Modulation of the visceromotor reflex by a lumbosacral ventral root avulsion injury and repair in rats

Huiyi H. Chang and Leif A. Havton

Department of Anesthesiology and Perioperative Care, University of California, Irvine, California; Department of Anatomy and Neurobiology, University of California, Irvine, California; and Reeve-Irvine Research Center, University of California, Irvine, California

Submitted 21 February 2012; accepted in final form 12 June 2012

Chang HH, Havton LA. Modulation of the visceromotor reflex by a lumbosacral ventral root avulsion injury and repair in rats. Am J Physiol Renal Physiol 303:F641–F647, 2012. First published June 13, 2012; doi:10.1152/ajprenal.00094.2012 — Increased abdominal muscle wall activity may be part of a visceromotor reflex (VMR) response to noxious stimulation of the bladder. However, information is sparse regarding the effects of cauda equina injuries on the VMR in experimental models. We studied the effects of a unilateral L6-S1 ventral root avulsion (VRA) injury and acute ventral root reimplantation (VRI) into the spinal cord on micturition reflexes and electromyographic activity of the abdominal wall in rats. Cystometrogram (CMG) and electromyography (EMG) of the abdominal external oblique muscle (EOM) were performed. All rats demonstrated EMG activity of the EOM associated with reflex bladder contractions. At 1 wk after VRA and VRI, the duration of the EOM EMG activity associated with reflex voiding was significantly prolonged compared with age-matched sham rats. However, at 3 wk postoperatively, the duration of the EOM responses remained increased in the VRA series but had normalized in the VRI group. The EOM EMG duration was normalized for both VRA and VRI groups at 8–12 wk postoperatively. CMG recordings showed increased bladder contraction duration at 1 and 3 wk postoperatively for the VRA series, whereas the contraction duration was only increased at 1 wk postoperatively for the VRI series. Our studies suggest that a unilateral lumbosacral VRA injury results in a prolonged VMR to bladder filling using a physiological saline solution. An acute root reimplantation decreased the VMR induced by VRA injury and provides earlier sensory recovery. Electromyography; cystometry; micturition reflex; external oblique muscle; cauda equina

LUMBOSACRAL VENTRAL ROOT AVULSION (VRA) injuries in experimental models may result in a combination of motor, sensory, and autonomic impairments, which mimic many of the neurological deficits encountered after cauda equina injuries. For instance, a lumbosacral VRA injury in the adult rat results in denervation of pelvic and hindlimb muscles, and the vast majority of axotomized motoneurons and preganglionic parasympathetic neurons undergo retrograde degeneration and death (16). Bilateral lumbosacral VRA injuries cause denervation of the lower urinary tract and are responsible for impaired reflex micturition and urinary retention in rats (5, 18). A lumbosacral VRA injury may also result in extensive sensory plasticity. For instance, at-level neuropathic pain with allodynia may develop after a VRA injury in rats (2). Interestingly, an acute surgical reimplantation of the avulsed ventral roots may protect axotomized motor and autonomic neurons against retrograde cell death (17), restore reflex micturition (5, 18), and ameliorate injury-induced neuropathic pain (2). However, the effects of lumbosacral VRA injury and repair on visceral sensory function have not been investigated previously in experimental models and are not well understood. In the present study, we performed a combination of cystometrogram (CMG) and electromyography (EMG) of the abdominal external oblique muscle (EOM). Previous studies (11, 35, 36) have demonstrated that electromyographic activation of the abdominal EOM is associated with normal micturition reflexes in rats. Interestingly, the EOM EMG activity is increased in response to bladder infusion of an acetic acid solution, a noxious stimulus to the bladder wall (11). This visceromotor reflex (VMR) may also be increased in response to bladder distension in the setting of bladder inflammatory conditions (12). In the present study, we infused the rat bladder with a normal saline solution as a physiological stimulus and to assess bladder contractions using a combination of CMG and abdominal EOM EMG recordings as an outcome measure for the VMR after a lumbosacral VRA injury and reimplantation of the avulsed ventral roots into the spinal cord. We demonstrate increased VMRs at 1 and 3 wk after the VRA injury, whereas the same EOM EMG responses did not differ from corresponding sham recordings at 8–12 wk after the VRA injury. Surgical reimplantation of the avulsed ventral roots did not aggravate the VRA-induced VMR at 1 wk postoperatively but provided amelioration of the VRA injury-induced VMR at 3 wk after surgery. At 8–12 wk postoperatively, no VMR suggestive of visceral pain was detected in the ventral root reimplantation (VRI) group.

METHODS

The studies included a total of 75 adult Sprague-Dawley female rats (180–220 g; Charles River Laboratories, Raleigh, NC). For each experimental group, studies were performed at 1, 3, and 8–12 wk postoperatively and divided into three experimental groups: 1) sham surgery (sham; n = 25) consisting of a unilateral lumbar laminectomy at L1-L2 vertebral level, opening of the dura, gentle root manipulation but no root lesion; 2) a unilateral L6-S1 VRA injury (VRA; n = 26); and 3) a unilateral lumbosacral VRA injury and repair of the avulsed ventral roots into the spinal cord (VRI; n = 25). All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals (National Academies Press, Eighth Edition, 2011) and were approved by the Institutional Animal Care and Use Committee. Surgical procedures. The VRA and VRI surgical procedures followed our established protocols (2, 16, 17). In short, a unilateral lumbar laminectomy was performed while the animals were under general gas anesthesia (isoflurane 2–2.5%). The dura was opened. For the sham animals, the surgical procedures were finished. For the VRA
and VRI groups, the left L6-S1 ventral roots were avulsed by gentle traction of roots along their normal course until the roots separated from the spinal cord surface. For the VRI groups, the L6-S1 ventral roots were surgically inserted into a small longitudinal incision, which had been made into the lateral funiculus at the corresponding spinal cord segmental levels (Fig. 1A). Gel foam was applied over the wound, and a titanium mesh cage was placed to protect the exposed spinal cord surface and to stabilize the spine in all rats (28). Rats were studied at 7 ± 1 days (1 wk), 21 ± 2 days (3 wk), and 8–12 wk postoperatively.

**Electrophysiological studies.** At 1, 3, and 8–12 wk postoperatively, rats of the VRA, VRI, and sham series underwent electrophysiological studies, which were performed as acute and terminal experiments. The rats were anesthetized with urethane (1.2 g/kg sc). A low abdominal incision was made, and a PE-50 catheter was inserted into the bladder dome and secured with a surgical suture. The bladder catheter was connected to a three-way connector for saline infusion (0.12 ml/min) and a pressure sensor to measure the intravesical bladder pressure (IVP). To record the abdominal EOM EMG, the two fine, insulated silver wire electrodes (0.05-mm diameter) with exposed tips were inserted into the ipsilateral EOM (11). The bladder pressure and EOM EMG activity were recorded using a BioPac MP150 system (BIOPAC Systems). The urodynamic recordings were conducted while the animals were under urethane anesthesia. The animal was terminated at the end of the experiment. The contraction duration, intercontraction interval, pressure threshold (PT), resting pressure, and maximum IVP were analyzed from three consecutive voiding cycles in each animal (Fig. 1B). In the same voiding cycles, the amplitude, duration and area under curve (AUC) measurements for the EOM EMG firing activity were determined. The threshold for EOM EMG firing (11) was calculated as the percentage of (threshold pressure − PT)/maximum IVP − PT, where threshold pressure was determined as the bladder pressure at the onset of EOM EMG firing.

**Statistical analysis.** Data are expressed as means ± SE. The Student t-test was applied to compare the VRA and sham groups as well as VRI and sham groups for each time point (1, 3, or 8–12 wk after surgical procedures). We used a value of P < 0.05 as indicating a significant change between groups. JMP (SAS) was applied to the statistical analyses.

**RESULTS**

EOM EMG activity and CMG were obtained from rats of sham, VRA, and VRI series at 1, 3, and 8–12 wk postoperatively. Reflex bladder contractions and associated bursts of EOM EMG activity were readily demonstrated in all rats of all experimental groups. EOM EMG measurements and quantitative studies of CMG recordings were used to determine the long-term effects of a unilateral L6-S1 VRA injury and repair on the VMR.

The VRA group showed a significant increase in the duration of EOM EMG activity at 1 wk (15.7 ± 1.8 s; n = 9; P < 0.05) and 3 wk (21.4 ± 5.2 s; n = 8; P < 0.05) after the L6-S1 VRA injury compared with sham-operated controls (6.7 ± 1.1 s, n = 10 for 1 wk; and 4.3 ± 1.2 s, n = 6 for 3 wk; Figs. 2 and 3A). However, the duration of the EOM EMG activity was not different from corresponding measurements in sham-operated rats at 8–12 wk after the L6-S1 VRA injury. The VRI group showed a prolonged duration of EOM EMG activity (12.4 ± 1.2 s; n = 8; P < 0.05) at 1 wk after the root replantation procedure, but the duration of the EOM EMG activity was not different from corresponding measurements in the control series at 3 and 8–12 wk postoperatively (Figs. 2 and 3A).

The VRA group showed a significant decrease in threshold for EOM EMG activation (26.9 ± 4.3%; n = 9; P < 0.05) compared with the sham-operated group (41.6 ± 8.1%; n = 10) at 1 wk after the L6-S1 VRA injury, but the threshold for EOM EMG activation was not different from corresponding measurements in sham-operated rats at 3 and 8–12 wk postoperatively (Figs. 2 and 3B). The VRI group showed no difference in threshold for EOM EMG activation compared with sham-operated rats at 1 and 3 wk after the root replantation procedure, but showed a higher threshold (45.9 ± 5.6%; n = 9; P < 0.05) compared with the control series (28.1 ± 4.4%; n = 9) at 8–12 wk postoperatively (Figs. 2 and 3B).

The VRA group showed a significant increase in the AUC (0.19 ± 0.04 mV/s; n = 8; P < 0.05) for the EOM EMG activity compared with the sham series (0.06 ± 0.03 mV/s; n = 6) at 3 wk after the L6-S1 VRA injury, but the AUC measurements were not different from sham-operated controls at 1 and 8–12 wk postoperatively (Fig. 3D). The VRI group demonstrated no effect of the VRA injury and replantation procedure compared with the control series at any of the three time points studied.
Both the VRA and VRI series showed absence of any significant change for the amplitude of the EOM EMG activity compared with sham-operated rats at 1, 3, and 8–12 wk postoperatively (Figs. 2 and 3).

CMG studies (Table 1) showed significantly prolonged contraction duration at 1 wk (25.9 ± 2.8 s; n = 9; P < 0.05) and 3 wk (27.8 ± 3.1 s; n = 8; P < 0.05) for the VRA series compared with sham-operated controls (21.2 ± 1.8 s; n = 10 for 1 wk; 20.3 ± 1.8 s; n = 6; for 3 wk; Figs. 2 and 4A). The VRI series also showed significantly a significant increase in contraction duration at 1 wk postoperatively (24.6 ± 2.7 s; n = 8; P < 0.05) compared with the sham series. Maximum IVP was significantly decreased in both the VRA (27.3 ± 1.4 cm H₂O; n = 9; P < 0.05) and VRI series (30 ± 2.3 cm H₂O; n = 9; P < 0.05) compared with sham-operated controls (37.6 ± 4.2 cm H₂O; n = 9) at 8–12 wk postoperatively (Figs. 2 and 4A). For both VRA and VRI series, comprehensive CMG recordings demonstrated absence of any significant change for resting pressure, pressure threshold, and intercontraction interval at 1, 3, and 8–12 wk postoperatively (Figs. 2 and 4, B and C).

DISCUSSION

The present study demonstrates that a unilateral lumbosacral VRA injury augments the VMR. Specifically, reflex voiding was associated with increased EOM EMG at 1 and 3 wk postoperatively with recovery of VMR responses at 8–12 wk after the ventral root lesion. An acute ventral root replantation provided an earlier recovery of the VMR with an amelioration of visceral pain already at the 3-wk postoperative interval.

Activation of the abdominal wall muscles is part of normal micturition in both rats (11, 35, 36) and humans (33). Such contractions of abdominal wall muscles promote micturition by increasing the intra-abdominal pressure and are detectable by EMG. An increase in abdominal wall EMG activity is an established marker for visceral pain responses to colonic distension (10, 27) and bladder filling with an acetic acid solution.

Fig. 2. Representative examples of CMG and EOM EMG recordings at 1, 3, and 8–12 wk postoperatively for all experimental groups. Top: bladder contraction during a voiding cycle. Bottom: EOM EMG activity associated with bladder filling and voiding. A1–A3: sham. B1–B3: VRA. C1–C3: ventral root reimplantation (VRI).
(11). In the present translational research, a continuous saline infusion was used as a physiologic stimulus to primarily address whether any potential adverse effects, such as pain, would emerge after the VRA and VRI procedures. Use of a graded bladder pressure approach may be less suitable, as it may cause bladder overdistension due to the VRA injury, which represents an incomplete lower motoneuron syndrome and may result in a flaccid bladder (5). Increased bladder contraction duration, as is demonstrated at 1 and 3 wk after VRA injury as well as at 1 wk after VRI in the present study, has also been associated with visceral pain in rats following the infusion of an acetic acid solution into the bladder (11). Previous studies (12) have demonstrated that visceral pain with augmented abdominal EMG activity may be induced by the induction of inflammation of the bladder wall. Although an enhanced VMR may be associated with visceral pain, it is possible that it may also reflect a compensatory mechanism related to decreased voiding function. Interestingly, our preliminary study has suggested that a unilateral L5-S2 VRA injury in the rats may result in decreased voiding efficiency at 12 wk postoperatively (6). However, our present study shows that the VMR normalizes at 8–12 wk postoperatively. Therefore, the VMR may primarily be a sign of pain, rather than a sign of associated with impaired voiding function, in the lumbosacral VRA injury model. In the present study, the increased VMR represents a neuropathic pain response, as the underlying lesion was a VRA injury and a normal saline solution was infused into the bladder as a physiologic stimulus.

For studies on neuropathic pain, allodynia and hyperesthesia are commonly induced by a spinal segmental nerve ligation (21), dorsal root ganglionectomy (22), or a peripheral nerve injury (13). These models all include a direct injury to axons of primary afferents. Interestingly, an injury to primary sensory afferents does not appear to be required for the induction of pain, as a ventral root transection injury, which spares the majority of primary afferent axons, may also induce hyperalgnesia and allodynia within the same segmental dermatome (22, 34). Although the ventral root transection studies suggest that a pure efferent injury may induce neuropathic pain, it is also possible that the observed pain may be in response to a transection injury of afferent fibers that make U-turn loops within the ventral root (9). However, an acute-to-chronic state of allodynia was demonstrated in rats after a combined L6-S1 VRA injury and sensory testing of the adjacent and intact L5

Table 1. Statistical analysis of cystometrogram in the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Contraction Duration, s</th>
<th>Intercontraction Interval, s</th>
<th>Resting Pressure, cmH2O</th>
<th>Maximum IVP, cmH2O</th>
<th>Pressure Threshold, cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>21.2 ± 1.8</td>
<td>141.3 ± 17.9</td>
<td>5.4 ± 0.7</td>
<td>32.1 ± 3.7</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>3 wk</td>
<td>20.3 ± 1.8</td>
<td>163.2 ± 22.3</td>
<td>5.8 ± 0.9</td>
<td>28.3 ± 1.9</td>
<td>11.3 ± 1.1</td>
</tr>
<tr>
<td>8–12 wk</td>
<td>28.6 ± 0.7</td>
<td>146.1 ± 21.2</td>
<td>5.5 ± 0.4</td>
<td>37.6 ± 4.2</td>
<td>13.3 ± 0.8</td>
</tr>
<tr>
<td>VRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>25.9 ± 2.8*</td>
<td>181.9 ± 36.8</td>
<td>5.1 ± 0.6</td>
<td>25.5 ± 2.8</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td>3 wk</td>
<td>27.8 ± 3.1*</td>
<td>147.9 ± 26.6</td>
<td>6.5 ± 0.7</td>
<td>30.2 ± 4.5</td>
<td>12.3 ± 0.9</td>
</tr>
<tr>
<td>8–12 wk</td>
<td>26.8 ± 2.1</td>
<td>115.9 ± 14.6</td>
<td>5.7 ± 1.1</td>
<td>27.3 ± 1.4*</td>
<td>15.8 ± 1.3</td>
</tr>
<tr>
<td>VRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>24.6 ± 2.7†</td>
<td>156.2 ± 28.2</td>
<td>5.6 ± 0.9</td>
<td>29.4 ± 2.4</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>3 wk</td>
<td>21.9 ± 3.1</td>
<td>122.8 ± 22.1</td>
<td>6.2 ± 0.6</td>
<td>30.6 ± 2.5</td>
<td>12.5 ± 0.7</td>
</tr>
<tr>
<td>8–12 wk</td>
<td>27.6 ± 2.1</td>
<td>98.6 ± 9.36</td>
<td>6.6 ± 0.6</td>
<td>30.0 ± 2.3†</td>
<td>14.8 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. IVP, intravesical bladder pressure. *P < 0.05 and †P < 0.05, significant change in ventral root avulsion (VRA) and ventral root reimplantation (VRI) series, respectively, compared with the sham series.
dermatome (2), thereby providing additional support to the notion that an axonal injury to primary afferents may not be required to evoke neuropathic pain. In the present studies, we used the L6-S1 VRA injury model for the testing of the VMR in rats.

The mechanism for VRA injury-induced pain is uncertain. Possible contributors to the development of pain include interactions between motoraxons undergoing Wallerian degeneration and intact sensory fibers (14, 34). Specially, the axonal injury results in fragmentation of the myelin sheath, activation of endoneurial macrophages, infiltration by hemogenous macrophages, Schwann cell proliferation, and an increased expression of immune signal molecules, including cytokines (8, 25, 29, 38). Here, an L5 ventral root transection injury resulted in a decrease in withdrawal thresholds of the paw and increased low-frequency spontaneous activity of nonlesioned C-fiber afferents of the adjacent L4 spinal nerve (41). In the present study, the L6 and S1 ventral roots were avulsed. Motor and autonomic neurons of the L6 and S1 spinal cord segments extend their axons into the segmental ventral roots and innervate the major pelvic ganglion or peripheral target organs, including the lower urinary tract (7, 23). It is therefore possible that the L6-S1 VRA injury results in Wallerian degeneration of efferent fibers, which may cause altered function of primary afferents involved with visceral function. In addition, the L6 ventral root contributes with some fibers to the sciatic nerve (1, 32). It is therefore possible that a common mechanism may exist for the acute-to-chronic allodynia, which was detected within the L5 dermatome after an L6-S1 VRA injury (2).

Injuries to ventral roots may induce both sustained and temporary pain. In studies on somatosensory pain responses, a significant decrease in the threshold for mechanical withdrawal in the middle of the ipsilateral hindlimb paw was detectable already on day 2 after an L5 ventral root transection injury and lasted ≥20 days postoperatively (34). A similar rapid onset mechanical allodynia was present at 24 h after an L5 ventral root transection injury and remained at 56 days after the injury (22). In contrast, cold allodynia and thermal hyperalgesia were limited to the first 2 wk after the L5 ventral root transection (22). Sensory testing of the L5 dermatome after a combined L6-S1 VRA injury resulted in acute onset of mechanical allodynia, which remained for at least the first 7 wk after the lesion, whereas no signs of thermal hyperesthesia was detected throughout the same postoperative interval (2). In the present study, we demonstrate visceral pain at 1 and 3 wk after a similar sensory-sparing avulsion of the L6-S1 ventral roots with a spontaneous recovery of the VMR at 8–12 wk postoperatively. Thus the presence and time course of neuropathic pain states after ventral root injury appear modality-specific and may also be affected by injury type.

The present study adds to previous reports suggesting a potential benefit of a surgical replantation of avulsed ventral roots. Somatosensory pain in the form of allodynia was ameliorated by an acute replantation of avulsed L6-S1 ventral roots in rats (2). In clinical cases, there has also been observations made that pain has improved in some patients with a brachial plexus injury after surgical replantation of avulsed ventral roots (3, 4). In the present study, surgical replantation of avulsed lumbosacral ventral roots results in a sooner recovery of visceral pain in a rat model for cauda equina injury. With regards to possible mechanism of action for the early amelioration of visceromotor reflexes by VRI, the observed plasticity is not likely due to reinnervation of peripheral targets. Specifically, functional reinnervation of the lower urinary tract is well established by 12 wk after VRI (5, 18). However, our previous preliminary observations suggest that functional reinnervation of the lower urinary tract takes place between 6 and 12 wk after a bilateral VRA injury followed by the VRI procedure in rats. Therefore, we conclude that the observed visceromotor reflex plasticity related to the VRI procedure at 3 wk postoperatively is more likely related to a direct effect of the root replantation on spinal cord reflex function.

It is not well understood how the replanted roots ameliorate VRA-induced pain. However, the properties of the avulsed ventral roots change markedly after the injury. Specifically, the avulsed ventral roots undergo Wallerian degeneration, which
includes a rapid elimination of the disconnected axonal segment (31, 37). In addition, a peripheral nerve or root lesion, denervated Schwann cells may divide and increase their expression of a variety of neurotrophic factors (15, 19, 20, 26, 39). Several growth factors have been associated with sensory plasticity and pain (24, 30, 40). It is therefore possible that such factors may be released by the reimplanted root and modulate sensory functions intramedullary.

To summarize, an L6-S1 VRA injury results in an augmented VMR response. The response is detected by EOM EMG studies during bladder filling and is suggestive of visceral pain. The increased reflex response normalizes to control levels at late time points. A surgical replantation of avulsed roots for repair purposes ameliorates the visceral pain with an earlier recovery of the VMR. The observed beneficial effect from acute ventral root replantation on visceral sensory function adds to previous studies that have shown functional reinnervation of the lower urinary tract after a bilateral lumbarosacral VRA injury and replantation of avulsed ventral roots (5, 18).

ACKNOWLEDGMENTS

We sincerely thank Dr. Jun Wu for helpful assistance with the surgical procedures.

GRANTS

This work is supported by National Institute of Neurological Disorders and Stroke NS-042719 and the Miriam and Sheldon G. Adelson Medical Research Foundation.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES