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Surgical Implantation of Avulsed Lumbosacral Ventral Roots Promotes Restoration of Bladder Morphology in Rats

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Abstract

Injuries to the cauda equina and conus medullaris of the spinal cord commonly result in paraplegia, sensory deficits, neuropathic pain, as well as bladder, bowel, and reproductive dysfunctions. In a recently developed lower motoneuron model for cauda equina injury and repair, we have demonstrated that an acute surgical implantation of avulsed lumbosacral ventral roots into the conus medullaris is neuroprotective, promotes regeneration of efferent spinal cord axons into the implanted roots, and may result in functional reinnervation of the lower urinary tract. Here, we investigated the effects of a bilateral lumbosacral ventral root avulsion (VRA) injury and re-implantation on the morphology of the rat bladder at twelve weeks post-operatively. We demonstrated a VRA-induced overall thinning of the bladder wall, which exhibited reduced thickness of both the lamina propria and smooth muscle. In contrast, the bladder epithelium markedly increased its thickness in the injured series. Quantitative immunohistochemical studies showed a selective increase in CGRP immunoreactivity in the lamina propria after the VRA injury. Interestingly, the injury-induced changes in bladder wall morphology were ameliorated by an acute implantation of the lesioned roots into the conus medullaris. Specifically, bladders of the implanted group showed a partial restoration of the thickness of the lamina propria and epithelium as well as a return of CGRP immunoreactivity to baseline levels in the lamina propria. Our results support the notion that surgical implantation of severed ventral roots into the spinal cord may promote the recovery of a normal morphological phenotype in peripheral end organs.

Keywords

CGRP; immunohistochemistry; epithelium; lamina propria; cauda equine

INTRODUCTION

Traumatic injuries to the conus medullaris (CM) and cauda equina (CE) commonly result in complex neurological deficits, which may include paraplegia, a sensory impairment, neuropathic pain, as well as bladder, bowel and reproductive dysfunctions (Maynard et al., 1997; Hoang and Havton, 2006). Impairments of the lower urinary tract (LUT) include urinary retention and loss of voluntary micturition (Pavakis et al., 1983). Presently, no treatments are available to reverse the deficits of a CM syndrome.

We have previously developed an experimental model, which mimics principal components of CM and CE injuries. In this model, lumbosacral ventral roots are separated from the surface of the spinal cord by means of an avulsion injury with subsequent retrograde degeneration of

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both motor and autonomic neurons by apoptosis, development of neuropathic pain, and denervation of the LUT (Hoang et al., 2003, 2006a, b; Bigbee et al., 2007). The lumbosacral ventral root avulsion (VRA) injury results in the loss of reflexive and voluntary micturition, urinary retention, as well as an increase in bladder size and weight (Hoang et al., 2006b). Interestingly, an acute surgical implantation of avulsed lumbosacral ventral roots into the CM is neuroprotective, promotes regeneration of efferent spinal cord axons into the implanted roots, ameliorates neuropathic pain, results in reinnervation of the LUT with return of reflexive micturition, as well as restores bladder size and weight (Hoang et al., 2006a, b; Bigbee et al., 2007; Chang and Havton, 2008). However, information is sparse with regards to the long-term consequences of pelvic end-organ denervation on the morphology of the LUT. Similarly, the microscopic anatomy of the LUT has previously not been explored following successful repair of lumbosacral ventral root injuries with functional reinnervation on the LUT.

Therefore, we performed quantitative assessments of the bladder wall morphology using histochemical and immunohistochemical techniques after a bilateral lumbosacral VRA injury and an acute implantation of lesioned roots into the CM of the adult rat. For these experiments, we used bladders previously obtained from rats in our recent urodynamic studies on lumbosacral ventral root injury and repair (Hoang et al., 2006b). Thus, all rats in the sham-operated series demonstrated normal micturition reflexes to bladder filling. In contrast, the VRA injury group exhibited a denervated LUT, and all rats in the implanted treatment group showed signs of functional reinnervation of the bladder and external urethral sphincter (EUS).

Here, we demonstrate a thinning of the lamina propria and smooth muscle layers of the bladder wall in combination with a markedly increased thickness of the bladder epithelium after a lumbosacral VRA injury. Also, quantitative immunohistochemical studies showed an increase in CGRP immunoreactivity in the lamina propria layer of the bladder wall after the VRA injury. Interestingly, the injury-induced changes in bladder wall morphology were ameliorated by an acute implantation of the lesioned roots into the CM. Specifically, bladders of the implanted group showed a partial restoration of the thickness of the lamina propria and epithelium layers as well as a return of CGRP immunoreactivity to baseline levels in the lamina propria. Our results are supportive of the notion that early repair of proximal lumbosacral root lesions, by surgical implantation of lesioned ventral roots into the CM, promotes the recovery of the LUT towards a normal functional and morphological phenotype.

MATERIALS AND METHODS

Animal preparations

19 adult female Sprague-Dawley rats (200–220 g, Charles River Laboratories, Raleigh, NC) were included in the study. The animals were divided into three groups: 1) Laminectomy and dura opening (sham-operated rats, n=6), 2) bilateral L5–S2 VRA (VRA rats, n=7) and 3) bilateral L5–S2 VRA and acute implantation of the L6 and S1 ventral roots into the spinal cord (implanted rats, n=6). All animal procedures were carried out according to the standards established by the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). The experimental protocols were approved by the Chancellor's Animal Research Committee at UCLA. All efforts were made to minimize the number of animals used and their suffering.

A bilateral lumbar laminectomy (L1–L4) and opening of the dura mater were performed in all rats under general gas anesthesia with 2–2.5% isoflurane (Abbott Laboratories, North Chicago, IL, USA). Anatomical landmarks were used to identify the L5, L6, S1 and S2 ventral roots under a surgical microscope. The sham group did not undergo any further surgery. In the implanted group, all four ventral roots were avulsed bilaterally. The functional contractions of the bladder (mediated by autonomic neurons in the L6 and S1 spinal cord segments) and the

EUS activity (innervated by motoneurons located primarily in the L6 segment) were thereby completely abolished (Schröder, 1980; Nadelhaft and Booth, 1984; Hoang et al., 2006a). By applying a constant traction with a pair of fine jeweler's forceps along the normal course of each individual root, all rootlets of each root were separated from the anterior surface of the spinal cord. The rootlets of the avulsed L6 and S1 ventral roots were trimmed and two small longitudinal scalpel incisions were made bilaterally into the lateral funiculus of the L6 and S1 spinal cord segments. The roots were implanted into the spinal cord in their respective incision sites. No tissue glue was needed to hold the roots in place. An 8-0 suture (Ethilon, Somerville, NJ, USA) was loosely placed around each implanted root to allow for easier identification during later dissections. For all animals, a titanium mesh cage was placed over the laminectomy site to stabilize the vertebral column and protect the spinal cord from compression by the overlying muscles (Nieto et al., 2005). The overlying paraspinal muscles and skin were subsequently sutured in layers, and all animals were allowed to recover. Bladders were manually expressed three times a day for two days after surgery, then twice a day until termination of the experiment at 12 weeks post-operatively. All animals underwent urodynamic experiments before the termination of studies (Hoang et al., 2006b).

Perfusion and histology

Animals were anesthetized with Nembutal (100 mg/kg; Abbott Laboratories, North Chicago, IL), their bladders completely expressed of residual urine, and all rats were intravascularly perfused with 200 ml of phosphate buffered saline followed by 500 ml of 4% paraformaldehyde (pF). The bladders were removed, postfixed in 4% pF for 24 hours and transferred to 30% sucrose for 24 hours. The length (L) and width (W) were measured over the surface of the flattened bladders, which had not been bisected. The trigone region was excluded. The formula $[(4/3) \times \pi \times L \times W^2]$ was used to estimate the bladder volume (Hoang et al., 2006b). In the present study, the total bladder volume was considered as the outer bladder volume (OV). The inner bladder volume (IV) was calculated by subtracting the averaged bladder wall thickness from both L and W in each animal. The formula $[(OV-IV)]$ was used to calculate the volume of the bladder wall. All bladders were frozen and segments of their walls were cut (10 μ m) transversely from the top of the dome. Serial bladder sections were stained by routine hematoxylin-eosin and Masson's trichrome staining. These slides, stained by Masson's trichrome staining, were studied in a Nikon E600 light microscope to measure the thickness of the epithelium, lamina propria and smooth muscle using a SPOT camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA). Quantitative measurements were performed using C-Imaging software (Compix, Inc., Brandywine, PA, USA) to determine the thickness of three layers of bladder wall. The outermost layer, or serosa, which is external to the smooth muscle was not included in the thickness of bladder wall.

Immunohistochemistry

Some serial bladder sections were immunohistochemically processed for CGRP (Chemicon International, Inc., Temecula, CA, USA). The primary antibodies were diluted in 0.3% Triton X-100/Phosphate Buffered Saline (PBS) to increase tissue permeability. The sections were rinsed in PBS, and incubated 1h with 5% normal donkey serum (Vector Laboratories, Burlingame, CA, USA) at room temperature as well as overnight with primary antibodies against CGRP (1:4000) at 4°C. All sections were then rinsed in PBS and placed in secondary antibody (Alexa Fluor 594; 1:500; Molecular Probes, Eugene, OR) for 1 h at room temperature. All sections were then rinsed in PBS, mounted with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA, USA), and examined in a Nikon E600 light microscope equipped for the detection of fluorescent markers. Quantitative immunohistochemical analyses were performed using C-Imaging software (Compix, Inc., Brandywine, PA, USA) to determine the total area of CGRP immunoreactivity within a 2500 μ m² region of interest in three layers of bladder wall.

Statistical Analysis

Quantitative data were expressed as mean $\pm$ standard error. For the thickness of bladder layers, an average was taken of all eighteen measurements in three bladder sections of each animal to represent the overall thickness of each layer. For the CGRP expression, fifteen measurements were taken in five bladder sections of each animal. To assess for differences between the three groups, the One Way Analysis of Variance was first applied. The Student-Newman-Keuls Method was used for the multiple comparison procedures. We considered $p < 0.05$ as indicating statistically significant differences between groups.

RESULTS

We have previously demonstrated that the bladders of the VRA series exhibited a larger size and weight compared to the bladders of the sham-operated and implanted series (Hoang et al., 2006b). In the present study, we investigated, using the bladders obtained from the earlier study (Hoang et al., 2006b), the effects of a bilateral lumbosacral (L5–S2) VRA injury and repair on the morphology of the rat urinary bladder at twelve weeks after sham surgery ($n=6$), a bilateral L5–S2 VRA injury ($n=7$), and a bilateral L5–S2 VRA injury followed by the surgical implantation of the lesioned L6 and S1 ventral roots into the spinal cord ($n=6$). For this purpose, histochemical methods were used to delineate layers of the bladder wall and immunohistochemistry was performed for CGRP detection.

Changes in bladder wall thickness and histology after VRA injury and repair

The histologic sections of the rat bladder wall were studied in the light microscope. The volume of the bladder wall in VRA series ($1.04 \pm 0.18 \text{ mm}^3$, $n=7$) showed a significant increase compared to both the sham ($0.58 \pm 0.06 \text{ mm}^3$, $n=6$) and implantation ($0.75 \pm 0.13 \text{ mm}^3$, $n=6$) series (Fig. 1). In all three groups, the bladder epithelium (urothelium), lamina propria and the underlying smooth muscle fibers were readily distinguished as separate layers in hematoxylin-eosin and Masson's trichrome stained sections (Fig. 2). Qualitatively, the bladders of the VRA series showed an overall thinning of the bladder wall and sparse mucosal fold formation when compared to the sham-operated and implanted series (Fig. 2).

Quantitative studies demonstrated a reduced thickness of the bladder wall in the VRA series ($639 \pm 56 \mu\text{m}$, $n=6$) compared to the bladder walls of sham-operated rats ($912 \pm 52 \mu\text{m}$, $n=7$, $p < 0.05$) and bladder walls of the implanted series ($785 \pm 23 \mu\text{m}$, $n=6$, $p < 0.05$) (Fig. 3A). Although the bladder walls of the implanted series were thicker than those of the VRA series, the bladder walls of the implanted series remained thinner than those in sham-operated rats ($p < 0.05$) (Fig. 3A). Interestingly, the various layers of the bladder wall demonstrated different responses to the VRA injury and root repair procedure. Specifically, in spite of an overall thinning of the bladder wall thickness in rats of the VRA series, the epithelium layer here showed a significant increase in thickness ($52 \pm 7 \mu\text{m}$, $n=7$) compared to the epithelium layer in sham-operated rats ($36 \pm 2 \mu\text{m}$, $n=6$, $p < 0.05$) and in rats of the implanted series ($43 \pm 3 \mu\text{m}$, $n=6$, $p < 0.05$) (Fig. 3B). Although the epithelium layer in the implanted series was thinner than the epithelium layer in VRA-operated rats, it remained thicker than the corresponding epithelium layer in sham-operated rats ($p < 0.05$) (Fig. 3B). Qualitatively, the epithelium layer of VRA-operated rats was not thickened in a uniform fashion but instead exhibited multiple areas with focal thickening of the epithelial layer, a feature not typically encountered in the sham-operated and implanted series (Fig. 4). The underlying lamina propria was thinner in VRA-operated rats ($163 \pm 28 \mu\text{m}$, $n=7$) compared to the sham-operated rats ($320 \pm 41 \mu\text{m}$, $n=6$, $p < 0.05$) and rats of the implanted series ($268 \pm 24 \mu\text{m}$, $n=6$, $p < 0.05$) (Fig. 3C). However, the lamina propria of the implanted series remained thinner than the corresponding layer if the sham-operated rats ($p < 0.05$) (Fig. 3C). Furthermore, the smooth muscle layer of the bladder was significantly thinner in the VRA series ($410 \pm 40 \mu\text{m}$, $n=7$) compared to the corresponding

smooth muscle layer in the sham-operated rats ($555 \pm 34 \mu\text{m}$, $n=7$, $p<0.05$) and animals of the implanted series ($464 \pm 27 \mu\text{m}$, $n=6$, $p<0.05$) (Fig. 3D). There was no difference in the thickness of the smooth muscle layer between the sham-operated and implanted series (Fig. 3D).

Plasticity of CGRP IR in the bladder wall after VRA injury and repair

Immunoreactivity for CGRP was detected in the form of thread-like fibers in the lamina propria and smooth muscle layers of the bladder wall but not within the urothelium in sham- and VRA-operated rats as well as in the implanted series (Fig. 5). Quantitatively, there was no overall difference in CGRP density within the bladder wall between the three experimental groups (Fig. 6A). However, when the lamina propria and smooth muscle layers were analyzed separately, a differential response pattern was detected. Specifically, the CGRP IR of the lamina propria of VRA-operated rats ($n=7$) was increased by 27% compared to the corresponding region in the sham-operated rats ($n=6$, $p<0.05$) and by 34% compared to the lamina propria of the implanted series ($n=6$, $p<0.05$) (Fig. 6B). However, there was no difference in CGRP IR in the lamina propria between the sham-operated and implanted series. In contrast, the underlying smooth muscle layer showed no difference in CGRP IR between any of the three groups (Fig. 6C).

DISCUSSION

In the present study, we investigated the effects of bilateral lumbosacral VRA injury and repair on the urinary bladder wall morphology and expression of CGRP immunoreactivity in the adult rat. The VRA injury represents a lower motoneuron type lesion and results in the loss of preganglionic parasympathetic innervation of pelvic ganglia and denervation of the EUS muscle. Functionally, the lumbosacral VRA injury is followed by the absence of reflex bladder contractions and EUS electromyographic activity, whereas the acute implantation of lesioned ventral roots into the CM promotes reinnervation of the LUT and return of reflexive micturition (Hoang et al., 2006b). Peripheral end organ effects are also present in this injury and repair model, as both the bladder size and weight increased following the VRA injury but normalized towards baseline values, when the VRA injury is combined with an acute implantation of the lesioned roots into the CM (Hoang et al., 2006b). Here, we demonstrate that the bladder wall also exhibits marked morphological changes and plasticity of CGRP IR afferents after a bilateral lumbosacral VRA injury and repair.

Effects of lumbosacral ventral root injury and repair on bladder wall morphology

Traumatic spinal cord injuries and other myelopathies result in urinary retention. As the normal urinary production in the adult rat measures about 15–30 ml per 24 hour period (Kerns et al., 2000; Krinke et al., 2000), frequent manual bladder expressions or intermittent drainage of urine through a chronic indwelling cannula is needed following spinal cord lesions (Pikov and Wrathall, 2001; Keirstead et al., 2005; Zinck et al., 2007). However, spinal cord injury-induced urinary retention and bladder distension in the rat also result in changes of the bladder morphology with an overall increase in the thickness of the bladder wall (Zinck et al., 2007).

In contrast, a lower motoneuron injury syndrome, which may follow lesions to the CM and cauda equina, results in an areflexic bladder in combination with a flaccid weakness and atrophy of the EUS (Pavlakis et al., 1983). Interestingly, a bilateral lumbosacral VRA injury in rats leads to urinary retention of the relatively modest amount of approximately 3–5 ml per 24 hour period, likely as a result of decreased urethral resistance and intermittent involuntary urinary leakage during movement or transient increases in intra-abdominal pressure (Hoang et al., 2006b). However, when a lumbosacral VRA injury is followed by an acute implantation of lesioned lumbosacral roots into the CM, the degree of urinary retention is initially similar

to what is encountered in the VRA injury series, but urine expression measurements approach baseline values in the implanted group after several weeks (Hoang et al., 2006b).

Here, the bilateral lumbosacral VRA injury resulted in an overall reduced thickness of the bladder wall with thinning of both the lamina propria and smooth muscle layer. This is in sharp contrast to the thickening of the bladder wall following a spinal cord transection (Zinck et al., 2007). Interestingly, fundamental functional differences in neuronal circuitry between the upper motoneuron injury of a spinal cord transection and the lower motoneuron injury of lumbosacral ventral root lesions may contribute to the apparent opposite bladder wall responses. Following a spinal cord crush or transection injury, there is loss of descending inputs to the CM with a series of functional changes affecting micturition, including decreased oscillating activity of bladder contractions and reduced phasic EUS EMG activity in response to bladder filling (Pikov and Wrathall, 2001; Cheng and de Groat, 2004). However, the spinal reflex loop is still anatomically intact with parasympathetic efferent fibers being able to induce a reflex bladder contraction in response to bladder distension and increased bladder afferent activity. As coordination between the bladder contraction and EUS activity is also impaired following a spinal cord injury in rats (Pikov and Wrathall, 2001; Cheng and de Groat, 2004; Chang et al., 2007), the reflex bladder contraction may occur in the setting of increased urethral resistance, which could further contribute to the hypertrophy of the detrusor muscle. In contrast, the parasympathetic efferent loop was interrupted by the VRA injury in the present study. The resultant pelvic ganglia denervation results in the loss of detrusor reflex contractions to bladder filling (Hoang et al., 2006b). As bladder distensions here take place unopposed by detrusor muscle contractions, stretching and thinning of the bladder wall appears as reasonable consequences of the lumbosacral VRA injury. Interestingly, we also demonstrate that implantation of lesioned ventral roots into the CM result in partial restoration of the bladder thickness and morphology. The latter finding is consistent with the notion that functional reinnervation of the LUT with return of reflexive micturition is important for the bladder to maintain a normal morphological phenotype.

Effects of lumbosacral ventral root injury and repair on the bladder epithelium

Recent studies have demonstrated that a spinal cord transection injury may result in an increase in the thickness of both the bladder epithelium and smooth muscle layers (Zinck et al., 2007). Here, we demonstrate that the bladder epithelium may exhibit a response pattern, which is independent from the rest of the bladder wall, as the urothelium increased in thickness in spite of the bladder distension and associated thinning of the lamina propria and smooth muscle layers following a bilateral lumbosacral VRA injury. Normally, the bladder epithelium provides a permeability barrier but its function may be affected by neurological injury (Birder and de Groat, 2007). For instance, a thoracic spinal cord transection injury in rats may result in an early but transient decrease in transepithelial resistance and increased epithelial permeability to water and urea (Apodaca et al., 2003). The effect of lumbosacral VRA injuries and forms of lower motoneuron lesions on the permeability of the bladder epithelium remains to be elucidated. However, the morphological phenotype of the urothelium appears to be responsive to the functional state of the bladder, as we here demonstrated that the thickness of the bladder epithelium is partially restored in association with successful functional reinnervation of the LUT following the acute implantation of lesioned ventral roots into the CM. We speculate that the thickening of the epithelium may be the result of proliferation or hypertrophy of the urothelium cells.

CGRP changes – interactions with urothelium

The rat bladder wall is innervated by different functional types of primary afferents, including Aδ- and C fibers (Namasivayam et al., 1998; Morrison, 1999). Immunohistochemical studies of bladder wall innervation in neurologically intact rats have demonstrated the presence of

several neuropeptides, including CGRP, substance P, vasoactive intestinal peptide (VIP), neuropeptide Y, galanin, and pituitary adenylate cyclase activating polypeptide (PACAP) (Mattiasson et al., 1985; Ferrandino and Grimaldi, 1996; Gabella and Davis, 1998; Zvarova et al., 2005). In the present study, we investigated CGRP IR as a marker of peptidergic primary afferents in the bladder wall. CGRP was chosen as it is prevalent and readily detectable in the rat bladder wall (Gabella and Davis, 1998). CGRP IR primary afferents also exhibit prominent inputs to the dorsal horn and autonomic nuclei of the lumbosacral spinal cord (Vizzard, 2001). We demonstrated a selective increase in the density of CGRP IR in the lamina propria of the bladder wall after a bilateral lumbosacral VRA injury. However, the lamina propria of the bladder demonstrated a normalized CGRP IR when the VRA injury was followed by an acute implantation of lesioned ventral roots into the CM. Furthermore, we did not identify any CGRP IR fibers within the epithelium, and these findings are consistent with previous studies on CGRP localization within the bladder wall of rats (Gabella and Davis, 1998).

It is interesting to note that the VRA injury results in an increase in CGRP IR within the bladder wall. Here it is important to note that primary afferents were not subjected to any direct lesion in our model (Fig. 7). However, a lesion to sensory afferents is not required for sensory plasticity to occur. Previous studies have demonstrated that neuropathic pain may occur following a ventral root transection or avulsion injury (Li et al., 2002; Bigbee et al., 2007). In the present model, it remains to be determined whether the VRA-induced increase in CGRP in the bladder wall may be associated with other pain patterns, e.g. visceral pain.

Interestingly, recent studies have suggested an extensive interaction between the bladder epithelium and the sensory afferents of the underlying lamina propria, as the urothelium may respond to a variety of different mechanical and chemical stimuli and release signaling molecules in the vicinity of the nerve endings of sensory afferents (Birder, 2005; Apodaca et al., 2007). A possible injury-induced protective effect provided by capsaicin-sensitive afferents, including primary afferents containing CGRP and/or substance P, has also been suggested, as capsaicin pretreatment to desensitize bladder afferents worsened the effects of a spinal cord transection on bladder epithelium permeability in rats (Apodaca et al., 2003). These reports raise the possibility that similar close interactions between the urothelium and primary afferents in the lamina propria may also exist following lumbosacral VRA injury and repair.

Overall effects of lumbosacral ventral root injury and repair

Surgical implantation of lesioned ventral roots into the spinal cord has been associated with a series of encouraging neural repair outcomes in experimental models and has thus emerged as a strategy of increasing translational research interest. Specifically, an acute implantation of lesioned ventral roots into the spinal cord may be neuroprotective, promote axonal regeneration as well as result in functional and anatomical reinnervation of extremity muscles and the LUT (Cullheim et al., 1989; Carlstedt et al., 1993; Hallin et al., 1999; Hoang et al., 2006a, b; Chang and Havton, 2008). Additional benefits from surgical implantation of lesioned ventral roots into the CM may include amelioration of neuropathic pain (Bigbee et al., 2007) and normalization of bladder weight and size (Hoang et al., 2006b). Here, we demonstrate that early repair of proximally lesioned ventral roots by an acute surgical implantation of the injured roots into the spinal cord may also promote restoration of the morphological phenotype of the peripheral end organ.

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Fig. 1.
The volume of the bladder wall in the VRA group was significantly increased compared to sham and implanted groups. * indicates $p < 0.05$.

Fig. 2.

The bladder wall morphology is partially restored when a lumbosacral VRA injury is followed by surgical implantation of lesioned roots into the conus medullaris (CM). The epithelium, lamina propria, and muscle layers are detected in sham operated, VRA, and implanted groups. Representative examples of hematoxylineosin (A–C) and Masson's trichrome (D–F) staining are shown for all three experimental groups. Calibration bar = 200 μ m. E: epithelium. L: lamina propria. M: smooth muscle.

Fig. 3.

The bladder wall thickness is reduced after a lumbosacral VRA injury and is partially restored after an acute implantation of lesioned ventral roots into the CM. The whole bladder wall thickness (A) was measured as was the thickness of the epithelium (B), lamina propria (C) and smooth muscle (D) layers in sham, VRA and implanted rats; * indicates $p < 0.05$.

A. Sham

B. VRA

C. Implantation

Fig. 4. The thickness of the bladder epithelium is increased after a lumbosacral VRA injury and partially restored following an acute implantation of lesioned roots into the CM. All sections were stained by Hematoxylin-eosin (A–C). Note that the VRA group demonstrated focal areas of increased epithelium thickness as indicated by arrow (B). Calibration bar = 100 μ m.

A. Sham

B. VRA

C. Implantation

Fig. 5.

Immunohistochemistry for CGRP shows absence of CGRP in the epithelium but presence of CGRP in both the lamina propria and smooth muscle layers. Note focal increase of CGRP immunoreactivity in the lamina propria after the VRA injury. Representative sections of the bladder wall in rats of sham (A), VRA (B) and implanted (C) series. E: epithelium. L: lamina propria. M: smooth muscle. Calibration bar = 50 μ m.

A. Whole bladder wall

B. Lamina propria

C. Smooth muscle

Fig. 6.

Immunoreactivity for CGRP is increased in the lamina propria of the bladder wall after lumbosacral VRA injury and restored to baseline levels following implantation of lesioned ventral roots into the CM. Analysis of CGRP expression was performed in whole bladder walls (A), the lamina propria (B) and the smooth muscle layer (C). * indicates $p < 0.05$.

Fig. 7.

Schematic presentation of the innervation of the lower urinary tract. Note that the L5–S2 VRA injury results in a lesion of all motor and autonomic efferent fibers in the pudendal and pelvic nerves, respectively, whereas efferent sympathetic fibers in the hypogastric nerve and all afferent fibers originating from the bladder remain intact. MPG: major pelvic ganglia. PPNs: parasympathetic preganglionic neurons. EUS: external urethral sphincter. MNs: motoneurons. SPNs: sympathetic preganglionic neurons. SCG: sympathetic chain ganglia. IMG: inferior mesenteric ganglia. E: epithelium. L: lamina propria. M: smooth muscle.