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URODYNAMIC FUNCTION AND EXPRESSION OF NEUROTROPHINS AFTER A MATERNAL CHILDBIRTH INJURY MODEL OF VAGINAL DISTENSION AND PUDENDAL NERVE CRUSH IN RATS

Hypothesis / aims of study

Clinically, during vaginal delivery of children, the muscles, nerves, ligaments and organs of the pelvic floor are compressed and injured. This includes the external urethral sphincter (EUS), the striated muscle of the urethra, which can become hypoxic during vaginal distension. In addition, the pudendal nerve, which innervates the EUS, courses through Alcock's canal and can be trapped and injured during vaginal childbirth. Thus, the injuries incurred during vaginal childbirth represent a unique neuromuscular injury: one in which both the target organ (the EUS) and its innervation are injured simultaneously. These two injuries, along with injuries to other tissues of the pelvic floor are strongly correlated with later development of stress urinary incontinence (SUI). Neurotrophins are upregulated after nerve injury and down regulated after muscle injury and may provide insight into the mechanism of later SUI development from pelvic floor injuries in childbirth. Animal models of the maternal injuries in childbirth that can lead to pelvic floor dysfunction have focused on either vaginal distension (VD) or pudendal nerve crush (PNC). However, since the pudendal nerve is trapped and injured in Alcock's canal simultaneous with pelvic floor muscles injuries during vaginal distension, a dual injury model may best represent the injuries seen clinically. The objective of this project was to investigate urethral function, urethral anatomy, and expression of neurotrophins after a dual injury model consisting of both PNC and VD.

Study design, materials and methods

Virgin Sprague-Dawley rats (n=140) were divided into 4 groups and underwent either VD using a modified Foley catheter that was inserted into the vagina and filled with saline (3 ml) for 4 hours (VD), PNC consisting of a standardized bilateral compression of the nerve in the ischiorectal fossa (PNC), both injuries (PNC+VD), or neither (C). One day after injury, neurotrophin (BDNF, NT-4, Trk-B and NGF) expression in the EUS was investigated by immunohistochemistry (n=20). Four, 10, and 21 days after injury, urethral function was assessed by leak point pressure in all 4 groups: the bladder was slowly depressed while bladder pressure was measured via a suprapubic catheter implanted 2 days prior. The increase in external abdominal pressure at leakage, the leak point pressure (LPP), was taken to indicate urethral resistance to flow. Data was analyzed using a two way ANOVA followed by a Student-Newman-Keuls test ($p=0.05$). Data is presented as mean ± standard error of the mean. Immediately after LPP measurement, the rats were euthanized and the urethra was dissected at each of the 4 time points and was used in qualitative histological analysis. Immunohistochemistry results were analyzed qualitatively.

Results

There were no significant differences in LPP in the C group over time. Four days after injury, all 3 injury groups had significantly decreased LPP compared to C rats (Figure 1). There were no significant differences in LPP between the injury groups. Ten days after injury, VD rats had recovered to C values but LPP of PNC and PNC+VD rats remained significantly lower than C rats. Three weeks after injury, all injury groups had recovered to C values. Histological evidence of injury was evident in the EUS of the VD and PNC+VD groups 1 day after injury. Four and 10 days after injury all injury groups showed evidence of striated muscle fiber disruption and atrophy in the EUS. Three weeks after injury the EUS had regenerated in the VD and PNC groups but remained disrupted in the PNC+VD group. All injury groups demonstrated evidence of connective tissue infiltration into the EUS. Immunohistochemistry demonstrated low expression of BDNF and NT-4 in the EUS of C rats. After VD, BDNF and NT-4 expression in the EUS was low and was not different from C animals. In contrast, BDNF and NT-4 expression was dramatically upregulated in the EUS of PNC rats. After PNC+VD, BDNF and NT-4 expression demonstrated downregulation in the region of EUS close to the vagina, although some animals showed upregulation in other regions in the urethra. Both NGF and Trk-4 showed either negative or slightly positive expression in the EUS of all 4 groups and had no significant difference between groups.

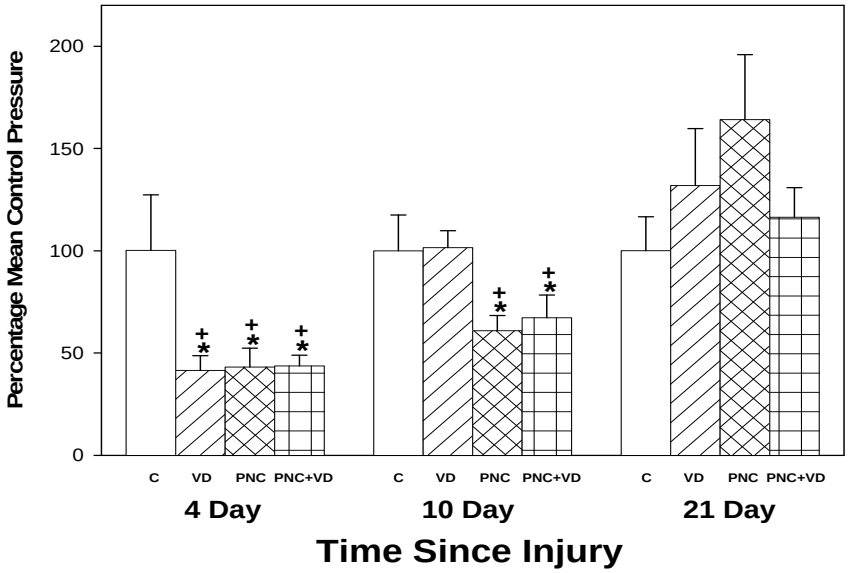

Figure 1. The leak point pressure (LPP). Data is presented as mean ± standard error of percent mean control value at each time point. * indicates a significant difference compared to control group at the same time point. + indicates a significant difference compared to similar injury group tested 21 days after injury.

Interpretation of results

LPP was significantly decreased in all 3 injury groups 4 days after injury, indicative of short-term decreased urethral resistance and urethral dysfunction. Ten days after injury, LPP was significantly decreased only in the PNC and PNC+VD groups, indicating that these injuries need longer time to recover than VD animals. Twenty-one days after injury, LPP was not significantly different from C, indicating a return to normal function.Histological results demonstrated muscle injuries in the urethra after either VD or PNC but these had recovered by 21 days, the dual injury may causes more severe injury to the EUS than either injury alone since it had not yet recovered 3 weeks after injury. Upregulation of BDNF & NT-4 after PNC indicates that BDNF & NT-4 are important for neuroregeneration. BDNF and NT-4 are not upregulated after VD suggesting that the lack of upregulation, possibly due to target organ (EUS) injury, may hamper neuroregeneration after simulated childbirth injuries. In addition, BDNF and NT-4 are not upregulated after PNC+VD suggesting that pudendal nerve regeneration may be impaired after a dual injury.

Concluding message

Simultaneous PNC+VD provides a more severe injury than PNC or VD alone as indicated primarily by histological assessment. The EUS may not upregulate neurotrophins enough for a strong neuro-regenerative response after PNC+VD, which may explain why the pudendal nerve does not appear to recover well in women with pudendal nerve injury after childbirth. Dual injury may provide a more clinically relevant injury model either PNC or VD alone.

FUNDING: NIH RO1 HD38679 and the Rehabilitation Research & Development Service of the Department of Veterans Affairs

ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by IACUC