to prestimulation level rapidly after the offset of stimulation. AP decreased slightly and transiently. Heart rate responses were small and variable in direction. *B*: effect of cervical stimulation between C3 and C5 on RSNA at intensities and frequencies indicated above bars. White bars represent responses in chronically intact rats with spinal cords also intact at C7-C8, $n = 9$. Responses evoked by stimuli of 75 μA/50 Hz and larger (except 125 μA/25 Hz) were larger than those evoked by stimuli of 25 μA/25 Hz and 25 μA/50 Hz ($P < 0.05$, one-way ANOVA, repeated measures, $n = 9$). Stippled bars represent responses in chronically intact rats with acute lesions of the CST at C7-C8, $n = 7$.

stimulus intensity, higher frequencies tended to evoke larger reductions in RSNA. Stimulation at an intensity of 125 μA and a frequency of 75 Hz evoked the largest average decrease in RSNA, 43.1 ± 8.8%. Stimulation also evoked a small, but highly reliable, decrease in AP of 1.1 ± 0.5 mmHg (averaged over-all stimulus paradigms, $P = 0.0022$, paired *t*-test). Stimulation did not significantly affect heart rate.

When the cervical CST was stimulated without first transecting the spinal cord at C2, responses ranged from small decreases to small increases (data not shown). These mixed responses in spinally intact rats likely resulted from simultaneous orthodromic stimulation of spinal systems and antidromic stimulation of medullary nuclei via brain stem CST collaterals (3, 41).

Rostral cervical CST stimulation after a caudal cervical CST lesion attenuated, but did not abolish, evoked decreases in RSNA. To determine whether decreases in RSNA were mediated only by CST input to cervical spinal cord, we repeated stimulation at C3-C5 after destroying the CST between C7 and C8 (see MATERIALS AND METHODS). Rostral cervical CST stimulation still evoked robust decreases in RSNA after this CST lesion (Fig. 1*B*, stippled bars). However, their magnitude was significantly reduced [$F(1, 168) = 12.68$, $P < 0.0005$].

In seven rats, stimulation lateral to the CST evoked decreases in RSNA similar to those evoked from the CST itself at all stimulus intensities. However, the magnitude of these decreases diminished with increasing distance from the CST. Stimulation just ventral to the CST in lamina-X in another seven rats decreased RSNA at low stimulus intensities but increased RSNA at greater intensities.

Caudal thoracic CST stimulation increased RSNA. In sharp contrast to responses evoked by stimulation of the cervical CST, stimulation of the thoracic CST at T10-T11 (following an acute spinal transaction at T8) increased RSNA activity (Fig. 2*A*). In each rat, increased stimulus intensities tended to evoke larger increases in RSNA (except at the maximum intensity of 150 μA). At each stimulus intensity, however, higher stimulation frequencies tended to evoke smaller excitations (Fig. 2*B*). Thoracic stimulation evoked small, but consis-

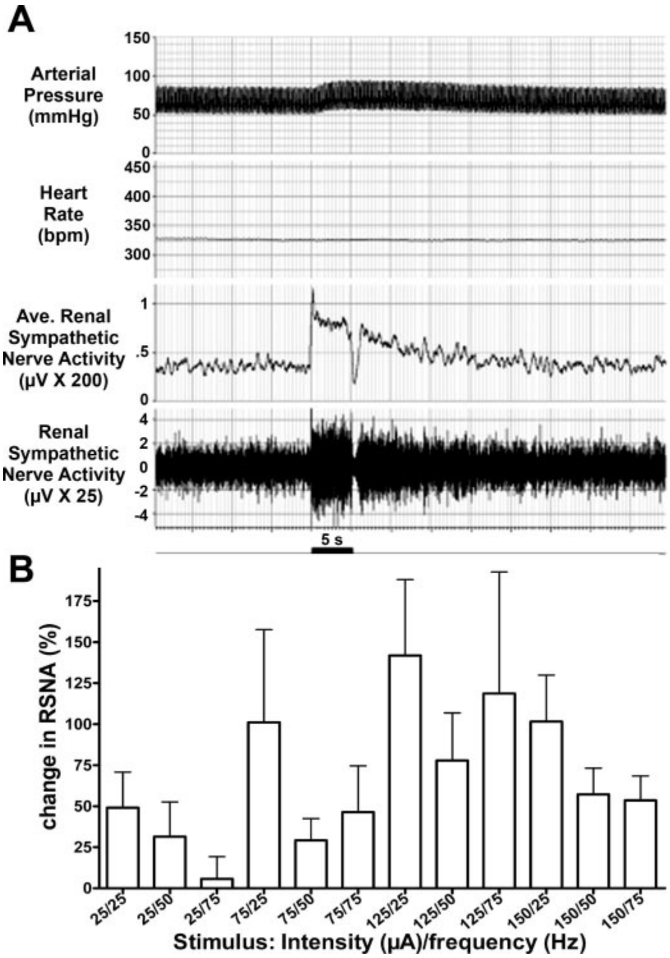

Fig. 2. *A*: representative response of RSNA, heart rate, and AP to stimulation of the thoracic CST (T10). Note rapid increase in RSNA with stimulation. At the offset of stimulation, after a transient silent period, RSNA recovered rapidly and remained elevated for ~15 s. AP increased significantly during stimulation, but heart rate was unaffected. *B*: thoracic CST stimulation (at intensities indicated below each bar) increased RSNA. However, increased frequency of stimulation at each stimulus intensity tended to decrease the magnitude of this response, $n = 5$.

tent, increases in AP of 3.8 ± 1.4 mmHg averaged over all stimulus paradigms ($P = 0.0197$, paired t-test). Heart rate was unaffected by thoracic stimulation.

Chronic lumbar spinal lesions did not significantly affect responses to CST stimulation. Immediately after surgery, all chronically injured rats demonstrated complete loss of use of the left hindleg and partial-to-complete loss of use of the right hindleg. Rats also either gained no weight or slightly lost weight during the first two postoperative weeks. Rats showed some recovery of their hindlimb motor function and a normal gain in weight after 3 wk.

Cervical CST stimulation reduced RSNA to an extent that was not significantly different from that in spinally intact rats (Fig. 3*A*) [$F(1,168) = 0.08$, $P = 0.78$]. Stimulation evoked decreases in AP of 1.1 ± 0.5 mmHg, identical to those evoked in spinally intact rats.

Similarly, thoracic CST stimulation in chronically lesioned rats increased RSNA to an extent that was not significantly different from that in spinally intact rats (Fig. 3*B*) [$F(1,120) = 0.03$, $P = 0.87$]. Note that even the positive relationship between stimulus intensity and response magnitude and the negative relationship between stimulus frequency and response magnitude were maintained after chronic spinal lesions. Stimulation-evoked increases in AP were nearly identical to those evoked in spinally intact rats, 3.7 ± 0.9 mmHg.

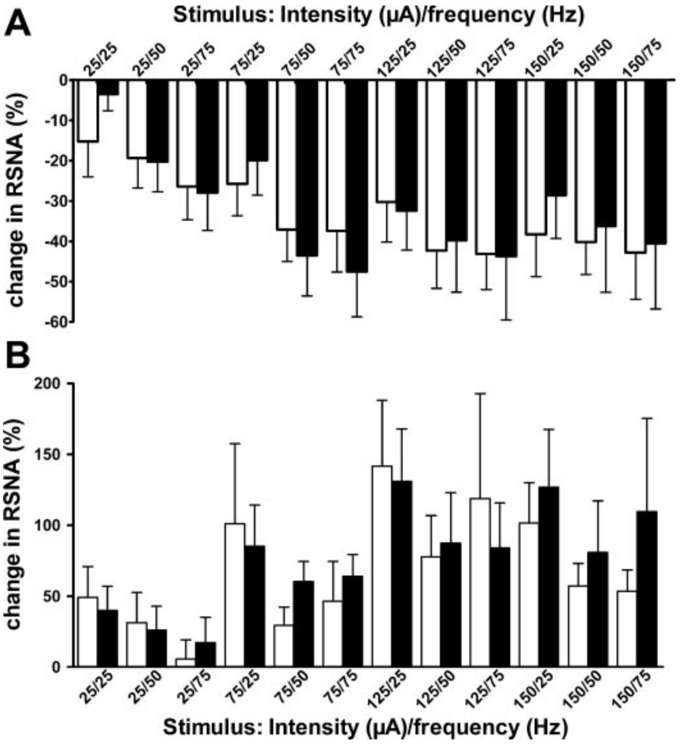

Fig. 3. *A*: decreases in RSNA evoked by cervical CST stimulation in chronically lesioned rats (black bars, $n = 7$) were not significantly different from those evoked in chronically intact rats (white bars are the same data as in Fig. 1*B*). *B*: increases in RSNA evoked by thoracic CST stimulation in chronically lesioned rats (black bars, $n = 6$) were not significantly different from those evoked in chronically intact rats (white bars are the same data as in Fig. 2*B*). See text for details.

DISCUSSION

Cervical CST stimulation decreased RSNA. Cortical (rather than CST) stimulation has been used in all previous studies of cortically evoked autonomic effects (8), and cortical efferents project to a broad range of brain stem nuclei that themselves affect sympathetic activity (3, 41). Therefore, we were unable to predict from the results of earlier experiments how electrical stimulation of the CST itself would affect sympathetic activity. We observed two major characteristics of responses in RSNA to cervical CST stimulation. First, cervical CST stimulation decreased RSNA. Second, these decreases were not mediated wholly by direct projections of the CST to lower thoracic segments because they could still be evoked after destruction of the dorsal CST between C7 and C8. Caudal cervical lesions did, however, significantly reduce the magnitude of cervically evoked decreases in RSNA. We hypothesize that the inhibitory system responsible for cervical effects on RSNA continues into rostral thoracic spinal cord. This hypothesis is supported by observations from a small number of rats in which we stimulated the CST in rostral thoracic spinal cord after transecting the spinal cord between C7 and C8 (Data not shown). In those rats, small but highly variable decreases (and occasionally small increases) in RSNA were evoked by CST stimulation, suggesting that we were stimulating at the transition between a rostral sympathoinhibitory system and a caudal sympathoexcitatory system.

Glutamate is the principal neurotransmitter in axons of the CST (18, 32), and the monosynaptic effect of activating these axons in somatic pathways is excitatory (for instance, see Ref. 35). However, cortical stimulation activates many IN within the terminal field of the cervical CST (13), and the CST mediates spinal inhibitory effects via projections to inhibitory IN in both somatic motor and sensory systems (1, 42). Therefore, the most likely mechanism for decreases in RSNA in response to cervical CST stimulation was glutamate-mediated activation of inhibitory IN. We hypothesize that these IN in turn inhibited caudal thoracic SPN and/or sympathoexcitatory IN.

Although our data do not identify the locus of these inhibitory IN, other data suggest that they reside in caudal thoracic, rather than cervical and upper thoracic, segments. Deuchars et al. (14) have identified thoracic GABAergic IN that likely project to both SPN and sympathoexcitatory IN. These neurons could be excited by long, propriospinal neurons that we and others have shown project from cervical spinal cord to caudal, thoracic, sympathetically related neurons (23, 37). Finally, to our knowledge no GABAergic or glycinergic neurons have been shown to project directly from cervical to caudal thoracic spinal cord.

The present study is not the first to implicate cervical neurons in spinal inhibitory processes. This laboratory showed that either chemical or electrical stimulation of cervical spinal cord reduced RSNA (24, 29, 31, 36). Although we did not rule out direct inhibition of thoracic SPN by cervical stimulation, our evidence suggested that reductions in RSNA were secondary to inhibition of thoracic sympathoexcitatory IN that received both nociceptive and discriminative afferent input (10, 11). This interpretation was supported by others who showed that systems residing in the cervical spinal cord inhibit input from primary afferents in response to a variety of somatic and visceral afferent input (9, 27, 33, 34).

Caudal thoracic CST stimulation increases RSNA. The anatomical substrate for the effects of thoracic CST stimulation on RSNA is better defined than that for the effects of cervical stimulation. Pan et al. (30) have shown that axons of the CST closely appose (presumably synapse upon) a small but significant number of caudal thoracic SPN and sympathetically related spinal IN (identified by transsynaptic retrograde tracing with pseudorabies virus). We suggest that the increased RSNA we observed in response to CST stimulation was mediated by these synapses. Because Pan et al. did not determine the transmitters expressed in the sympathetically related IN, they were unable to determine how many of the sympathetic IN contacted by CST axons were excitatory or inhibitory. However, both excitatory and inhibitory sympathetic IN are known to reside in thoracic spinal cord (14, 38). If the CST projects to both categories of IN, based on our observations the effects of direct stimulation of thoracic CST projections to SPN and excitatory IN outweigh the effects of stimulation of thoracic CST projections to inhibitory IN.

Many credible models could account for the opposite effects on RSNA of stimulating the cervical and thoracic CST. However, any model must account for the fact that, because the cervical CST is not somatotopically organized (12), cervical CST stimulation also stimulates thoracic CST axons. The model shown in Fig. 8 is consistent with our results.

Neurons 4 and 5 are sympathetic pre- and postganglionic neurons, respectively. The SPN 4 is shown receiving excitatory input from the CST and a sympathoexcitatory IN 3. That excitatory IN receives information from primary afferent neurons (represented by neuron 6) as well as from the CST. We have documented the existence of these sympathoexcitatory neurons and their somatic and visceral afferent input (10, 11, 24, 29). Neuron 2 is an inhibitory IN that inhibits both thoracic sympathoexcitatory neurons 3 and SPN 4. On the basis of the data of Deuchars et al. (14), in our model the inhibitory IN 2 resides in the thoracic spinal cord. Finally, neuron 1 is an excitatory propriospinal neuron that is excited by the cervical CST and that, in turn, excites a thoracic inhibitory IN. We and others have identified candidates for these cervical IN. Propriospinal neurons in the rostral, cervical, dorsolateral funiculus project to SPNs and IN labeled by renal injections of PRV (37). Furthermore, using anterograde tracing, Jansen and Loewy (23) have shown that the projections of these propriospinal neurons include the medial regions of lower thoracic segments. Deuchars et al. (14) have shown that GABAergic IN that project to SPNs reside in these medial regions.

Our data are consistent with the hypothesis that, in addition to inhibiting the ongoing RSNA, the inhibitory effects of the CST on excitatory IN 3 and SPN 4 prevent the excitatory effects mediated by CST projections to those excitatory IN and SPN. Activity in primary afferents 6 may be responsible for much of the ongoing RSNA after spinal cord lesions (10, 11), and Fig. 4 indicates that inhibition of IN 3 would reduce that afferent input as well as that from the CST. We do not propose that the opposite effects of stimulation of the cervical and thoracic CSTs mimic a plausible physiological process. It is more likely that cervical and thoracic CST axons and their related pathways are separately and specifically regulated by the cortex and rarely would be coactivated.

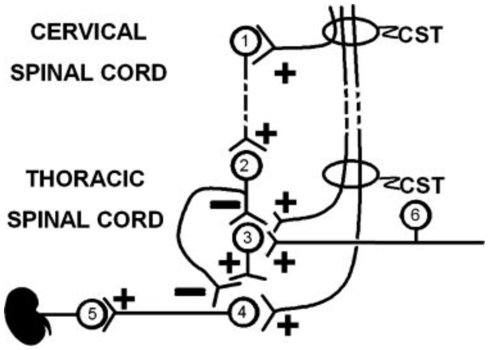

Fig. 4. Hypothetical model for cervical and thoracic projections of the CST affecting RSNA. 1 = Excitatory cervical propriospinal neuron, 2 = inhibitory thoracic interneuron, 3 = sympathoexcitatory thoracic interneuron, 4 = sympathetic preganglionic neuron (SPN), 5 = sympathetic postganglionic neuron, 6 = primary afferent neuron. Note that the inhibitory interneuron 2 inhibits both the excitatory interneuron 3 and the SPN 4 and that the thoracic CST excites both the excitatory interneuron 3 and the SPN 4. See text for detailed description.

Chronic lumbar spinal lesions did not significantly affect responses to CST stimulation. Our original hypothesis was that additional lesion-induced synapses of CST axons on spinal sympathetic neurons would increase the effects of CST stimulation on sympathetic activity and AP. However, the responses to neither cervical nor thoracic CST stimulation were affected by chronic spinal lesions. There are three explanations for our failure to observe differences. First and most likely, either lesion-induced synaptic changes were quantitatively insufficient to produce measurable changes in responses to stimulation or lesion-induced synapses were nonfunctional. We would not be able to distinguish between these possibilities without studying the effects of CST input to spinal sympathetic neurons intracellularly. Unfortunately, studying the nature of synaptic input between the CST and even relatively large motoneurons has proven difficult and controversial in the rat (2, 21).

Second, lesions in the present study may not have caused a degree of CST collateralization and an increase in close appositions similar to those observed in our previous study. We were unable to examine CST collateralization and close appositions in the present experiments because we were concerned that damage to the cortex necessary for anterograde tracing of the CST and damage to spinal sympathetic systems necessary for retrograde pseudorabies virus tracing of spinal sympathetic neurons would confound the results of our physiological experiments. Nevertheless, we consider it safe to assume that the CST collateralized to a similar degree in the present study. Rats with chronic spinal lesions were treated identically in this and our previous study that documented lesion-induced increases in close appositions.

Third, despite efforts to produce uniform animal preparations, responses to CST stimulation were surprisingly variable among rats. This variability is manifested by the relatively large standard errors exhibited by some of our data. We cannot exclude the possibility that small differences in responses in chronically intact and chronically lesioned rats were not detected because of this variability. If this were the case, however, the differences would be much smaller than would be predicted by the five- to sevenfold lesion-induced increase in anatomical input from the CST to spinal sympathetic neurons.

Methodological considerations. Because of the proximity of the CST to the dorsal horn and lamina-X, we were particularly careful to ensure that we were directly stimulating only the left CST. Responses could be evoked at very small stimulus intensities (25 μA), and in exploratory experiments the polarity of responses sometimes could be reversed by small dorsoventral movements of electrodes from the CST into lamina-X. In addition, all stimulation tracks were marked with fluorescent stain with which electrodes were coated, and stimulation sites were marked by small electrolytic lesions. We stimulated at only one site at the bottom of each electrode track. The locations of all electrode tracks and stimulation sites were verified histologically, and data were rejected from tracks in which the electrolytic marking lesion was not contained within the CST.

We recognize that RSNA represents only one of many separately regulated sympathetic pathways (7, 19, 28, 39, 40) and that sympathetic pathways could be affected differentially both by the CST and by chronic spinal lesions. Nevertheless, our original discovery of increased close appositions between CST axons and spinal sympathetic neurons focused on neurons that regulate renal sympathetic activity. Therefore, we felt that the renal sympathetic pathway would be the most appropriate for testing our hypothesis.

We limited our stimulation to the dorsal CST. In fact, the CST descends in three regions of the spinal cord of the rat, dorsal, dorsolateral, and ventral (5, 6). We chose the dorsal division of the CST for two reasons. First, our previous observations indicated that many of the collaterals of the CST that closely apposed spinal sympathetic neurons were derived from the dorsal CST (30). Second, the dorsal CST is a compact bundle of axons, nearly all of which belong to the CST, whereas both the dorsolateral and ventral divisions of the CST are diffuse and intermingled with other descending and ascending pathways. Therefore, the dorsolateral and ventral divisions of the CST cannot be stimulated specifically.

Perspective

A rarely considered consequence of spinal cord regeneration is the formation of inappropriate synapses between spinal somatic and spinal sympathetic systems. Such interconnections could, if they were robust, result in severe autonomic dysfunction. A previous study from this laboratory indicated that spinal injury-induced sprouting of collaterals of the CST substantially increased the anatomical synaptic input from this excitatory pathway to spinal sympathetic neurons. Our observation that CST effects on sympathetic activity were unaffected by chronic spinal cord lesions is encouraging because it demonstrates that even a substantial, injury-induced, anatomical change in synaptic input to spinal sympathetic systems does not necessarily predict a dysfunctional change in the effects of that input. Whether this lack of change was caused by an insufficient number of new synapses to mediate a change or a deficiency in the function of new synapses remains to be determined. We also need to determine whether these new synapses are only transient or, alternatively, whether they become more effective after recovery periods of more than 6 wk. Nevertheless, these experiments demonstrate that the functional significance of synaptic changes in the spinal cord after injury, and therefore after treatments for spinal cord injury can be tested quantitatively. Although the results of the present study are encouraging, the potential danger of inappropriate somatic-autonomic cross wiring is great enough that similar experiments should be conducted as regenerative therapies for spinal cord injury become more effective and widespread.

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GRANTS

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