JOURNAL OF NEUROTRAUMA
Volume 24, Number 7, 2007

Pp. 1219–1227
DOI: 10.1089/neu.2007.0276

Urinary Bladder Irritation Alters Efficacy of Vagal Stimulation on Rostral Medullary Neurons in Chronic T8 Spinalized Rats

EZIDIN G. KADDUMI and CHARLES H. HUBSCHER

ABSTRACT

The presence of pelvic visceral inputs to neurons in the rostral medulla that are responsive to electrical stimulation of the abdominal branches of the vagus nerve (VAG-abd) was investigated in a complete chronic T8 spinal transection rat model. Using extracellular electrophysiological recordings from single medullary reticular formation (MRF) neurons, 371 neurons in 15 rats responsive to pinching the ear (search stimulus) were tested for somato-visceral and viscero-visceral convergent responses to stimulation of the following nerves/territories: VAG-abd, dorsal nerve of the penis, pelvic nerve, distention of urinary bladder and colon, penile stimulation, urethral infusion, and touch/pinch of the entire body surface. In addition to these mechanical and electrical stimuli, a chemical stimulus applied to the bladder was assessed as well. Of the total neurons examined, 205 were tested before and 166 tested beginning 20 min after application of a chemical irritant (2% acetic acid) to the urinary bladder (same rats used pre/post irritation). As with intact controls, many ear-responsive MRF neurons responded to the electrical stimulation of VAG-abd. Although MRF neuron responses failed to be evoked with direct (mechanical and electrical nerve) pelvic visceral stimuli, acute chemical irritation of the urinary bladder produced a significant increase in the number of MRF neurons responsive to stimulation of VAG-abd. The results of this study indicate a central effect that potentially relates to some of the generalized below level pelvic visceral sensations that have been documented in patients with complete spinal cord injury.

Key words: inflammation; pelvic; spinal cord injury; urinary bladder; vagus

INTRODUCTION

THERE ARE REPORTS that some paraplegic patients suffer from severe pain (phantom pain) in denervated somatic and visceral organs (Melzack and Loeser, 1978). For example, visceral pain produced from urinary bladder and bowel distention and sensation of fullness are evident in patients with "functionally complete" spinal cord injuries (SCI) (as determined with criteria set by the American Spinal Injury Association [ASIA]) (Komisaruk et al., 1997). In addition, a study on 52 "complete" SCI patients (both above and below T10 spinal level) showed that 67% of them retain partial sensations of bladder filling (Ersoz and Akyuz, 2004). Many women with complete SCI also feel stimulation applied to the vagina and cervix (some of them even experience orgasm) and most still experience menstrual pain (Komisaruk et al., 1997). Cordectomy (removal of one or more spinal cord segments) that were performed in para-

Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, Kentucky.

plegic SCI patients (in the 1960's and 1970's) failed to relieve phantom pain sensations, which was at first thought to be due to the presence of some remaining undamaged fibers (Melzack and Loeser, 1978). The sympathetic chain was examined as a potential alternative route to convey some of the afferent fibers (which could transmit nociceptive inputs) from the spinally denervated areas; however, sympathetic blocks as well as sympathectomy failed to relieve phantom pain in the subjects receiving cordectomy (Melzack and Loeser, 1978). One possible alternative route, which has been hypothesized for sensory information to bypass a clinically complete SCI, is via afferents within the vagus nerve. The pelvic viscera are known to be innervated both by spinal nerves as well as the vagus (Altschuler et al., 1993; Jancso and Maggi, 1987; Tanaka et al., 2002; Vera and Nadelhaft, 1992).

In the present study, electrophysiological recordings were made in chronic T8 spinalized rats to determine if viscero-visceral convergent neurons in the rostral medulla (Kaddumi and Hubscher, 2006) respond to mechanical and/or chemical stimulation of below level pelvic/visceral organs as well as electrical stimulation of the nerves that innervate them. As shown previously, single medullary reticular formation (MRF) neurons receive convergent inputs from various pelvic visceral organs such as the urinary bladder, colon, and urethra (Hubscher and Johnson, 2004; Hubscher et al., 2004; Kaddumi and Hubscher, 2006). About 84% of those MRF neurons responding to bladder and colon stimulation respond to electrical stimulation of the abdominal branches of the vagus nerve (VAG-abd) (Hubscher et al., 2004; Kaddumi and Hubscher, 2006). Neuroanatomical tracing studies have shown that VAG-abd provides innervation to the rat descending colon and urinary bladder (Altschuler et al., 1993; Jancso and Maggi, 1987). Multiple stimulation techniques were employed in the present study to maximize the chances of evoking responses in the chronic SCI model. These techniques included electrical nerve (pelvic nerve, dorsal nerve of the penis and VAG-abd) and mechanical (bladder and colon distention, urethral infusion) stimulation. The effect of chemically irritating the urinary bladder was also tested. Urinary tract infection is a common complication of SCI and has been shown in longitudinal examination to increase in number with time post injury (Charlifue et al., 1999). Symptoms include pelvic discomfort, fever, autonomic dysreflexia, increased spasms, and headache (see "Bladder Care and Management Fact Sheet 11" at *www.spinalcord.uab.edu*).

METHODS

A total of 15 male Wister rats (120 days of age) were used to investigate the MRF neuronal responses to electrical stimulation of VAG-abd, dorsal nerve of the penis (DNP), and pelvic nerve (PN), urinary bladder and colon distention, and urethral infusion 4-6 weeks after T8 spinal cord transection. In 12 of these 15 animals, neuronal responses were examined prior to and then again 20 min after infusing 2% acetic acid in the urinary bladder.

For spinal cord transection, each animal was anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), injected intraperitonealy. Just before the surgery, each animal was injected (SC) with 0.5 mL of dual penicillin (Ambi Pen®, Butler Co., Columbus, OH) as a preventive measure for possible infection from the surgical procedure.

All surgeries were done under aseptic conditions and the body temperature was controlled throughout the surgery and recovery period. A dorsal longitudinal incision was made to expose the T7 vertebra. A laminectomy was performed in order to expose the underlying T8 spinal cord. The dura was incised, reflected laterally and the spinal cord cut using a pair of surgical microdissecting scissors. Complete transection was visualized through a surgical microscope. Gentle suction with an air vacuum was used to carefully elevate the cut stump in order to verify the completion of the lesion. Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) soaked in topical hemostat solution (Henry Schein Inc., Melville, NY) was placed in the lesion cavity. The incision was closed using 4-0 nylon suture for the muscle layers and fascia and surgical clips for the skin.

Each animal received 5 mg/kg of gentamicin (Abbott Laboratories, North Chicago, IL) once/day for five days after the surgery, to control for possible bladder infections, and 2.5 mg/kg of ketoprofen (Ketofen®, Fort Dodge Laboratories, Fort Dodge, IA) once/day for two days after the surgery in order to alleviate potential post-surgical pain. Each animal was housed individually. The urinary bladder was expressed every 8 h until the micturition reflex occurred automatically, 6–12 days (Hubscher and Johnson, 2000). A behavioral and medical record was kept for each animal during the recovery period.

After 4-6 weeks of recovery following the spinal cord transection, each animal was anesthetized with 50% urethane (1.2 g/kg) in preparation for the terminal electrophysiological experiment. The jugular vein, carotid artery and trachea were exposed and intubated for anesthetic supplement (5% urethane, as needed), blood pressure monitoring, and respiratory rate/end expired pCO_2 level monitoring, respectively. The animal temperature was monitored throughout the experiment by an esophageal heat sensor probe connected to a thermometer. The animal temperature was maintained at around 37°C throughout the experiment using a circulating water-heating pad.

A dorsal incision was then made in each animal for the nerve exposure (the PN and DNP bilaterally). The nerves were separated from the connective tissues and prepared to be placed on specially fabricated bipolar electrodes just prior to the brainstem recording (Hubscher and Johnson, 1996). For electrical stimulation of the abdominal branches of the vagus, a bipolar electrode was introduced along side the esophageal probe just caudal to the esophageal hiatus (Hubscher et al., 2004; Kaddumi and Hubscher, 2006).

A midline abdominal incision was then made for insertion of the urinary bladder and urethral catheters through the proximal urethra as described previously (Kaddumi and Hubscher, 2006). The configuration of the catheters avoids leakage from the bladder into the urethra (Kaddumi and Hubscher, 2006, 2007). A 10-mm-long latex balloon was inserted intra-anally for distal colon distention (Hubscher et al., 2004). The pressure in the urinary bladder and colon balloon was monitored by connecting their respective catheters to a pressure monitor. Chemical irritation of the bladder was done by infusing 1 mL of 2% acetic acid through the catheter just after emptying its contents (Kaddumi and Hubscher, 2007; Mitsui et al., 2001). Electrophysiological recordings were made prior to and then continued 20 min after the acetic acid infusion (Kaddumi and Hubscher, 2007) in order to allow time for chemical irritation to occur (Mitsui et al., 2001).

After mounting the animal onto a stereotaxic device, a dorsal incision was made to gain access to the brainstem. The dorsal surface of rostral medulla was exposed by removing part of the occipital bone and suctioning the caudal midline portion of the overlying cerebellum (Hubscher and Johnson, 1996).

Electrophysiological recordings were done by lowering a tungsten microelectrode with $\approx 7 \pm 1$ MOhms impedance (Fredrich Haer and Co., Bowdoinham, ME) from the dorsal surface of the brainstem with a motorized drive (Fredrich Haer and Co., Bowdoinham, ME) into the MRF. Stereotaxic coordinates were 3400 μm rostral to obex, and 400 and 800 μm lateral to midline on the left side of the brainstem (two tracks/animal) in 15 animals. Another two equivalent tracks were done on the right side of the brainstem (post-UB irritation) in 12 out of the 15 animals. The search area for each dorso-ventral track covered a length of 2800–3000 μm, which covered the rostral part of dorsal pargigantocellular nucleus, nucleus reticularis gigantocellularis (Gi), Gi pars alpha, and the medial part of the lateral paragigantocellular nucleus (see Fig. 1 in Hubscher and Johnson, 1999).

Both pinching the ear (above level) and bilateral PN stimulation (below level) were used as search stimuli (Hubscher and Johnson, 2006; Hubscher et al., 2004). Each neuron found responsive to one or both of these search stimuli was tested for responses to VAG-abd and DNP. The stimulus intensity and duration of the electrical stimulation of the nerves were set as in previous protocols (Hubscher and Johnson, 1996; Hubscher et al., 2004). In addition, the responses of the MRF neurons were further examined to urinary bladder and colon distention and urethral infusion, as well as touch/pinch of the entire surface of the rat's body. Responses of the MRF neurons were recorded on videotape and analyzed offline using Datawave software (*www.dwavetech.com*).

At the end of the terminal electrophysiological experiment, the urinary bladder from each animal was removed, then subsequently dried out and weighed. Each animal was then perfused with 0.9% normal saline followed by 4.0% paraformaldehyde injected into the left ventricle. The brainstem was removed and later sectioned at 100 μm thickness on the vibratome and stained with cresyl violet. The electrophysiological tracks were identified in these sections under the light microscope and the location of each neuron along these tracks was reconstructed (Paxinos and Watson, 1998) based on the stereotaxic distance below the dorsal surface, as previously described (Berkley et al. 1993; Johnson and Hubscher, 1998).

The spinal cord containing the lesion area was removed and sectioned sagittally at 18 μm thickness in the cryostat and stained with both luxol fast blue and cresyl violet (Kluver-Barrera Method). The sections were viewed under the light microscope to confirm the completion of the spinal cord transection.

Statistical Analysis

Data was analyzed for significance using either the Student *t*-test or the chi-square test. Results were considered significant when $p < 0.05$. Spike histograms were generated using Datawave software program. The present study was performed in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Louisville School of Medicine and with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, publication no. 0-309-05377-3).

RESULTS

The average weight of the urinary bladder following chronic spinal transection at T8 was 0.33 ± 0.03 g, which is twice the weight of the urinary bladder obtained from intact animals (0.15 ± 0.01 g) of equivalent size (350–450 g) and age matched. In addition, the average latency for restoring automatic reflex micturition after the

Table 1. Characteristics and Distribution of MRF Neurons

	Chronically transected rats		*Intact rats*[a]
Group	*Pre-UB irritation*	*Posts-UB irritation*	*Post-UB irritation*
No. of MRF neurons (no. of rats)	205 (15)	166 (12)	75 (8)
Percentage with spontaneous background activity	36.1	38.6	32.0
Mean background (spike/sec)	17.5 ± 1.3	16.3 ± 1.7	19.0 ± 2.9
MRF neuronal responses			
% excitatory (+)	73.2	80.7	80.0
% inhibitory (−)	20.5	14.5	14.7
% complex (+/−)	6.3	4.8	5.3
Responses to vagus nerve			
% responses	65.4	75.3*	61.3
Response latency (msec)	270.4 ± 7.8**	268.7 ± 8.8**	191.4 ± 21.4

[a]Kaddumi and Hubscher, 2007.

*Significantly different (χ^2, $p < 0.05$) compared to pre-bladder irritation in transected and intact rats.

**Significantly different (t, $p < 0.05$) compared to intact controls.

spinal cord transection was 5.9 ± 0.5 days, compared to about 11 days after severe contusion (Hubscher and Johnson, 2000).

A total of 205 MRF neurons responsive to ear pinching in 15 animals (two tracks per animal) without bladder irritation plus an additional 166 MRF neurons in 12 of the 15 animals (two equivalent tracks per animal on the opposite side of the brainstem) post-bladder irritation were examined in this study. The neuronal characteristics of the MRF neurons in the transected (with and without urinary bladder irritation) and normal animals with bladder irritation (Kaddumi and Hubscher, 2007) are presented in Table 1. There were no significant differences (χ^2, $p > 0.05$) in the background activity and neuronal response properties between the groups. The distribution of the different neuronal response modalities (excitatory, inhibitory and complex) throughout the various MRF subregions (Gi, dorsal Gi, and Gi pars alpha) was not affected by spinal cord transection. As seen in normals with MRF neurons responding to both pinching of the ear and electrical stimulation of the PN (Kaddumi and Hubscher, 2006b), the ear-responsive neurons that were inhibited with the various stimuli also tended to be more ventrally located in the MRF search zone post-transection.

The MRF neurons did not respond to any of the mechanical pelvic visceral stimuli (urinary bladder and colon distention and urethral infusion) following chronic transection at T8. MRF neurons also did not respond to electrical stimulation of the DNP or PN. However, 65.4% (134 out of 205) of the MRF neurons responded to electrical stimulation of VAG-abd prior to chemical irritation of the urinary bladder. This amount was not significantly different from the responses previously seen in spinally intact animals without irritation (61.3%, or 173 out of 282 neurons at equivalent MRF locations: total from three previously published articles (Hubscher et al., 2004; Kaddumi and Hubscher, 2007; Kaddumi and Hubscher, 2006). The latency of MRF neuronal response to VAG-abd was 270.4 ± 7.8 msec, which is significantly (t, $p <$

FIG. 1. Example of a single MRF neuron recording following chronic transection at the T8 spinal level. (**A**) A diagram showing the level of the electrophysiological recordings. Cross-section is adapted from *Atlas* (Paxinos and Watson, 1998) Figure 65. (**B**) A sagittal section of the spinal cord showing a complete transection. Note the presence of gelfoam in the lesion cavity. (**C**) Example of electrophysiological recordings of a single MRF neuron (location is shown in A) to pinching the ear (search stimulus), electrical stimulation of pelvic nerve (other search stimulus), dorsal nerve of the penis, and abdominal branches of the vagus (VAG-abd), distal urethral infusion, and urinary bladder and distal colon distention. As shown, this neuron responded to ear and vagus nerve stimulation. This neuron also responded to pinching the face, eyelid, trunk (above level of lesion only), and forepaw (not shown). Bars indicate the onset and duration of the mechanical stimulus. Note electrical artifacts in records of nerve stimulation. Arrows indicate the beginning of the stimulus. C, caudal; Gi, gigantocellular reticular nucleus; GiA, Gi pars alpha; R, rostral; RMg, raphe magnus nucleus.

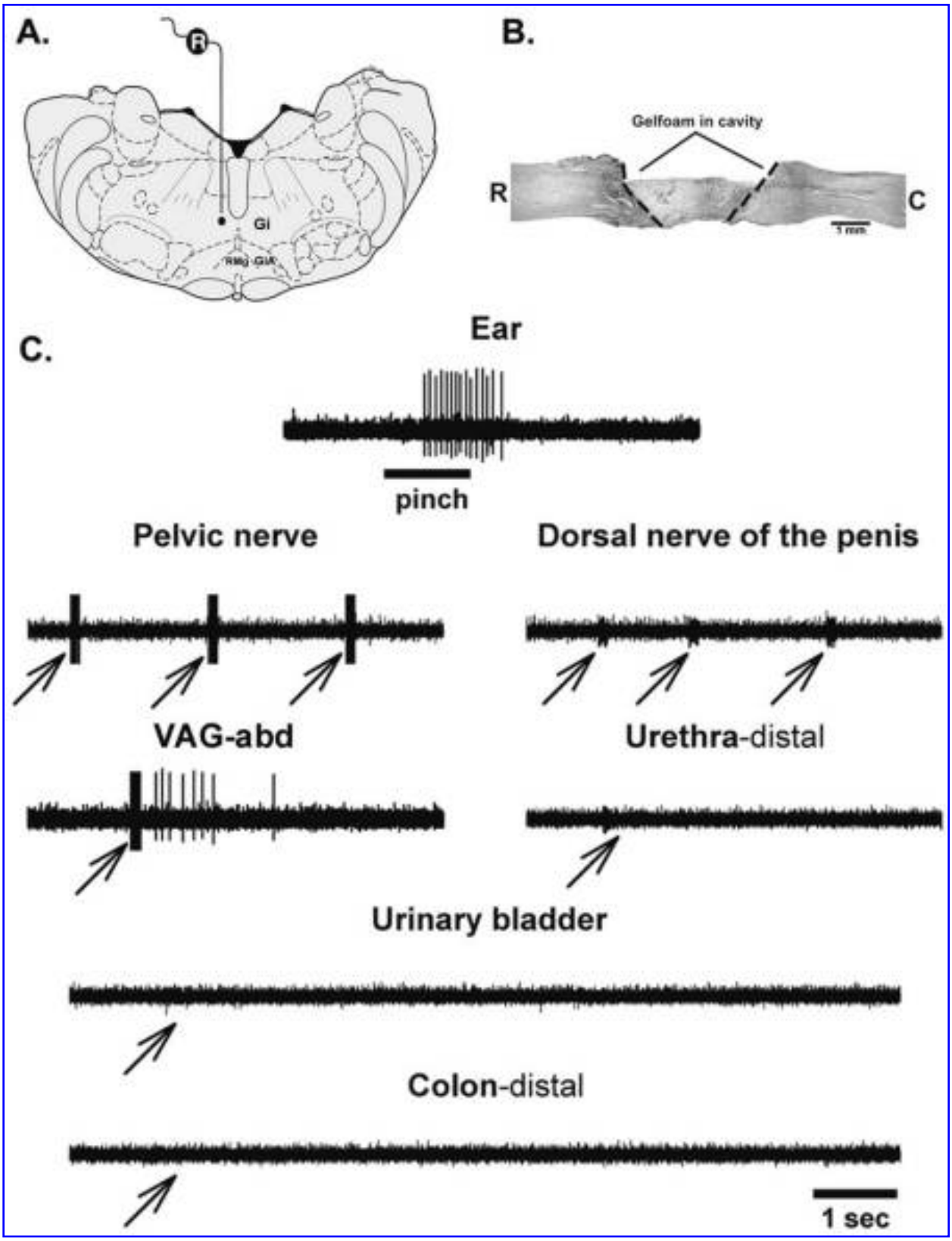
A.
R
GI
B.
Gelfoam in cavity
R
C
1 mm
C.
Ear
pinch
Pelvic nerve
Dorsal nerve of the penis
VAG-abd
Urethra-distal
Urinary bladder
Colon-distal
1 sec

FIG. 1.

0.0001) higher than normal animals (196.1 ± 16.6 msec). An example of MRF neuronal responses to VAG-abd is shown in Figure 1.

Although urinary bladder chemical irritation did not evoke MRF responses to mechanical pelvic visceral stimuli, there was a significant (χ^2, $p < 0.05$) increase in the number of MRF neurons that were responsive to VAG-abd (Table 1). The response latency of the MRF neurons to VAG-abd after bladder irritation was 268.7 ± 8.8 msec, which is not significantly different from the latency without bladder irritation.

DISCUSSION

Chemical irritation of the urinary bladder in T8 transected rats significantly increased the percentage of supraspinal MRF neurons responding to electrical VAG-abd stimulation. Mechanical stimulation (distention) of the bladder and other pelvic viscera, however, failed to evoke responses in MRF at 4–6 weeks post-transection. The ability of a below level chemical stimulus to produce an effect above level could explain why poorly localized pelvic visceral sensations remain in patients following "complete" SCI (Ersoz and Akyuz, 2004; Komisaruk et al., 1997).

The above level increase in MRF neuronal responsiveness to VAG-abd stimulation in chronically transected rats produced by below level urinary bladder irritation could be explained by direct activation of vagal afferents innervating the bladder. Some neuroanatomical tracing studies have shown that VAG-abd provide innervation to the rat descending colon and urinary bladder (Altschuler et al., 1993; Jancso and Maggi, 1987). For example, injection of horseradish peroxidase conjugated with wheat germ agglutinin in the urinary bladder wall labels some nodose ganglion neurons (Jancso and Maggi, 1987). Other evidence that the pelvic viscera could be innervated by afferents conveyed via vagus nerve includes indirect evidence from electrophysiological studies in female rats. These studies indicate that bilateral vagotomy either eliminates or alters the response properties to inputs from the female rat reproductive organs (uterine horn, cervix, and vagina) onto the solitary nucleus in the caudal medulla (Hubscher and Berkley, 1995). The limited evidence from these studies along with the lack of responses in the present study to mechanical pelvic visceral stimuli, however, suggests that the VAG-abd innervation may not be functionally significant.

Alternative (and more likely) explanations for the effect produced by urinary bladder irritation could be through the activation of vagal afferents indirectly. One possibility is activation through a potential connection between the vagus and PN. Several studies have shown that the viscera in the vagal and PN territories interact with each other. For example, electrical stimulation of the afferent fibers in the vagus nerve and/or distention of esophagus, stomach, and duodenum inhibit urinary bladder contractions in dogs (Moda, 1992). On the other hand, urinary bladder distention in dogs decreases the activity of the efferent fibers in the vagus nerve innervating the carotid sinus (Hassan et al., 1987). These effects are thought to be due either to indirect connections (by affecting supraspinal centers controlling these viscera) or direct connections (i.e., dual innervation).

Heightened excitability in other viscera innervated by VAG-abd following bladder irritation, such as the stomach, liver, pancreas, and proximal, ascending and transverse colon (Altschuler et al., 1993; Prechtl and Powley, 1985), may likely have contributed to the increased responses produced by electrical stimulation of VAG-abd afferents. Many cross-organ effects have been documented in recent years (Dmitrieva et al., 2001; Pezzone et al., 2005; Kaddumi and Hubscher, 2006; Winnard et al., 2006). These cross-organ effects have been shown to involve the hypogastric nerve (Winnard et al., 2006), which is composed of unmyelinated sympathetic afferents which branch to some extent (Hulsebosch and Coggeshall, 1982). Five mechanisms, including two possibilities involving only afferent fibers (branching sensory afferents; dorsal root reflexes) have been proposed by Berkley and colleagues (see Fig. 3 in Winnard et al., 2006). Note that the electrical stimulus in the present study could also have produced an effect on organs with spinal inputs above T8 through activation of vagal efferents (the nerve was not cut in the present study).

The vigorous afferent stimulation induced by chemical stimulation in the electrophysiological study likely increases sympathetic tone below the level of injury. The increased sympathetic activity likely involves circuitries that have undergone plastic changes in a way similar to those observed alterations in animals and humans with lesions cranial to T6 that are faced with cardiovascular challenges (i.e., generation of autonomic dysreflexia) (see Fig. 5 in Rabchevsky, 2006). Descriptions of the reorganization of below level circuitries have been well described in several recent reviews (Schramm, 2006; Weaver et al., 2006). Previous studies demonstrated significant increases in the expression of Fos-labeled spinal cord neurons, a proto-oncogene used as a marker of central neuronal activity resulting from a peripheral stimulus, below the level of a complete transection (after 4–6 weeks) in response to pelvic visceral stimulation (Vizzard, 2000; Landrum et al., 2002). This increase in Fos

was three fold greater and had a wider laminar distribution following chronic versus acute (1-day) transection (Landrum et al., 2002). Thus, the additional stimulation from the chemical irritant in the present study likely affects a very complex network of interconnected neurons involved in autonomic function that have reorganized in such a way that makes it possible for input to re-route itself to the brainstem.

Many studies have shown that vagal afferents innervating other viscera, such as the esophagus, respond to chemical agents, but fail to respond to mechanical stimuli (Page et al., 2002). The lack of responses from direct pelvic visceral stimulation in the present study is consistent with these findings. The results indicate that mechanical sensory inputs from the urinary bladder, distal colon, and urethra are conveyed to MRF neurons through the spinal cord only and not via the vagus nerve. The pelvic viscera are known to be innervated by pelvic and hypogastric nerve fibers that are conveyed through the L5-S1 and T13-L4 spinal nerves, respectively (Tanaka et al., 2002; Vera and Nadelhaft, 1992). It is possible, however, that visceral mechanical inputs from bladder, colon, and urethra through the vagus nerve could be conveyed to supraspinal nuclei other than the MRF. Vagal afferent input to MRF is likely via its connection with the solitary nucleus (Jean, 1991), which also projects to many other nuclei, including for example the parabrachial nucleus (Karimnamazi et al., 2002). Vagal afferents also project to the supraoptic nucleus, paraventricular nucleus, lateral parabrachial nucleus and others (Carlson and Osborn, 1998).

Since the third and fourth electrode tracks examined post bladder irritation in spinally intact animals (see right hand column in Table 1; data from Kaddumi and Hubscher, 2006a) did not show the same increase relative to the equivalent two tracks through the MRF post-transection, the increased number of MRF neuronal responses in the present study could be due to plasticity during the 4–6-week recovery period. Possible mechanisms include sprouting of vagal afferents to innervate the urinary bladder and/or recruiting potentially already existing silent fibers (in order to compensate for the lost ascending spinal pathways) (Page et al., 2002). Future experiments involving acute transection lesions will address this possibility. In the present study, the response latency of MRF neurons to electrical stimulation of VAG-abd increased significantly after the spinal cord transection. The same results were also observed with chronic dorsal column lesions (Hubscher and Johnson, 2004). These results also likely reflect the plasticity that occurs following SCI. Vagal afferents do not normally respond to visceral stimuli within the noxious range (Ozaki et al., 1999). The activation of slow conducting vagal afferents (c-fibers) could be responsible for the observed increase in latency following SCI (Hubscher and Johnson, 2004). Expansion of the VAG-abd afferent field is possible as bladder weight increased. Bladder weight and volume are known to increase significantly following SCI (Pikov and Wrathall, 2001).

In the present study, the weight of the urinary bladder almost doubled after spinal cord transection compared with normal controls. However, this increase is much less than the increase observed in other studies (Kruse et al., 1993), which have shown urinary bladder weight to increase about fivefold after spinalization. The difference with these other studies is likely due to the recovery protocols post-SCI for expressing the bladder (three times per day in the present study compared to two times per day in the other study). The recovery of the micturition reflex results from the reorganization of the reflex circuitry in the weeks that follow the SCI (de Groat et al., 1990). With the recovery of the micturition reflex, the urinary bladder increases in weight, volume, and residual volume (Kruse et al., 1993). The results of the present study indicate that better management post-SCI by emptying the urinary bladder as frequently as possible (minimum of 3 times daily for male rats) until the bladder reflex becomes automatic could help in decreasing side effects and complications of SCI. Decreasing the urinary bladder weight means decreasing the volume of the bladder and thus decreasing the residual volume. The fact that all animals survived for the duration of the chronic experiment (up to 6 weeks) likely relates to this aggressive management of the injured animals beginning immediately post-transection.

All responses to cutaneous stimulation were also lost below the T8 level of the SCI (all below level dermatomes). Many of the remaining responses to cutaneous stimulation (touch and/or pinch) above the level of the injury were to all cutaneous areas. These findings are consistent with previous studies where all regions below level were lost following severe contusion injury at T8 (Hubscher and Johnson, 1999) and many MRF neurons receive convergent inputs from cutaneous regions across the entire body (Hubscher et al. 2004).

In conclusion, although vagal afferents were not found to transmit mechanical information from the urinary bladder, distal colon, and urethra to MRF neurons following chronic complete mid-thoracic spinal transection, they may transmit chemical inputs by any one of several possible ways. More work is needed to better understand the role the vagus nerve in transmitting sensory information from below the level of a severe SCI, as the vagus is a potential target for managing visceral dysfunctions and phantom pain following SCI.

ACKNOWLEDGMENTS

We wish to thank James Armstrong for excellent technical assistance. This study was supported by the NIH (grant NS40919).

REFERENCES

ALTSCHULER, S.M., ESCARDO, J., LYNN, R.B., and MISELIS, R.R. (1993). The central organization of the vagus nerve innervating the colon of the rat. Gastroenterology **104,** 502–509.

BERKLEY, K.J., HUBSCHER, C.H., and WALL, P.D. (1993). Neuronal responses to stimulation of the cervix, uterus, colon, and skin in the rat spinal cord. J. Neurophysiol. **69,** 545–556.

CARLSON, S.H., and OSBORN, J.W. (1998). Splanchnic and vagal denervation attenuate central Fos but not AVP responses to intragastric salt in rats. Am. J. Physiol. **274,** R1243–R1252.

CHARLIFUE, S.W., WEITZENKAMP, B.A., and WHITENECK, G.G. (1999). Longitudinal outcomes in spinal cord injury: aging, secondary conditions, and well-being. Arch. Phys. Med. Rehabil. **80,** 1429–1434.

DE GROAT, W.C., KAWATONI, M., HISAMITSU, T. et al. (1990). Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. J. Auton. Nerv. Syst. **30,** S71–S77.

DMITRIEVA, N., JOHNSON, O.L., and BERKLEY K.J. (2001). Bladder inflammation and hypogastric neurectomy influence uterine motility in the rat. Neurosci. Lett. **313,** 49–52.

ERSOZ, M., and AKYUZ, M. (2004). Bladder-filling sensation in patients with spinal cord injury and the potential for sensation-dependent bladder emptying. Spinal Cord **42,** 110–116.

HASSAN, A.A., HICKS, M.N., WALTERS, G.E., and MARY, D.A. (1987). Effect on efferent cardiac vagal nerve fibres of distension of the urinary bladder in the dog. Q. J. Exp. Physiol. **72,** 473–481.

HUBSCHER, C.H., and BERKLEY, K.J. (1995). Spinal and vagal influences on the responses of rat solitary nucleus neurons to stimulation of uterus, cervix and vagina. Brain Res. **702,** 251–254.

HUBSCHER, C.H., and JOHNSON, R.D. (1996). Responses of medullary reticular formation neurons to input from the male genitalia. J. Neurophysiol. **76,** 2474–2482.

HUBSCHER, C.H., and JOHNSON, R.D. (1999). Changes in neuronal receptive field characteristics in caudal brain stem following chronic spinal cord injury. J. Neurotrauma **16,** 533–541.

HUBSCHER, C.H., and JOHNSON, R.D. (2000). Effects of acute and chronic midthoracic spinal cord injury on neural circuits for male sexual function. II. Descending pathways. J. Neurophysiol. **83,** 2508–2518.

HUBSCHER, C.H., and JOHNSON, R.D. (2004). Effects of chronic dorsal column lesions on pelvic viscerosomatic convergent medullary reticular formation neurons. J. Neurophysiol. **92,** 3596–3600.

HUBSCHER, C.H., and JOHNSON, R.D. (2006). Chronic spinal cord injury induced changes in the responses of thalamic neurons. Exp. Neurol. **197,** 177–188.

HUBSCHER, C.H., KADDUMI, E.G., and JOHNSON, R.D. (2004). Brain stem convergence of pelvic viscerosomatic inputs via spinal and vagal afferents. Neuroreport **15,** 1299–1302.

HULSEBOSCH, C.E., and COGGESHALL, R.E. (1982). An analysis of the axon populations in the nerves to the pelvic viscera in the rat. J. Comp. Neurol. **211,** 1–10.

JANCSO, G., and MAGGI, C.A. (1987). Distribution of capsaicin-sensitive urinary bladder afferents in the rat spinal cord. Brain Res. **418,** 371–376.

JEAN, A. (1991). The nucleus tractus solitarius: neuroanatomic, neurochemical and functional aspects. Arch. Int. Physiol. Biochim. Biophys. **99,** A3–52.

JOHNSON, R.D., and HUBSCHER, C.H. (1998). Brainstem microstimulation differentially inhibits pudendal motoneuron reflex inputs. Neuroreport **9,** 341–345.

KADDUMI, E.G., and HUBSCHER, C.H. (2007). Changes in rat brainstem responsiveness to somatovisceral inputs following acute bladder irritation. Exp. Neurol. **203,** 349–357.

KADDUMI, E.G., and HUBSCHER, C.H. (2006). Convergence of multiple pelvic organ inputs in the rostral medulla. J. Physiol. **572,** 393–405.

KARIMNAMAZI, H., TRAVERS, S.P., and TRAVERS, J.B. (2002). Oral and gastric input to the parabrachial nucleus of the rat. Brain Res. **957,** 193–206.

KOMISARUK, B.R., GERDES, C.A., and WHIPPLE, B. (1997). 'Complete' spinal cord injury does not block perceptual responses to genital self-stimulation in women. Arch. Neurol. **54,** 1513–1520.

KRUSE, M.N., BELTON, A.L., and DE GROAT, W.C. (1993). Changes in bladder and external urethral sphincter function after spinal cord injury in the rat. Am. J. Physiol. **264(6 Pt 2),** R1157–1163.

LANDRUM, L.M., JONES, S.L., and BLAIR, R.W. (2002). The expression of Fos-labeled spinal neurons in response to colorectal distension is enhanced after chronic spinal cord transection in the rat. Neurosci. **110,** 569–578.

MELZACK, R., and LOESER, J.D. (1978). Phantom body pain in paraplegics: evidence for a central "pattern generating mechanism" for pain. Pain **4,** 195–210.

MITSUI, T., KAKIZAKI, H., MATSUURA, S., AMEDA, K., YOSHIOKA, M., and KOYANAGI, T. (2001). Afferent fibers of the hypogastric nerves are involved in the facilitating effects of chemical bladder irritation in rats. J. Neurophysiol. **86,** 2276–2284.

MODA, Y. (1992). Effects of afferent vagal stimulation and distention of the upper digestive tract on the micturition reflex and activity of the pontine micturition center in dogs. Nippon Hinyokika Gakkai Zasshi **83,** 2005–2014.

OZAKI, N., SENGUPTA, J.N., and GEBHART, G.F. (1999). Mechanosensitive properties of gastric vagal afferent fibers in the rat. J. Neurophysiol. **82,** 2210–2220.

PAGE, A.J., MARTIN, C.M., and BLACKSHAW, L.A. (2002). Vagal mechanoreceptors and chemoreceptors in mouse stomach and esophagus. J. Neurophysiol. **87,** 2095–2103.

PAXINOS, G., and WATSON, C. (1998). The rat brain in stereotaxic coordinates. Academic Press, San Diego.

PEZZONE, M.A., LIANG, R., and FRASER, M.O. (2005) A model of neural cross-talk and irritation in the pelvis: implications for the overlap of chronic pelvic pain disorders. Gastroenterology **128,** 1953–1964.

PIKOV, V., and WRATHALL, J.R. (2001). Coordination of the bladder detrusor and the external urethral sphincter in a rat model of spinal cord injury: effect of injury severity. J. Neurosci. **21,** 559–569.

PRECHTL, J.C., and POWLEY, T.L. (1985). Organization and distribution of the rat subdiaphragmatic vagus and associated paraganglia. J. Comp. Neurol. **235,** 182–195.

RABCHEVSKY, A.G. (2006). Segmental organization of spinal reflexes mediating autonomic dysreflexia after spinal cord injury. In Weaver L.C., Polosa C. (Eds.), Progress in Brain Research **152,** pp. 256–274.

SCHRAMM, L.P. (2006). Spinal sympathetic interneurons: their identification and roles after spinal cord injury. In Weaver L.C., Polosa C. (Eds.), Progress in Brain Research **152,** pp. 27–37.

TANAKA, K., MATSUGAMI, T., and CHIBA, T. (2002). The origin of sensory innervation of the peritoneum in the rat. Anat. Embryol. (Berl) **205,** 307–313.

VERA, P.L., and NADELHAFT, I. (1992). Afferent and sympathetic innervation of the dome and the base of the urinary bladder of the female rat. Brain Res. Bull. **29,** 651–658.

VIZZARD, M.A. (2000). Increased expression of spinal cord Fos protein induced by bladder stimulation after spinal cord injury. Am J Physiol Regulatory Integrative Comp Physiol **279,** R295–R305.

WEAVER, L.C., MARSH, D.R., GRIS, D., BROWN, A., and DEKABAN, A. (2006). Autonomic dysreflexia after spinal cord injury: central mechanisms and strategies for prevention. In Weaver L.C., Polosa C. (Eds.), Progress in Brain Research **152,** pp. 245–263.

WINNARD, K.P., DMITRIEVA, N. and BERKLEY, K.J. (2006). Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve. Am J Physiol Regulatory Integrative Comp Physiol **291,** R1592–R1601.

Address reprint requests to:
Charles H. Hubscher, Ph.D.
Department of Anatomical Sciences and Neurobiology
University of Louisville
Louisville, KY 40292

E-mail: chhubs01@louisville.edu