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NERVE REGENERATION AND VOIDING BEHAVIOR PATTERNS AFTER PUDENDAL NERVE CRUSH IN FEMALE RATS

AIMS OF STUDY

Stress Urinary Incontinence (SUI) is a significant medical problem affecting approximately 25 million Americans [1]. Among the causes of SUI is pudendal nerve injury secondary to childbirth or pelvic surgeries [2]. However, there is no therapy currently available to treat those with pudendal nerve injury. The objective of this study was to determine if changes in urinary behavior are temporally related to neuroregenerative activity after bilateral pudendal nerve crush in female rats. We hypothesized that initiation of pudendal nerve regeneration, as indicated by increased mRNA levels of $\exists_{II}$ tubulin, a cytoskeletal protein, would occur prior to normalization of urinary behavior after pudendal nerve crush.

METHODS

Surgery. Eighteen virgin female Sprague-Dawley rats underwent bilateral pudendal nerve crush. Using an operating microscope, the pudendal nerve was identified bilaterally in the ischiorectal fossa and was crushed twice for 30 sec. using the same microtip forceps in all animals. Seventeen rats were used as unoperated controls.

Voiding Behavior. Each animal was housed in a modified metabolic cage for 24 hours with 12 hours of light and 12 hours of darkness, pre-operatively and 2, 6, and 13 days post-operatively (dpo). A beaker on a force transducer (Grass, FT 10) was placed under each cage to quantify urination pattern. Mean urinary volume and urinary frequency were calculated separately for light and dark periods and were normalized to the amount of water drunk to minimize differences in voiding patterns due to differences in drinking patterns.

Spinal Cord Harvest and Preparation. At either 7 or 14 dpo the lumbar spinal cord was removed and frozen at –80^{o}C. The spinal cords were sectioned transversely (20 :m) through the dorsolateral motoneurons (DLM), which innervate the pudendal nerve and the retrodorsolateral motoneurons (RDLM), which innervate the sciatic nerve. The sections were mounted on chrome-alum subbed slides and were fixed.

In Situ Hybridization. For *in situ* hybridization, sections were hybridized with denatured hybridization solution and a radio-labeled rat cDNA probe specific for $\exists_{II}$ tubulin, RBT-1 3'UT [3]. After posthybridization washes, the slides were dipped in Kodak NTB-2 emulsion, exposed in light tight boxes, and developed. The sections were stained in fresh thionin and coverslipped for light microscopic evaluation.

Grain Counts and Data Analysis. Each $\exists_{II}$ tubulin mRNA was represented as a grain observed under light microscopy. Background grain density was subtracted from each DLM grain density. RDLM cells were not injured and function as an internal control. To reduce variation due to different hybridization levels, DLM grain densities were normalized by the mean of 3 RDLM grain densities on each slide. One to six sections from each animal were analyzed. Results are presented as mean $\forall$ standard error of the mean. Students t-test was used to compare results at each time period.

RESULTS

There was no significant difference in urinary frequency or mean urinary volume in the dark cycle between the two groups at any time point. At 2 dpo, there was no significant difference in normalized mean urinary volume between the nerve crush (0.023 $\forall$ 0.013; n=16) and control (0.027 $\forall$ 0.003; n=15) groups. At 6dpo, normalized mean urinary volume during the light cycle in the nerve crush group (0.021 $\forall$ 0.003; n=17) was decreased compared to the control group (0.032 $\forall$ 0.004; n=16), although not significantly (p=0.053), suggesting the potential for altered urinary behavior. At 7dpo, normalized DLM $\exists_{II}$ tubulin mRNA grain density was significantly increased in the nerve crush group (2.49 $\forall$ 0.38; n=10) compared to the control group (1.01 $\forall$ 0.10; n=9; p=0.002). At 13 dpo, there was no difference in mean urinary volume in the light cycle between nerve crush (0.029 $\forall$ 0.006; n=6) and control (0.023 $\forall$ 0.003; n=6) groups, suggesting a return to normal behavior. At 14 dpo, there was no significant difference in normalized DLM $\exists_{II}$ tubulin mRNA grain density between nerve crush (1.47 $\forall$ 0.28; n=8) and control (1.13 $\forall$ 0.12; n=8) groups, indicating an end to that stage of nerve

regeneration. There was no difference in RDLM $\exists_{II}$ tubulin mRNA grain density between nerve crush (0.09 $\forall$ 0.03 grains/:m) and control (0.09 $\forall$ 0.02 grains/:m) groups, indicating that the sciatic nerve was not injured and that RDLM grain densities serve as a good internal control.

CONCLUSIONS

Voiding behavior in the rat is highly variable and difficult to study. However, this research suggests that initiation of nerve regeneration occurs prior to normalization of voiding behavior after pudendal nerve crush. Urodynamic studies are needed to determine if pudendal nerve injury causes incontinence. This animal model could be useful for developing treatments to accelerate nerve regeneration and improve functional recovery after pudendal nerve injury.

REFERENCES

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COMPLEX REPETITIVE DISCHARGES DURING URETHRAL SPHINCTER ELECTROMYOGRAPHY: CLINICAL CORRELATES

Aims of Study

Abnormal urethral EMG activity including complex repetitive discharges (CRDs) and decelerating bursts has been reported in patients with and without symptoms of lower urinary tract dysfunction and with a variety of neurologic disorders[1,2]. It has also been linked with a poor response to biofeedback techniques used to treat voiding dysfunction[3]. We sought to characterize patients with these EMG abnormalities in our referral urogynecolgy practice.

Methods

Needle EMG of the urethral sphincter is typically performed during multichannel urodynamic (MCUD) studies at our center. The Nicolet Viking IIE electrodiagnostic instrument processes the signal from a 30-gauge concentric EMG needle is placed approximately 5mm ventral to the urethral meatus. Optimal needle placement is confirmed by sound and by morphology of the motor unit action potential. Recording parameters include a bandwidth of 20-10,000 Hz, sensitivity of 50 μV and sweep speed of 10ms per division. EMG activity is continuously recorded during fill and void attempts with the patient seated at a 45 degree angle. Abnormal discharges at the time of needle placement or readjustment are discounted as likely artifact. Urodynamic diagnoses are made in accordance with the definitions of the International Continence Society. All patients with CRDs during any stage of their study were characterized by age, vaginal parity, history of neurologic disorder, lower urinary tract symptoms, and diagnoses during MCUD testing. The relationship between symptoms of strain voiding, and the finding of urethral CRDs was tested with a Chi-squared test of association and considered significant at the 1% level.

Results

The EMG data from 351 consecutive studies was reviewed. A subgroup of twenty-seven (8%) of the patients demonstrated CRDs during some phase of their study. The average age of this subgroup was 49.4 yrs (range 18-78) and mean vaginal parity was 2.3 (range 0-5). Whereas the majority of patients with CRDs were free of previously diagnosed or grossly detectable neurologic abnormalities, six (22%) of the patients had known or suspected neurologic disorders (4 spinal cord lesions, 1 with seizure disorder, 1 with possible multiple sclerosis). Strain voiding was reported in 13 (48%) of patients with CRDs, and in 18% of the entire population of 351 patients. A statistically significant association between the symptom of strain voiding and the finding of CRDs was present (χ^2=17.56, p<0.001). The mean spontaneous postvoid residual prior to urodynamic testing was 57cc (range 5-250). CRDs were recorded only at rest in 2 (7%) patients, only during bladder filling in 10 (37%) patients, only