

TIME COURSE OF NERVE REGENERATION AND URETHRAL DYSFUNCTION AFTER BILATERAL PUDENDAL NERVE INJURY IN FEMALE RATS

Aims of Study

Stress urinary incontinence (SUI) is a significant medical problem affecting approximately 25 million Americans of all ages [1]. One of the epidemiologic factors most strongly associated with development of SUI in women is vaginal delivery of children [2], which can injure the nerves, muscles, and connective tissues responsible for maintaining continence [1]. In particular, the pudendal nerve, which innervates the external urethral sphincter (EUS), can be damaged during childbirth [3]. It has been shown previously that a bilateral pudendal nerve crush injury induces symptoms of SUI in female rats [4,5]. The purpose of this study was to determine the time course and extent of pudendal nerve regeneration and urethral dysfunction after a bilateral pudendal nerve crush in female rats.

Methods

Pudendal Nerve Crush. 53 rats were anesthetized with a mixture of ketamine and xylazine. The pudendal nerve was located in the ischiorectal fossa in 42 of these rats and the pudendal nerve was crushed twice, just proximal to the branch point of the obturator nerve [5]. Nerve injury was observed immediately afterward by transparency of the nerve sheath at the crush site. Eleven rats were used as sham controls and underwent a sham surgery.

Bladder Catheter Implantation. Two days prior to leak point pressure (LPP) functional testing, the rats were anesthetized as above and a circular purse-string suture was placed on the bladder wall. A small incision was made and the catheter (PE-50 tubing with a flared tip) was implanted. The catheter was tunneled subcutaneously to the neck where it exited the skin.

Leak Point Pressure (LPP) Testing. Four days, 2 weeks, or 6 weeks after nerve crush, the animals were anesthetized with urethane and placed supine for LPP testing [6]. The bladder was palpated to empty, then filled with saline at 5 ml/hr. Gentle pressure was applied to each rat's abdomen and was slowly increased until the first leakage of saline through the urethra, when the pressure was rapidly removed. Peak pressure was taken as LPP. The procedure was repeated at least 3 times and mean values of LPP and the external abdominal pressure, P_{diff} , were calculated for each rat and for each group.

Pudendal Nerve Histology. Immediately after LPP testing, the animals underwent intracardiac perfusion fixation. Rats were perfused with fixative and the pudendal nerve was dissected 5mm distal to the lesion site and prepared for light microscopy [4]. Myelinated axons and nuclei were counted in a cross-section of all fascicles of the EUS branch of the pudendal nerve to quantitatively measure nerve regeneration. Selected regions were trimmed for ultrathin sectioning and qualitative analysis under electron microscopy.

Data Analysis. Data is presented as mean \pm standard error of the mean for each experimental group. Statistical comparisons were made using a one-way ANOVA followed by a Student-Newman-Keuls posthoc test with $p < 0.05$ indicating a significant difference.

Results

LPP. P_{diff} was significantly decreased 4 days after pudendal nerve crush (20.3 ± 2.1 cmH₂O), compared to sham animals (38.3 ± 3.2 cmH₂O), as well as animals tested 2 weeks (32.0 ± 2.1 cmH₂O) or 6 weeks (31.4 ± 2.6 cmH₂O) after nerve crush. There were no significant differences in P_{diff} between animals tested 2 weeks or 6 weeks after nerve crush and sham animals, suggesting that urethral function had returned nearly to normal values. LPP results demonstrated the same trends (decrease 4 days after nerve crush with a return to near normal values 2 weeks after nerve crush) but there were no significant differences, due to variations in baseline pressure.

Histology. Four days after pudendal nerve crush, the EUS branch of the pudendal nerve contained many myelin figures distal to the crush site and no normal spared myelinated

axons, indicating that the crush injury was complete. Two weeks after nerve crush, the pudendal nerve had many myelinated axons (62 ± 5), but significantly fewer than the sham group (81 ± 2). Six weeks after nerve crush the number of myelinated axons (75 ± 6) was not significantly different from the sham group, indicating that by this time, nerve regeneration had substantially progressed. The number of nuclei significantly increased 2 weeks after nerve crush (29 ± 4) compared to the sham group (6 ± 1). The number of nuclei decreased 6 weeks after nerve crush (14 ± 2) but were still significantly higher than sham values. Electron microscopic analysis confirmed the results observed using light microscopy and revealed ultrastructural details not observable at the light microscopic level, particularly at 4 days after nerve injury.

Conclusions

The pudendal nerve is among those tissues injured during vaginal childbirth, which can lead to EUS denervation, urethral dysfunction, and symptoms of SUI. We have demonstrated that a direct pudendal nerve crush injury in female rats leads to decreased urethral resistance 4 days after injury. By 2 weeks after injury, urethral resistance had returned nearly to normal values. However, histological indicators of neuroregenerative processes were significantly different from controls: the numbers of nuclei from Schwann cells, macrophages, fibroblasts, and mast cells in the pudendal nerve were 5 times normal values. In addition, myelinated axons were present, although their number and maturation were less than normal. The 2 week time point is consistent with EUS reinnervation [7] and functional recovery. The subsequent maturation, therefore seems to be primarily structural. However, it may be that other urethral structures, potentially smooth muscle, can compensate for denervation of the EUS.

While there was no improvement in function from 2 weeks to 6 weeks after the nerve crush, the number of nuclei in the nerve decreased to approximately twice that of control values and the number of myelinated axons increased nearly to control values. This suggests that the neuroregenerative process was nearing completion by 6 weeks. Therefore, it may be that as the EUS reinnervates during this time period and improves its functional ability, urethral smooth muscle decreases its functional contribution accordingly.

Future directions for this work will be aimed at clarifying the functional, anatomic, and molecular events occurring at the urethra in response to pudendal nerve injury. When properly characterized, this animal model could be useful for preclinical testing of new treatments for urethral dysfunction and SUI.

References

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