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Elevated renal nerve activity after spinal transection: effects on renal function

OSBORN, JOHN W., JR., RENEA H. LIVINGSTONE, AND LAWRENCE P. SCHRAMM
Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

SPINAL CORD INJURY causes a variety of autonomic and cardiovascular dysfunctions. The fall in arterial blood pressure that follows either complete or subtotal cervical spinal cord transection is often considered a manifestation of a generalized decrease in sympathetic activity. Indeed there seems to be general agreement that spinal cord injury reduces sympathetic activity to the tissues of the body wall (see Ref. 20 for review).

On the other hand, the response of abdominal sympathetic activity to spinal transection is more controversial. Fealy et al. (6) have suggested that the gastric hypomotility observed in humans after spinal lesions might be a manifestation of elevated gastric sympathetic activity. Meckler and Weaver (13) found that, although renal sympathetic activity was reduced by spinal transection in cats, splenic activity was not. In spinally transected rats, Taylor and Schramm (24) found that lumbar sympathetic activity was markedly reduced. However, both renal and splenogastric sympathetic activity were approximately doubled. They concluded that spinal systems exist that are capable of generating substantial renal and splenogastric activity and that these systems are under strong, tonic, supraspinal inhibition in the intact rat. However, they did not determine the spinal course of these descending sympathoinhibitory pathways.

Taylor and Schramm (24) also suggested that the autoregulatory properties of the abdominal vasculature might prevent the elevation in abdominal sympathetic activity from compensating for the reduction in sympathetic activity to the body wall, resulting in the observed reduction in arterial pressure. However, they made no direct observations of vascular resistance.

There are several reasons to believe that acute spinal transaction would substantially alter renal function. First, transection invariably reduces renal arterial pressure. The renal circulatory responses to this decrease, alone, could reduce salt and water excretion. Second, increases in renal sympathetic activity can reduce renal salt and water excretion, independent of overall renal circulatory changes (see Ref. 5 for review), and spinal transection in the a-chloralose-anesthetized rat substantially increases renal sympathetic activity.

The present experiments were conducted to answer three questions. First, what are the effects of subtotal transections of the spinal cord on renal sympathetic activity and what can be concluded from these effects about the loci of descending pathways that modulate renal sympathetic activity? Second, after spinal transection, what is the effect of the elevated renal sympathetic activity on renal hemodynamics. Finally, what is the role of the increased sympathetic activity in modifying renal function after spinal transection?

METHODS

General Surgical Methods

Charles River Sprague-Dawley rats were anesthetized with ether. After femoral venous cannulation, a-chloralose (100 mg/kg) was administered. Supplemental doses of a-chloralose (25 mg/kg) were administered as necessary to maintain a moderately deep level of anesthesia. Tracheal and carotid arterial cannulas were implanted for administration of artificial respiration and measurement of arterial pressure, respectively. Heart rate was monitored with aortic arch catheters.
measured in some rats with a cardiotachometer driven by the electrocardiogram.

Rats were placed in a stereotaxic apparatus. The cervical spinal cord was exposed via a laminectomy. Through a left flank incision, the left renal nerve was approached following ventral reflection and lateral retraction of the left kidney. All lesions or transections of the spinal cord were made with microdissecting scissors or scalpel blades. Body temperature was maintained between 36 and 38°C. Gallamine triethiodide (Flaxedil) was administered periodically to induce skeletal muscle paralysis. Rats were artificially respired at rates and minute volumes that maintained arterial PCO₂ at normal levels. The level of anesthesia was determined from the corneal reflex before each supplemental dose of Flaxedil and by autonomic responses to noxious stimuli at other times.

**Renal Nerve Recording and Signal Processing**

Renal nerves were dissected from the renal artery or from the junction of the renal artery and the aorta and kept under warm mineral oil. For studies of renal function, the nerves were left intact. In other experiments, they were cut distally. Multiunit recordings from bipolar electrodes were amplified ~100,000 times in two stages by Grass P15 and either Grass P511 or 7P5A amplifiers. Nerve activity was full-wave rectified, integrated, and expressed in arbitrary units proportional to volt-seconds. In most experiments nerve activity was recorded on magnetic tape along with arterial pressure.

Quantification of total efferent sympathetic activity was accomplished in one of two ways. For recordings made on nerves that had been sectioned distally (total recorded activity was equal to the sum of efferent activity plus electrical noise), the nerves were cut proximal to the recording electrode, and the electrical noise remaining after nerve section was recorded. For recordings made on intact nerves (total recorded activity equal to the sum of efferent and afferent activity plus electrical noise), the signal remaining after intravenous administration of hexamethonium (20 mg/kg) was assumed to represent electrical noise and afferent activity. The electrical activity that remained after nerve section or ganglionic blockade was recorded and processed in a manner identical to that described above. The magnitude of this signal was considered to be an estimate of “zero” efferent nerve activity, and it was subtracted from previously quantified activity to arrive at true estimates of efferent sympathetic activity.

**EXPERIMENTAL PROTOCOLS**

**Sequential Lesions of Spinal Cord**

Rats were prepared as described in General Surgical Methods above. Average renal sympathetic activity was measured while the spinal cord was intact. Then between two and four transverse lesions were made between C1 and C2. Each lesion destroyed either the right or left half of the spinal cord or a single quadrant (left dorsal, right dorsal, left ventral, or right ventral) of the spinal cord. These lesions were purposely made at several adjacent transverse levels to facilitate histological reconstruction.

Renal nerve sympathetic activity was measured after each lesion.

At the end of each of these experiments rats were perfused with saline followed by 10% Formalin. Spinal cords were removed and stored for at least 1 wk in Formalin. They were then embedded in paraffin and sectioned serially in a horizontal plane. Sections were stained by the Weil technique for myelinated axons and counterstained with neutral red for neuronal somas. Lesions were reconstructed and projected onto a standardized coronal section of cervical spinal cord by microscopic inspection of these sections.

**Studies of Renal Circulation and Renal Function**

Experiments were conducted to examine indices of renal function, before and after spinal transection, in relation to changes in renal sympathetic nerve activity. Subsequent to preparation for renal nerve recording, rats were instrumented for measurement of glomerular filtration rate and renal blood flow as well as for sodium, potassium, and urine excretion rates. Glomerular filtration rate was estimated using standard inulin clearance techniques. A 2 g% (wt/vol) solution of inulin (Sigma, St. Louis, MO) in 0.9% saline was infused intravenously at a rate of 33 μl/min. The ureter was cannulated with PE-10 tubing for timed urine collections into preweighed vials for gravimetric determination of urine flow rate.

Renal blood flow was measured using a 0.5-mm pulsed Doppler flow probe (Valpey Fisher, Hopkington, MA) and a Doppler flowmeter (University of Iowa, Iowa City, IA). Special care was taken to prevent damage to the renal nerve during placement of the probe. The nerve was first dissected free from the aorta ~1-5 mm rostral to the renal artery and tested for activity by recording. The course of the nerve along the renal artery was noted, and the artery was carefully separated from the renal vein with the nerve attached. The flow probe was then placed on the renal artery and stabilized by taping the probe leads to the stereotaxic apparatus. At the end of the experiment, to test whether the nerve was damaged during the dissection, the recording electrode was connected to a Grass SD9 stimulator, the nerve was stimulated (20 V, 15 Hz), and the renal blood flow response was noted. Only those rats in which a renal vasoconstriction was observed were used for data analysis. Plasma and urine inulin concentrations were assayed using the anthrone method (8), and sodium and potassium concentrations were measured by flame photometry (Beckman model FES 1000). Three groups of rats were studied.

**Group 1 (n = 8).** The effect of spinal transection on renal sympathetic nerve activity, mean arterial pressure, renal blood flow, glomerular filtration rate, and sodium, potassium, and urine excretion rates were determined. Renal vascular resistance was calculated as the quotient of mean arterial pressure and renal blood flow. After surgical preparation the animals were allowed to stabilize for a period of 15–30 min before control measurements were taken. All variables were measured at steady state during a 15-min clearance period. Arterial blood samples (150 μl) were collected at the midpoint of each clearance period for measurement of hematocrit and plasma inulin.
concentration. Measurements were made immediately before and 30–40 min after spinal transection at a time when arterial pressure, renal blood flow, and renal nerve activity had reached steady state.

Group 2 (n = 9). The protocol for this group was identical to group 1, with the exception that an adjustable clamp was placed around the aorta, distal to the renal arteries. Once arterial pressure had stabilized after spinal transection, the clamp was adjusted to return arterial pressure to pretransected levels. Between 10 and 15 min later, posttransection measurements were made.

Group 3 (n = 5). The protocol for this group was identical to that for group 2. However, these experiments were conducted in rats which, 7–10 days previously, had undergone denervation of the left kidney. Denervation was accomplished by cutting all visible nerves, stripping the renal artery and vein of adventitia and, then, painting the vessels with a solution of 10% phenol in ethanol.

Statistical Analysis

The probability that spinal lesions were responsible for observed changes in renal sympathetic activity was determined by the sign test (22). The probability that different lesions caused different changes in sympathetic activity was determined by Fisher’s exact probability test (22). In the studies on renal function, groups were compared by unpaired t tests, paired t tests, or analysis of variance, as appropriate (23). Values of P < 0.05 were considered statistically significant. Data are expressed as means ±SE.

RESULTS

Effects of Sequential Spinal Lesions

Ideally, we would have conducted an entire series of experiments using each of the 44 possible sequences by which a spinal cord can be transected using combinations of quadrant lesions and hemisections. This was not practicable. Therefore we chose several sequences that held the greatest promise of elucidating the roles of spinal pathways. Histological analysis indicated that the lesions in 22 rats were well enough defined to be categorized as having destroyed either single spinal quadrants or the right or left half of the spinal cord. The average renal sympathetic activity before any spinal lesions in this series of rats was 62 ± 9 arbitrary units. After completion of all lesions, activity had increased in 21 of the 22 rats to an average of 134 ± 24 U (P < 0.02, paired t test).

We measured heart rate in 10 of these 22 rats. In these animals average heart rates increased from 323 ± 10 to 361 ± 12 beats/min after transection. Although some of the posttranssection increments in heart rate were small, some were as large as 20%, and rates increased in 9 of these 10 rats (P < 0.02, sign test).

Under identical conditions (including the amount of spinal cord left intact during a sequence of lesions), there were often large variations in renal sympathetic activity between rats. Because of these differences it was most appropriate to test for the effects of lesions using nonparametric tests that were sensitive only to the direction of change of renal activity caused by each lesion. The results of this analysis are presented in Fig. 1.

When the left dorsal quadrant (ipsilateral to the recorded renal nerve) was lesioned first, renal sympathetic activity increased (Fig. 1A). When the first lesion was a left hemisection, activity also increased (Fig. 1A). On the other hand, a right hemisection most often reduced left renal sympathetic activity.

When data were analyzed for the effects of the final quadrant lesion (meaning that the other three quarters of the spinal cord had been destroyed prior to this lesion), the results were less clear (Fig. 1B). Nevertheless, the most common changes in the direction of renal sympathetic activity were consistent with those observed after the first lesions. A left dorsal lesion tended to elevate renal sympathetic activity. A lesion in either ventral quadrant tended to reduce activity.

Analysis of the effects of quadrant lesions, without regard for the extent of previous lesions, provided additional evidence that dorsal lesions increased, and ventral lesions decreased, renal nerve activity (Fig. 1C). Only the effects of left ventral quadrant lesions were ambiguous. Note that, since the sympathetic effects of these lesions were analyzed regardless of the order in which they were made, some rats appear in more than one of the four groups in Fig. 1C.

The distinction between the effects of dorsal and ventral lesions was most clearly shown by an analysis of the effects of making a quadrant lesion that completed either a dorsal or a ventral hemisection (Fig. 1D). Completion of a dorsal hemisection elevated activity in every rat. Completion of a ventral hemisection reduced activity in most rats. Again, because the effects of these lesions were analyzed without respect to previous lesions, some rats are represented in both of these populations.

Effects of Spinal Transection on Renal Hemodynamics

The effects of spinal cord transection on renal sympathetic nerve activity and renal hemodynamics for the three groups studied are shown in Fig. 2. In rats whose arterial pressure was not controlled (group 1), mean arterial pressure fell 20 mmHg from a control level of 97.4 ± 3.0 mmHg, and renal sympathetic activity increased twofold after spinal transection. Despite the significant increase of renal sympathetic activity, renal vascular resistance decreased by 17% and renal blood flow was not significantly different from pretransection levels.

Responses in rats whose arterial pressure was controlled after spinal transection (group 2) were different. In this group arterial pressure fell only 3 mmHg from a control level of 100 mmHg. Renal sympathetic activity increased significantly 1.5-fold, and a 12% fall of renal arterial blood flow was observed. Renal vascular resistance increased 12%. However, this increase was not statistically significant. Finally, when arterial pressure was maintained at pretransection levels in rats with denervated kidneys (group 3), spinal transection had no significant effect on either renal arterial blood flow or renal vascular resistance.
Effect of Spinal Transection on Salt and Water Excretion

The effects of spinal transection on salt and water excretion for the three groups are shown in Fig. 3. Although glomerular filtration rate decreased ~30%, from a control of 1,010.0 ± 203.8 to 727 ± 94.8 μl/min after spinal transection when arterial pressure was not controlled, this change was not statistically significant due to the variability of the control measurements. Urine flow rate decreased by 50% from ~7.0 to 3.5 μl/min. Sodium excretion decreased from 0.579 ± 0.262 to 0.134 ± 0.024 μeq/min (a 77% fall), and potassium excretion dropped by almost 60% from 1.705 ± 0.112 to 0.736 ± 0.095 μeq/min. Changes in sodium and potassium excretion were not due to transection-induced alterations in plasma concentrations. Plasma sodium concentrations before and after transection were 148 ± 4 and 144 ± 4 meq/l, respectively. Potassium concentrations before and after transection were 4.6 ± 0.3 and 4.3 ± 0.4 meq/l, respectively.

Salt and water excretion rates also decreased after spinal transection in rats whose arterial pressure was maintained at control levels (group 2). However, magnitudes of the changes were smaller than those observed in rats whose arterial pressure was not controlled. There was no significant change of glomerular filtration rate. However, urine flow decreased by 36%, sodium excretion decreased by 28% (P > 0.05), and potassium excretion decreased by 45%. Plasma concentrations before and after transection of sodium (150 ± 3 and 149 ± 2 meq/l) and potassium (4.4 ± 0.2 and 4.4 ± 0.2 meq/l) were not significantly different. Spinal cord transection had no significant effect on the excretion rates of salt and water in the group with denervated kidneys (group 3).

DISCUSSION

Sequential Lesions of Spinal Cord

Confirming previous reports from this laboratory (21, 24), renal sympathetic activity was approximately doubled after spinal transection in the present experiments. This effect was seen whether transection was accomplished as a single surgical maneuver or as a sequence of lesions made over several hours. Posttransection sympathetic hyperactivity supports the hypothesis that, in the intact rat, some spinal generators of sympathetic activity are under tonic descending inhibition.

There is considerable confusion about the spinal loci of descending sympathomodulatory pathways. Many earlier studies relied on changes in arterial pressure after spinal lesions to map these pathways. It is now clear that changes in arterial pressure do not adequately represent changes in sympathetic activity, even after complete spinal transection (13, 24). Recent studies employing direct measurement of sympathetic activity have not greatly clarified these anatomical problems, and descending sympathoexcitatory and sympathoinhibitory pathways have been described in all four spinal quadrants. This complicated issue has recently been reviewed by Barman (1).
The present study is the first to investigate the effects of subtotal spinal lesions on tonic renal sympathetic activity. Together the results suggest that spinal systems capable of generating renal sympathetic activity are under tonic inhibition from pathways that descend bilaterally in the dorsal spinal cord. These observations are supported by an earlier report from this laboratory that electrical stimulation of the dorsolateral spinal cord reduces renal sympathetic activity after, but not before, spinal transection (21).

Of course, dorsal lesions destroyed ascending as well as descending pathways (25). It is possible that elevations in renal sympathetic activity after dorsal lesions resulted from supraspinal disinhibition of sympathoexcitatory systems descending in the ventral spinal cord. However, this mechanism is unlikely, for dorsal lesions were followed by similar increments in renal sympathetic activity, whether these lesions were made in otherwise intact cords (164% increment) or whether they were made in cords in which all other quadrants had been destroyed previously (154% increment). Renal sympathetic activity was very large in the one rat that exhibited a decrease in activity after a final dorsal lesion (Fig. 1B), and activity in this rat decreased by only 1%.

Subtotal cervical lesions destroyed some descending sympathomodulatory pathways while leaving others intact. Thus addition of the excitatory and inhibitory effects of sequential lesions never equaled the effect of complete transection. For instance, of the 22 rats whose renal sympathetic activity was increased from 62 to 134 units by total transection, eight rats exhibited an increase from 72 to 164 units after a lesion of only the dorsal quadrant of their previously intact spinal cords. Although a lesion of just one quadrant produced approximately the same percentage change in renal sympathetic activity as total transection, it would be incorrect to assume that this lesion altered the overall distribution of sympathetic activity in the same way as complete transection. Nevertheless, the discovery that limited cervical lesions may cause profound changes in renal sympathetic activity is significant, for these lesions are clinically more common than complete transections.

**Effect of Spinal Transection on Renal Hemodynamics**

Posttransection elevations in renal nerve activity had a negligible effect on renal vascular resistance. Indeed, despite a doubling of renal sympathetic activity, renal vascular resistance actually fell by 17% in those rats whose arterial pressure was not controlled after transection. Maintenance of renal blood flow in the face of both a 20-mmHg fall of renal arterial pressure and a doubling of renal sympathetic activity suggests that the autoregulatory properties of the abdominal viscera are dominant over sympathetic influences. This observation supports the hypothesis (24) that the depressor response to spinal
transection is primarily the result of a decrease in sympathetic activity to vascular beds with relatively weak autoregulatory properties (skin and skeletal muscle).

Interactions between the neural and autoregulatory regulation of the renal circulation have been studied for many years. The very large sympathetic discharge elicited by cerebral ischemia compromises renal autoregulation (7). However, renal autoregulation successfully competes with lesser degrees of sympathetic activation (4) and with elevated circulating catecholamines (12). Recently, responses of renal nerve activity and renal blood flow to hemorrhage (15, 17) and volume expansion (16) were measured in conscious dogs. Although a 200% increase of renal sympathetic activity was observed after hemorrhage, renal vascular resistance did not increase. Similarly, acute volume expansion decreased renal sympathetic activity by almost 90%, but renal vascular resistance actually increased due to an autoregulatory response to elevated renal arterial pressure (16).

Effects of Spinal Transection on Salt and Water Excretion

Although changes in renal nerve activity had little effect on renal hemodynamics, renal function was influenced, in part, by alterations in renal sympathetic activity. Spinal transection in our rats resulted in a marked decrease of sodium, potassium, and water excretion. However, when arterial pressure was not controlled, it was impossible to determine whether these responses were mediated by the increased renal sympathetic activity, by the decreased renal arterial pressure, or by both. A second group of rats, with controlled arterial pressure, also exhibited a significant increase in renal sympathetic activity. In this group, salt and water excretion decreased after transection. However, the magnitude of these responses was less than that seen in rats in which renal arterial pressure decreased after spinal transection. Most likely, this difference can be explained by the fact that arterial pressure did not fall in this series of rats. However, it must be noted that renal sympathetic activity did not increase as much in this group as it did in group 1. The observation that renal denervation, combined with controlled renal perfusion pressure, abolished the effects of spinal transection on salt and water excretion, excludes the possibility that these responses were the result of alterations in circulating hormones such as vasopres- sin. We conclude that the decreased salt and water excretion observed after spinal transection was caused both by the increase in efferent renal nerve activity and by the fall of renal arterial pressure.

These findings are in agreement with previous studies in anesthetized animals that have demonstrated an effect of renal nerves on salt and water excretion independent of changes in renal hemodynamics (2, 19). Furthermore, in the studies in unanesthetized dogs cited above (16), the decreased renal sympathetic activity seen with volume expansion did not alter renal vascular resistance but was responsible for an increase of sodium and water excretion.

Although, at the present time there is no direct evidence that renal sympathetic nerve activity is elevated chronically in animals or humans with spinal lesions, there is indirect evidence that supports this hypothesis. Plasma renin activity is elevated in quadriplegic patients (9, 11), and it is either normal or low in patients with progressive autonomic failure (18). Activation of the renin-angiotensin system in quadriplegics cannot be attributed to their low renal arterial pressure, for patients with progressive autonomic failure, whose pressures are also low, would then also be expected to have elevated plasma renin activity. Perhaps the increased activity of the renin-angiotensin system in spinaly transected humans is, in part, dependent on chronically elevated renal nerve activity.

Previous studies in dogs with chronically transected spinal cords indicated that the sympathetic nervous system was not an important determinant of resting arterial pressure. This was based on the fact that plasma levels of norepinephrine were negligible (14) and that sympathetic blocking drugs did not affect arterial pressure (10, 14). However, if renal nerve activity is elevated chronically and acts via salt and water retention to maintain arterial pressure, sympathetic blockade would not have an immediate effect on pressure. In other words, although sympathetic vasoconstrictor activity may contribute minimally to the maintenance of arterial pressure in spinal subjects, sympathetic influences on salt and water balance may be very important. Indeed, relative to normal subjects, the extracellular fluid compartment of spinal patients is expanded (3). The mechanism of this volume expansion is not known. However, the results of the present study suggest that elevated renal sympathetic nerve activity is one possibility. The plasma levels of nor epinephrine after spinal transection may simply reflect reduced sympathetic activity to nonvisceral vascular beds (24).

In summary, the elevations in renal sympathetic activity after spinal transection in anesthetized rats most likely result from destruction of inhibitory pathways which descend, at cervical levels, in the dorsal spinal quadrants. Lesions smaller than complete transsections may also elicit large increases in renal sympathetic activity. This renal sympathetic hyperactivity does not increase renal vascular resistance because it is counteracted by powerful renal autoregulatory responses to a transection-elicited depressor response. Renal sympathetic hyperactivity does contribute to posttranssection decreases in salt and water excretion. If a similar hyperactivity exists in humans after spinal transection, it may affect arterial pressure, both by promoting retention of salt and water and via effects on the renin angiotensin system.

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