Identification of the spinal pathways involved in the recovery of baroreflex control after spinal lesion in the rat using pseudorabies virus

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Castillo DG, Zahner MR, Schramm LP. Identification of the spinal pathways involved in the recovery of baroreflex control after spinal lesion in the rat using pseudorabies virus. Am J Physiol Regul Integr Comp Physiol 303: R590–R598, 2012. First published July 18, 2012; doi:10.1152/ajpregu.00008.2012.—Neurons in the rostroventrolateral medulla (RVLM) mediate baroreflex regulation (BR) of sympathetic preganglionic neurons. Previously, our laboratory has shown that recovery of BR occurs in the rat after spinal hemisection, (Zahner MR, Kulikowicz E, and Schramm LP. Am J Physiol Regul Integr Comp Physiol 301: R1584–R1590, 2011). The goal of these experiments was to determine whether the observed recovery of BR is mediated by the reorganization of ipsilateral pathways or by compensation through spared contralateral pathways. To determine this, we infected the left kidney in rats with the retrograde transynaptic tracer, pseudorabies virus (PRV), either 1 or 8 wk after left spinal hemisection at either T3 or T8, or after a sham lesion. In sham-lesioned rats, PRV infection of RVLM neurons was bilateral. In all rats with a left hemisection, regardless of the location of the lesion (T3 or T8) or postlesion recovery time (1 or 8 wk), PRV infection of left RVLM neurons was significantly reduced compared with sham-lesioned rats (P < 0.05). In a separate group of rats, we performed BR tests by measuring responses of left renal sympathetic nerve activity to pharmaco logically induced decreases and increases in arterial pressure. In rats with T8 left hemisection and 8-wk recovery, BR was robust, and acute right upper thoracic hemisection abolished all BR of left renal sympathetic nerve activity. Collectively, these data suggest that the recovery of BR is not mediated by reorganization of ipsilateral bulbo spinal connections, but instead by improved efficacy of existing contralateral pathways.

Neurons in the rostroventrolateral medulla (RVLM) are responsible for the excitability of sympathetic BR via projections to sympathetic preganglionic neurons located in the intermediolateral (IML) cell column of the thoracic and lumbar spinal cord (8, 13). Destruction of the descending pathways responsible for excitation of sympathetic preganglionic neurons impairs the regulation of AP. Previously, we have reported that the BR-related sympathoexcitatory pathways in rats descend bilaterally throughout the dorsal and ventral spinal cord (35).

Several studies have documented the partial recovery of somatic motor function after SCI in rats and mice (3, 7, 12, 20, 33). Similarly, we have shown that BR in spinally lesioned rats improves over time, and that the degree of recovery is dependent on the rostrocaudal location of the lesion (34). In that study, surgical left hemisection at T3 caused modest impairment of BR after 1 wk, and full recovery was observed after 8 wk. Rats with a T8 hemisection showed severe impairment after 1 wk and modest improvement after 8 wk.

The mechanism for restoration of somatic motor function is attributed, in part, to sprouting and reorganization of new and spared brain stem and spinal pathways. In rats, transected cervical corticospinal tract (CST) axons sprout and create new contacts with propriospinal neurons within the cervical gray matter. Over time, the propriospinal neurons create de novo spinal circuits, which circumvent the lesion and contact lumbar motoneurons (3). Based on those findings, we hypothesized that the recovery for BR function in the rat is due to spinal reorganization similar to that which occurs after lesion of the CST. However, the sprouting of bulbo spinal axons is not as robust as that of the CST (23). Therefore, an alternative hypothesis is that the recovery of BR is accomplished by improved efficacy of existing contralateral pathways that, in the absence of the hemisected ipsilateral pathways, are better able to regulate BR.

To test our hypothesis, we used transynaptic pseudorabies virus (PRV) to anatomically trace the supraspinal pathways impinging on sympathetic preganglionic neurons in rats 1 or 8 wk after either a T3 or T8 left hemisection or sham lesion. Using this tracing method, we determined the number of sympathetic preganglionic neuron-projecting RVLM neurons in the right and left brain stem and the degree of lateralization before and after spinal hemisection. Additionally, in another group of rats with T8 left hemisections, we studied BR performance 8 wk after the initial lesion and compared the BR of left renal sympathetic nerve activity (RSNA) (ipsilateral to the lesion) before and after acute contralateral upper thoracic hemisection.

METHODS

Adult, female Sprague-Dawley rats (Charles River, Raleigh, NC), weighing between 100 and 250 g, were surgically prepared in accor-
dance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) using procedures approved by the Johns Hopkins University Committee on Animal Care and Use. A total of 45 rats underwent left spinal hemisection. Six rats died during, or shortly after surgery. Of the 39 surviving lesioned rats, 7 were used for acute physiological BR experiments. Although all of the remaining 32 lesioned rats underwent PRV injection, 11 rats were eliminated from analysis due to either over- or under-infection.

Thoracic spinal cord hemisections. For left hemisection experiments, we anesthetized rats, first in a plastic chamber and then with a nose cone, using 2% isoflurane in O2. Before surgery, we shaved and depilated the dorsal surface of the rat and treated this region with Betadine. We made a 2-cm cutaneous incision centered on the vertebra overlying the site of the intended lesion (T3 or T8). The T3 spinal level underlies the T3 vertebra, and the T8 spinal level underlies the T6 and T7 vertebrae. We used a small retractor to maintain a clear surgical field. We removed the paraspinal muscles from the dorsal surface of the exposed vertebra using a septal elevator. After the dorsal surfaces of the vertebrae were free of muscle and connective tissue, we removed the dorsal portion of the vertebra of choice using a microsurgical tool to access the underlying spinal segment. We opened the dura with dura scissors and cut the left hemisphere of the spinal cord with a 1-mm sapphire blade microknife (World Precision Instruments, Sarasota, FL). We closed the dorsal musculature and skin with sterile sutures and wound clips. We used an identical technique, but without opening the dura, for sham-lesioned rats. We manually expressed rats’ bladders, when necessary, following surgery. We monitored food and water intake daily, and saline was administered subcutaneously, if dehydration was suspected.

PRV injection. Following hemisections, rats were randomly selected for either the 1- or 8-wk recovery period. Rats in each group were euthanized at the end of their respective recovery periods. Three rats in each group (PRV-152/GFP, 4.02 × 10⁶ plaque-forming units/ml, kindly supplied by Dr. Patrick J. Card, University of Pittsburgh, Pittsburgh, PA). The first injection was made at the cranial pole, and the second at the caudal pole of the kidney. Each injection lasted ∼1 min. Care was taken to avoid PRV exposure to the surrounding tissue. The musculature and skin were closed with sterile sutures and wound clips. After surgery, 3 ml of saline were administered subcutaneously. Rats survived for 72 h after inoculation to allow for proper infection of sympathetic preganglionic neurons at the spinal level and spinally projecting neurons at the brain stem level, as previously demonstrated by our and other laboratories (4, 23). During this time, rats were carefully observed several times a day to ensure that there were no signs of discomfort. Rats ate, drank, and groomed normally during this postinoculation period.

Data from this and other laboratories indicate that the degree of PRV infection is closely related to the volume and site of injection and to survival time (4, 27). For the chosen 72-h survival period after PRV inoculation, our criterion for an appropriate degree of infection was that both sympathetic preganglionic neurons and RVLM neurons were infected with minimal infection of α-motoneurons (≤10 neurons).

Tissue preparation. At 72 h postinoculation, rats were anesthetized with 2% isoflurane and perfused transcardially with 400 ml of physiological saline, followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer. Spinal cords and brains were removed and postfixed in the perfusion fixative for 48 h at 4°C and then transferred to 30% sucrose in 0.1 M phosphate buffer overnight. The cerebellum was removed from the brains to allow a clear view of the brain stem, which was sectioned from the caudal pole of the facial nucleus to the obex to include the RVLM. Serial coronal sections of brain stem and horizontal sections of spinal cord from T1 to T12 were cut with a freezing microtome (40 μm). For each rat, two sets of brain stem and spinal cord sections were collected. Only one set of sections (i.e., every other 40-μm section) was used for quantification of PRV-labeled neurons. Sections were stored in 0.1 M PBS at 4°C before immunohistochemical processing. After processing, sections were mounted on gelatin-coated glass slides. Spinal hemisections were examined microscopically to verify the completeness of the hemisections (Fig. 1).

Immunohistochemistry. Immunohistochemistry was used to increase the GFP signal of PRV-infected neurons. Sections were rinsed three times in 0.1 M PBS and incubated with slow agitation for 3 h at room temperature in a blocking solution containing 5% normal goat serum, 3% bovine serum albumin, and 0.2% Triton (R) X-100. After

![Fig. 1. Histological horizontal sections of a representative left surgical hemisection (T3). Thick horizontal lines represent the horizontal plane of the respective histological sections. A: corticospinal tract (CST). B: lateral funiculus (LF). C: ventrolateral funiculus (VLF). The left hemisection completely severed the left LF and VLF. Scale bar = 1 mm.](http://ajpregu.physiology.org/doi/abs/10.1152/ajpregu.00008.2012)
removal of blocking solution, sections were incubated overnight at room temperature in a solution containing 3% bovine serum albumin, 0.2% Triton X, and a rabbit anti-GFP primary antibody conjugated to Alexa 594 (1:1,000; Invitrogen, Carlsbad, CA). Sections were then washed three times for 10 min with 0.1 M PBS and mounted on glass slides before being studied with fluorescence microscopy.

Quantification of PRV-infected cells. We identified the RVLM using the criteria of Moon et al. (18). For quantification of PRV-labeled neurons, the dorsolateral extent of the RVLM area was defined by a triangular region enclosed by the apex of the nucleus ambiguus, the lateral border of the pyramidal tract, and the ventral border of the spinal trigeminal nucleus. Only PRV-infected cells within this area were counted. Similarly, only PRV-infected cells in the IML, located between T1 and L1, were counted. Although renal inoculation of PRV for identification of spinal sympathetic pathways is a reliable and common tool, not all PRV-infected cells in the spinal cord are sympathetic preganglionic neurons (29). Therefore, we refer to PRV-positive neurons located in the IML as sympathetically related neurons.

We counted the number of PRV infected neurons in the right and left RVLM and the right and left IML to determine the strength of descending projections, either 1 or 8 wk after left spinal hemisection. Neuronal cell bodies were distinguished and counted as previously reported (29). Because we counted cells from every other 40-μm section, there was little likelihood of double counting of cells. We determined the changes in the lateralization of the descending spinal projections by calculating the ratio of the number of PRV-infected neurons in the left or right RVLM to PRV-infected neurons (sympathetically related neurons) in the left IML. The histological data (number of PRV-labeled neurons and all ratios) are expressed as means ± SE. Statistical analysis employed one-way ANOVA (with Bonferroni’s posttests) to compare the ratio of five different treatment groups, while a two-way ANOVA (with Bonferroni’s posttests) was used to compare the ratio of five different treatment groups. Values of P < 0.05 were considered significant.

Acute surgical preparation. In a separate group of rats with T8 left hemisection and an 8-wk recovery period, but which were not infected with PRV, we tested BR regulation of left RSNA before and after acute right upper-thoracic hemisection. For these acute BR experiments, we initially anesthetized rats with isoflurane, as previously described. We discontinued isoflurane after administration of α-chloralose (100 mg/kg iv, Sigma) via a left jugular cannula. This cannula was also used to administer gallamine triethiodide (see below). We maintained the depth of anesthesia at a surgical plane by supplemental doses of α-chloralose (25 mg/kg). We determined the depth of anesthesia either by corneal reflexes before and during the recovery from paralysis, or by the variability of RSNA and AP when rats were paralyzed. We monitored body temperature with a rectal probe and maintained it between 35 and 37°C with a heating lamp. We cannulated the trachea for mechanical ventilation using a rodent ventilator (CWE, Ardmore, PA), and we cannulated the right femoral artery for measurement of AP. We recorded AP and heart rate (HR) simultaneously with Cambridge Electronic Design Micro1401 hardware and Spike 2 software. We cannulated the left and right femoral veins for the separate administration of depressor and pressor drugs, respectively.

After baseline BR tests in the rats with chronic (8 wk) T8 left hemisection, we performed an acute contralateral upper thoracic hemisection and tested BR function again. To acutely hemisect the spinal cord, we made a 2-cm cutaneous incision at the T1 spinal level. We used a small retractor to maintain a clear surgical field and removed the paraspinal muscles from the dorsal surface of the exposed vertebrae using a separtal elevator. We performed a laminectomy, using a micro-rongeur, to access the underlying spinal segment, and we opened the dura with dura scissors and cut the right hemisphere of the T1 spinal cord with a 1-mm sapphire blade, microknife (World Precision Instruments, Sarasota, FL).

The T1 right hemisection was chosen to ensure that all thoracic contralateral projections from the right RVLM were destroyed.

Renal sympathetic nerve recording. Preparation for RSNA recording has been described elsewhere in detail (5). We approached the left kidney via a left flank incision and carefully dissected the renal nerve from the renal vasculature and surrounding tissue with the aid of an operating microscope. Then we immersed the renal nerve in mineral oil and mounted it on a bipolar hook electrode connected to a differential amplifier with a bandpass of 300–3,000 Hz. We cut the distal end of the renal nerve to avoid recording afferent activity. We further processed sympathetic activity by rectification and low-pass filtering at a time constant of 0.5 s and recorded both the unprocessed and the rectified/filtered activity with the AP and HR.

Baroreflex measurement. We obtained baroreflex function curves by plotting the reflex change in RSNA to increases and decreases in AP caused by the vasodilator sodium nitroprusside (SNP; 50 μg/ml) and the α-adrenergic agonist phenylephrine (PE; 125 μg/ml), respectively, in successive ramped infusions. We administered SNP first, beginning at a rate of 2.5 ml/h, and increased the rate by 2.5 ml/h every 30 s until an AP of 60 mmHg below baseline or a maximum rate of 25 ml/h was reached. Following SNP administration, we administered PE, beginning at a rate of 2.5 ml/h and increasing the rate by 2.5 ml/h every 30 s. These infusions produced an approximately linear increase in AP from 60 mmHg below baseline AP to 60 mmHg above baseline AP at a rate of ~1.5 mmHg/s. We analyzed the RSNA from the SNP-induced nadir of AP (60 mmHg below baseline) to the PE-induced peak of AP (60 mmHg above baseline). We fit baroreflex function curves to a sigmoidal function (17). We used the maximum slope of the sigmoidal curves as our measurement of the gain of the baroreflex.

Data analysis. We fit each RSNA response to changes in AP to either a sigmoidal or linear function, as appropriate, using Prism software (Graphpad, version 4.0). The sigmoidal function is described by the following equation

\[ y = A_1 \left(1 + \exp\left(A_2(x - A_3)\right)\right) + A_4 \]

where y is the RSNA, x is AP, A1 is the range of RSNA, A2 is the gain coefficient, A3 is the value of x at the midpoint, and A4 is the minimum RSNA of the reflex curve (17). For all rats, this sigmoidal function was best fit to the RSNA-AP relationship prior to acute contralateral hemisection. After acute hemisection, a linear function was the best fit to the relationship. We calculated the maximum gain (maximum slope of the AP-RSNA relationship) and change in RSNA according to each fitted function.

To construct grouped BR relationship curves, we averaged AP and corresponding RSNA data in 10-mmHg bins. To account for variations in baseline AP, we used the change from baseline as the reference for the AP bins when constructing the BR curves. Thus the data are expressed as mean change in AP (in mmHg) from baseline and the percent change in RSNA. Stable plateau values were determined when RSNA for a particular 10-mmHg AP bin did not vary by >5% from the previous RSNA value in that 10-mmHg AP bin. The maximum RSNA plateau occurred typically at least 50 mmHg below baseline AP, and the minimum RSNA plateau at least 30 mmHg above baseline AP. All grouped data are expressed as means ± SE. Statistical analyses employed one-way ANOVA (with Bonferroni’s posttests). Values of P < 0.05 were considered significant.

RESULTS

PRV labeling of sympathetically related neurons after chronic left spinal hemisection. The degree of PRV infection of sympathetically related neurons was independent of the locations of hemisections (T1 or T8), or the interval after hemisections (1 or 8 wk). A representative image of the thoracic spinal cord showing PRV infection is shown in Fig. 2A. Inoculation of the
left kidney infected mainly cells in the left IML of the spinal cord.

The mean number of PRV infected cells in the left IML and right IML in lesioned rats was not significantly different from that of the sham-lesioned rats (Fig. 2, B and C). In sham-lesioned rats (n = 6), the mean number of PRV-infected cells was 449.2 ± 55.1 in the left IML and 36.8 ± 9.3 in the right IML. In rats infected with PRV 1 wk after T3 hemisection (n = 5), the mean number of PRV-infected cells was 533.2 ± 112.1 in the left IML and 40.4 ± 14.0 in the right IML. In rats infected with PRV 8 wk after T3 hemisection (n = 5), the mean number of PRV-infected cells was 350.4 ± 29.1 in the left IML and 23.6 ± 7.5 in the right IML.

PRV labeling of RVLM neurons after left spinal hemisection. In sham-lesioned rats, PRV inoculation of the left kidney labeled spinally projecting RVLM neurons bilaterally (Fig. 3, A and B). In addition to neurons in the RVLM, we also...
observed PRV labeling of neurons in the caudal medullary raphe nuclei, the caudal ventrolateral medulla, and the solitary nucleus.

In sham-lesioned rats, PRV inoculation labeled 94.2 ± 18.3 neurons in the left RVLM and 92.0 ± 16.6 in the right RVLM (Fig. 3B). In all lesioned rats, PRV labeling of left RVLM (ipsilateral to the left spinal hemisection) was significantly less ($P < 0.05$) compared with that of the sham-lesioned rats. With the exception of the rats euthanized 8 wk after T8 hemisection, the number of PRV-labeled neurons in the left RVLM in all lesioned rats was significantly less than the number of PRV-labeled neurons in the right RVLM ($P < 0.05$). In rats euthanized 1 wk after T3 hemisection, 9.4 ± 1.8 neurons in the left RVLM and 41.2 ± 11.3 neurons in the right RVLM were PRV labeled. In rats euthanized 8 wk after T3 hemisection, 8.2 ± 1.0 neurons in the left RVLM and 41.8 ± 9.9 neurons in the right RVLM were PRV labeled. In rats euthanized 1 wk after T8 hemisection, 31.6 ± 4.0 neurons in the left RVLM and 64.2 ± 15.7 neurons in the right RVLM were PRV labeled. In rats euthanized 8 wk after T8 hemisection, 16.2 ± 1.9 neurons in the left RVLM and 30.6 ± 4.1 neurons in the right RVLM were PRV labeled.

Laterality of spinally projecting RVLM neurons after left spinal hemisection. In the sham-lesioned rats, PRV inoculation of the left kidney produced symmetrical infection of the RVLM (Fig. 3C). The ratio of PRV-labeled neurons in the left RVLM to the number in the right RVLM was significantly different from control ($*P < 0.05$).

Fig. 3. A: representative coronal sections of the medulla at the level of the rostroventrolateral medulla (RVLM). The boxed areas show PRV infection of the left and right RVLM from rats with either T3 or T8 left hemisection and either 1- or 8-wk recovery or sham lesion. B: grouped data showing the number of PRV-infected neurons in the left and right RVLM either 1 wk ($T_3: n = 5$, $T_8: n = 5$) or 8 wk ($T_3: n = 5$, $T_8: n = 5$) after either $T_3$ or $T_8$ left hemisection (or sham lesion, $n = 6$). C: grouped data showing the ratio of the number of PRV-infected neurons in the left RVLM to the number in the right RVLM. Values are means ± SE. *Significantly different from control; #significantly different from right RVLM: $P < 0.05$. 

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RVLM to those in the right RVLM was 1.0 ± 0.07. In all hemisectioned rats, the ratio of PRV-labeled neurons in the left RVLM to those in the right RVLM was significantly less ($P < 0.05$) compared with that in sham-lesioned rats. However, the laterality of RVLM neurons 1 and 8 wk after either a T3 or a T8 left hemisection were not significantly different. In rats infected with PRV 1 wk after T3 hemisection, the ratio of PRV-labeled neurons in the left RVLM to the number of PRV-labeled neurons in the left spinal cord was 0.28 ± 0.07, and, in rats infected with PRV 8 wk after T3 hemisection, this ratio was 0.25 ± 0.17. In rats infected with PRV 1 wk after T8 hemisection, the ratio of PRV-labeled neurons in the left RVLM to those in the right RVLM was 0.56 ± 0.20, and in rats infected with PRV 8 wk after T8 hemisection this ratio was 0.56 ± 0.07.

In sham-lesioned rats, the ratio of the number of PRV-labeled neurons in the left RVLM to the number of PRV-labeled neurons in the left spinal cord and the ratio of the number of PRV-labeled neurons in the right RVLM to the number of PRV-labeled neurons in the left spinal cord were identical (0.21 ± 0.04, Fig. 4). With the exception of the ratio from the right RVLM to the left spinal cord in rats 1 wk after a T8 hemisection, the ratios in all lesioned rats were significantly smaller compared with those of sham-lesioned rats. Additionally, in all lesioned rats, the ratio of the number of PRV-labeled neurons in the left RVLM to the number of PRV-labeled neurons in the left spinal cord was significantly smaller than the ratio of the number of PRV-labeled neurons in the right RVLM to those in the right RVLM. In rats infected with PRV 1 wk after T3 hemisection, this ratio was 0.25 ± 0.01, and the ratio of the number of PRV-labeled neurons in the right RVLM to those in the left RVLM was 0.56 ± 0.02. In rats infected with PRV 8 wk after T3 hemisection, this ratio was 0.12 ± 0.02. In rats infected with PRV 8 wk after T8 hemisection, the ratio of the number of PRV-labeled neurons in the left RVLM to those in the right RVLM was 0.05 ± 0.01, and the ratio of the number of PRV-labeled neurons in the right RVLM to the number of PRV-labeled neurons in the left spinal cord was 0.09 ± 0.01.

Baroreflex function after chronic T8 left spinal hemisection. To determine whether improvement of BR was mediated via increased efficacy of contralateral pathways, we assessed BR after making an acute T1 right hemisection in rats with an existing chronic (8 wk) T8 left hemisection. This hemisection removed the fraction of BR mediated by contralateral pathways and, therefore, removed compensation by contralateral pathways. In this group of rats ($n = 6$), the mean baseline AP was 120 ± 3 mmHg and HR was 403 ± 25 beats/min. During BR testing, a 60-mmHg decrease in AP produced an increase in RSNA to a maximum plateau of 120 ± 5% of baseline, and a 60-mmHg increase in AP decreased RSNA to a minimum plateau of 38 ± 4% of baseline. The mean maximum gain of the BR was $-1.8 ± 0.4 \Delta%RSNA/\Delta AP$ ($\Delta%RSNA/\Delta AP$, the change in the percentage of RSNA to the change in the AP).

The acute T1 right hemisections did not significantly affect baseline BP (118 ± 4 mmHg), HR (392 ± 5 beats/min), or ongoing RSNA (94 ± 10% of baseline RSNA prior to right hemisection). However, acute T1 right hemisections abolished all BR (Fig. 5, A and C, $P < 0.05$). The maximum gain of the BR was $-0.12 ± 0.11 \Delta%RSNA/\Delta AP$ and was significantly reduced compared with BR gain prior to right hemisection ($-1.8 ± 0.4 \Delta%RSNA/\Delta AP$, Fig. 5D, $P < 0.05$).

**DISCUSSION**

This study provides important new anatomical and physiological information on the pathways involved in the recovery of BR after SCI. Based on the anatomical and physiological data from this study, the improved BR following spinal hemisection is caused by an improved efficacy of existing contralateral pathways, which cross the spinal cord caudal to the lesion. Our results do not support the hypothesis that recovery of BR is mediated by de novo crossing and recrossing ipsilateral descending pathways.

In the spinally intact rat, the excitatory BR pathways descend bilaterally, and left hemisection severely impairs BR, which then improves over time (34). Because this improvement is contingent on the rostrocaudal location of the lesion and because rats recover more completely from rostral lesions, we chose to investigate the PRV labeling after both T3 and T8 left hemisections. We identified spinally projecting RVLM neurons using PRV injections into the left kidney of rats either 1 or 8 wk after left spinal hemisection (T3 or T8) or after a sham lesion. We show that thoracic left hemisection significantly reduces the number of ipsilateral spinally projecting RVLM neurons, regardless of the rostrocaudal location of the thoracic lesion, and that this reduction does not change over time. We also show that a right T1 hemisection abolished the
BR, which had recovered 8 wk after a left T8 hemisection. Thus our results suggest that, although BR in rats improves over time (34), this recovery is not mediated by “new” connections from ipsilateral RVLM neurons, which circumvent the spinal lesion contralaterally to recross and synapse on sympathetically related neurons on the ipsilateral side.

In rats with intact spinal cords, renal PRV injections infect RVLM neurons bilaterally (4, 28). Surgical lesion experiments in rat show that RVLM axons decussate mainly at the level of the spinal cord (28). In the present experiments, we found that nearly all PRV labeling in the left RVLM was abolished in rats infected with PRV 1 wk after left hemisection at either T3 or T8. This observation was not different from that in rats infected with PRV 8 wk after left hemisection. Conversely, in the same rats infected with PRV 1 wk after left hemisection, PRV labeling in the right RVLM was reduced, but this decrease in PRV labeling only reached statistical significance in the rats infected 8 wk after T8 hemisection. That there was no improvement in the number of spinally projecting neurons in the left RVLM in rats 1 wk compared with the number at 8 wk after hemisection strongly suggests that any improvement in BR was not mediated by reorganization of ipsilateral spinal pathways. The number of spinally projecting RVLM neurons on the right side was smaller 8 wk after only the T8, but not the T3, spinal hemisection. Thus, although BR was modestly improved 8 wk after T8 left hemisection, the number of spinally projecting RVLM neurons did not increase. This suggests that the improvement of BR regulation was due to increased efficacy of the remaining RVLM neurons.

Recovery of somatic motor function after spinal lesions has been reported in the rat and mouse (2, 3, 7, 12, 33). We have recently shown that BR also improves after spinal hemisection (34). The mechanism for restoration of somatic motor function is attributed, in part, to robust sprouting and reorganization of...
new and spared connections. In rats, for example, transected cervical CST axons sprout and create new contacts to long and short propriospinal neurons within the cervical gray matter. Over time, the long propriospinal neurons create de novo spinal circuits, which circumvent the lesion and contact lumbar motoneurons (3). Based on those observations, we hypothesized that the recovery of BR function in the rat might be mediated by spinal reorganization similar to that which occurs after lesion of the CST. However, the sprouting of bulbospinal axons after spinal lesions is not as robust as that of the CST (23). Therefore, an alternate explanation for the observed recovery of BR function is that, rather than establishing “new” connections that circumvent the lesion, existing contralateral pathways might be strengthened. Before lesion, the main functional descending pathways responsible for the reduction in sympathetic activity on increases in AP are unilateral (35). After hemisection and in the absence of these pathways, previously weak contralateral pathways might strengthen, mediating the improvement of BR.

A similar mechanism for functional recovery of respiration after upper cervical hemisection has been known for well over a century, the “crossed phrenic phenomenon” (24, 25). In this case, cervical hemisection that interrupts descending bulbospinal respiratory pathways leads to ipsilateral hemiparalysis of the diaphragm. However, sectioning the contralateral phrenic nerve restores ipsilateral phrenic activity and diaphragm contractility. Neurons of the medullary rostral ventral respiratory group make monosynaptic connections to phrenic motoneurons, and propriospinal networks are not involved in the crossed phrenic phenomenon (19). The recovery of phrenic activity ipsilateral to the spinal hemisection is due to compensation by contralateral pathways that cross caudal to the spinal hemisection (for review, see Ref. 11).

We found that, 1 wk after left surgical spinal hemisection, the number of PRV-positive neurons in the left RVLM was substantially reduced compared with the number in sham-lesioned rats. No increase in the number of neurons or in the ratio of left RVLM neurons to left sympathetically related neurons in the IML was observed in rats infected 8 wk after left hemisection, regardless of the rostrocaudal location of the lesion. Therefore, the improvement of BR is most likely mediated by increased strength of existing contralateral pathways that cross the midline of the spinal cord between the level of the lesion and the level of the renal sympathetic preganglionic neurons, not reorganization of descending ipsilateral pathways.

To confirm our observations that strengthening of contralateral pathways mediates the improvement of BR, we performed BR tests before and after an acute right upper (T1) thoracic hemisection in rats that had undergone a T8 left hemisection 8 wk prior. In the rat, a complete spinal transection removes only supraspinal regulation of the sympathetic activity. In the spinal intact rat, tonically active sources of descending inhibition suppress spinal sources of activity to thereby suppress the spinally generated sympathetic activity, whereas, in the transected rat, spinal sources of activity are unimpeded (21, 26). Thus, even after acute right hemisection in rats with chronic left hemisection, basal sympathetic outflow driven by spinal sources exists. However, as predicted, acute T1 contralateral lesion abolished all supraspinally mediated BR.

We did not observe the impairment of PRV infection of sympathetically related neurons after spinal lesions that has been reported by others (9). One reason for this discrepancy is that the degree of infection at the spinal level is contingent on postinoculation time (27). Based on previous data published from this laboratory and others, we used a 72-h postinoculation time. This duration allows for optimal infection and retrograde transport of PRV to the brain stem. Studies using longer postinoculation time may risk lysis of sympathetic preganglionic neurons and underestimation of infected neurons. This phenomenon has previously been observed in a study that employed a 96-h postinoculation time (4). Consistent with the literature (4, 28), we found that, in sham-lesioned rats, PRV distribution in the RVLM is symmetrical. Thus projections of ipsilateral and contralateral projections from the RVLM to sympathetically related neurons in the left spinal cord are equal.

Perspectives and Significance

Loss of BR after SCI seriously degrades sympathetic cardiovascular regulation. This degradation leaves SCI patients at risk for orthostatic intolerance or autonomic dysreflexia, often including hypertensive crises. The findings in this study have more specifically identified important pathways responsible for BR recovery after SCI in rats. Discovering methods to improve and accelerate the efficacy of contralateral pathways could lead to treatments that improve BR recovery and increase the quality of life for SCI patients.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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