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

Deborah G. Castillo, Matthew R. Zahner, and Lawrence P. Schramm

1Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland; and 2Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Submitted 5 January 2012; accepted in final form 3 July 2012

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 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 pharmacologically induced decreases and increases in arterial pressure. In rats with T8 left hemisection and 8-wk recovery, BR was robust, and sympathetically induced decreases and increases in arterial pressure. In rats with T8 left hemisection and 8-wk recovery, BR was robust, and these experiments were 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 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 pharmacologically 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 bulbospinal connections, but instead by improved efficacy of existing contralateral pathways.

BAROREFLEX REGULATION (BR) of sympathetic and parasympathetic activity is an important mechanism for the moment-to-moment regulation of arterial pressure (AP) (8, 13). Loss of BR after spinal cord injury (SCI) can seriously impair sympathetic cardiovascular regulation. After SCI, the decreased ability to regulate sympathetic activity leaves patients at risk to varying degrees of orthostatic intolerance or autonomic dysreflexia with hypertensive crises. Orthostatic intolerance results from insufficient BR-mediated sympathetic outflow to maintain an upright posture without syncope (1, 6, 14, 15, 30). Autonomic dysreflexia occurs due to diminished suppression of spinal sources of sympathetic activity caudal to the lesion (10, 14, 16, 22, 31, 32). Both conditions can be attributed to limited regulation of the activity of spinal sympathetic preganglionic neurons caudal to the lesion.

Neurons in the rostral ventrolateral 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 brainstem 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 bulbospinal 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 (T1 or T8). The T1 spinal level underlies the T2 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 vertebrae 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 microrongeur 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 the 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 days prior to euthanasia, rats were anesthetized using 2% isoflurane in O2, as described above. Before surgery, we cleaned the skin overlying the left kidney with Betadine. We made a 3-cm cutaneous incision just below the left rib cage, followed by a left flank laparotomy to expose the left kidney. We used a 26-gauge Hamilton syringe (Hamilton, Reno, NV) to make two injections (1.0 μl each) of the recombinant Bartha strain of PRV constructed to express green fluorescent protein (PRV-152/GFP, 4.02 × 10⁸ 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 for several days 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 RVL 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 RVL. 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 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 (T8). 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.](image-url)
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 fluorescent 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 laterofacialization 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 applied to compare the ratio of five different treatment groups.

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 (T3 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 441.8 ± 60.1 in the left IML and 34.3 ± 10.7 in the right IML. In rats infected with PRV 1 wk after T8 hemisection (n = 5), the mean number of PRV-infected cells was 555.0 ± 71.3 in the left IML and 37.4 ± 6.7 in the right IML. In rats infected with PRV 8 wk after T8 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

Fig. 2. A: representative horizontal sections of thoracic spinal cord at the level of the intermediolateral (IML) column. The boxed area (T8 to T10) shows a representative pseudorabies virus (PRV) infection of neurons in the IML in rats with either T3 or T8 left hemisection and after either 1- or 8-wk recovery or sham lesion. R, right; L, left. Note the largely ipsilateral PRV infection of sympathetically related neurons. Scale bar = 1.0 mm (top) and 200 μm (bottom). Grouped data show the number of PRV-infected neurons in the left IML (B) and right IML (C), either 1 wk (T3: n = 5, T8: n = 5) or 8 wk (T3: n = 5, T8: n = 5) after either T3 or T8 left hemisection (or sham lesion; n = 6). Values are means ± SE.
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 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.
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 lateralities 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 the number of PRV-labeled neurons in the left spinal cord. In rats infected with PRV 1 wk after T3 hemisection, 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 0.02 ± 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 0.08 ± 0.04. In rats infected with PRV 8 wk after T3 hemisection, 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 0.02 ± 0.003, 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 ± 0.02. In rats infected with PRV 1 wk after T8 hemisection, 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 0.06 ± 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 0.12 ± 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 the number of PRV-labeled neurons in the left spinal cord 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 $-1.8 ± 0.4 \% \text{RSNA}/\text{AP}$ ($\% \text{RSNA}/\text{AP}$, 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 $-0.12 ± 0.1 \% \text{RSNA}/\text{AP}$ and was significantly reduced compared with BR gain prior to right hemisection ($-1.8 ± 0.4 \% \text{RSNA}/\text{AP}$, 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 in rats 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 T6 left hemisection 8 wk prior. In the rat, a complete spinal transection removes only supraspinal regulation of the sympathetic activity. In the spinally 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 unimpeeded (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.

GRANTS

This research was supported by National Heart, Lung, and Blood Institute Grant HL-16315 (to L. P. Schramm). M. R. Zahnner was supported by National Heart, Lung, and Blood Institute Training Grant 2-T32-HL-007581. Pseudorabies virus (PRV-152/GFP) was kindly supplied by Dr. Patrick J. Card, University of Pittsburgh, Pittsburgh, PA, and Dr. Lynn Enquist, Princeton University, Princeton, NJ., Co-Directors of the Center for Neuroanatomy with Neurotropic Viruses.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES
