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Short-term timecourse of bilateral pudendal nerve injury on leak-point pressure in female rats

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Abstract—The pudendal nerve innervates the external urethral sphincter and, when injured, can contribute to incontinence development. This experiment was designed to study the time course of functional changes in the urethra after pudendal nerve crush in rats. Leak-point pressure (LPP) was measured 2, 4, 7, or 14 days after bilateral pudendal nerve crush and was compared to that of a control group. LPP at all four time points after nerve injury was significantly decreased compared to control values. A minimum was reached 4 days after injury, and LPP appeared to trend upward with increasing time after injury, suggesting that nerve function may begin to recover or compensatory changes in the urethra may occur. Pudendal nerve crush induces decreased LPP in female rats, mimicking the clinical symptoms of stress incontinence. When fully characterized, this model could be useful for preclinical testing of treatment and rehabilitation protocols.

Key words: female, incontinence, leak-point pressure, nerve injury, pudendal nerve, rat, urethra.

INTRODUCTION

Stress urinary incontinence (SUI) is the leakage of urine when intravesical pressure exceeds urethral resistance as a result of increased intra-abdominal pressure in the absence of a detrusor contraction [1]. In women, vaginal delivery, parity, menopause, and pelvic surgery contribute to this common condition, which affects approximately 25 million Americans [2–3]. The pudendal nerve, which innervates the external urethral sphincter (EUS), is among those tissues injured during childbirth [4]. Since the EUS normally responds to increases in abdominal pressure by contracting to maintain continence [2], denervation and the resultant atrophy can lead to SUI [5]. The symptoms, however, often do not appear until menopause [6], suggesting a complex etiology in which multiple reinjuries and biochemical changes with age contribute.

SUI is currently diagnosed during a urodynamics exam by determination of abdominal leak-point pressure (LPP). Once diagnosed, SUI is treated by pelvic floor rehabilitation exercises (Kegels), surgery, or urethral injections aimed at adding bulk to the urethra [7]. Although SUI is known to be multifactorial in origin, no current treatments address its neurogenic causes. Since the timing of nerve damage is known (i.e., vaginal delivery or surgery), treatments or rehabilitation protocols aimed at

Abbreviations: EUS = external urethral sphincter, i.p. = intraperitoneal, LPP = leak-point pressure, P_{abd} = external abdominal pressure, SUI = stress urinary incontinence.

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accelerating neuroregeneration and preventing symptomatic development are possible. Testing pharmacologic and other interventions on an animal model would be a key step to development of novel methods of treating and preventing this common condition.

We have developed an animal model of incontinence due to pudendal nerve injury, useful for preclinical testing [8–10]. We have also developed and tested a method of mimicking clinical LPP measurement to determine urethral resistance in the rat [11]. Bilateral pudendal nerve crush results in symptoms of SUI, including decreased LPP 4 days after injury and alterations in voiding behavior within 1 week of nerve injury [9–10]. Our aim in this project was to determine the time course and extent of changes in LPP after pudendal nerve injury, with the expectation that this animal model could be used in the future to test rehabilitation therapies aimed at prevention and/or treatment of SUI.

MATERIALS AND METHODS

The Hines Department of Veterans Affairs Hospital Institutional Animal Care and Use Committee approved this project. Fifty-four virgin female Sprague-Dawley rats (180–210 g body weight) underwent a bilateral pudendal nerve crush. To determine the time course of functional effects, we conducted LPP testing on the rats 2 (n = 12), 4 (n = 16), 7 (n = 15), or 14 (n = 12) days after nerve injury. On a sham-operated control group (n = 13), we performed a skin incision only, followed by LPP testing 7 days later. We implanted a suprapubic bladder catheter 2 days prior to LPP testing to enable bladder filling and measurement of bladder pressure.

Pudendal Nerve Crush

We performed the pudendal nerve injury as previously described [10]. In brief, the rats were anesthetized with a mixture of ketamine (100 mg/kg, intraperitoneal [i.p.]) and xylazine (15 mg/kg, i.p.). We made a dorsal midline incision in the skin and bilateral dorsal incisions in the muscle, then located the pudendal nerve in the ischiorectal fossa using an operating microscope. We then identified the pudendal nerve on each side in the ischiorectal fossa and crushed it twice for 30 s bilaterally in the identical location (proximal to the branching of the obturator branch) using the same Castro-Viejo microsurgical clamps in each surgery. We observed nerve injury immediately afterward by a marked transparency of the nerve sheet at the crush site. The muscle and skin incisions were closed separately with 3-0 vicryl sutures. Upon awakening from anesthesia, animals were given buprenorphine (0.1 ml/100 g body weight) subcutaneously for pain control.

Catheter Implantation

All rats underwent suprapubic bladder catheter implantation 2 days prior to LPP testing, as previously described [10]. In brief, we anesthetized the rats as just described and made a longitudinal abdominal incision. A circular purse string suture (4-0 silk) was placed on the bladder wall. A small incision was made in the bladder wall in the center of the purse string and the catheter (PE-50 tubing with a flared tip) implanted. The purse string was tightened around the catheter and the catheter tunneled subcutaneously to the neck, where it exited the skin.

Leak-Point Pressure Testing

LPP testing was performed as previously described [10–11]. Even though no anesthetic agent maintains neural reflexes identical to those of a conscious state [11–12], we anesthetized the animals because movement is highly disruptive to LPP measurement. We selected urethane (1.2 g/kg, i.p.) as an anesthetic agent for LPP measurement because it is among the best at maintaining micturition reflexes [11,13–14].

We connected the bladder catheter to both a pressure transducer (model P300, Grass Instruments, West Warwick, RI) and a flow pump (model 100, KD Scientific, Holliston, MA). The transducer, connected to an amplifier, polygraph (model MT 9500, Astro-Med, Inc., West Warwick, RI), and computer, digitized pressure data at a rate of 10 samples per second. The rat was placed supine and underwent a 30 min accommodation period of filling (5 cc/h) and voiding. The bladder was then palpated (Creded) to empty and filled with saline to 0.3 cc (approximately half the bladder capacity of a 200 g rat [11]). While bladder pressure was recorded and digitized, gentle pressure was applied externally over the bladder (a gentle Crede maneuver) to slowly increase pressure until the rat leaked saline through the urethra. At the first indication of leakage, the externally applied abdominal pressure was rapidly removed. By definition, SUI occurs in the absence of bladder contractions [15]. Therefore, if a bladder contraction occurred during LPP measurement, those data were not included, the bladder was drained and refilled,

and the LPP test began again. The bladder was drained and refilled, and the study repeated at least three times in each rat.

We calculated peak bladder pressure at leakage (LPP) for each LPP test. We also calculated the increase in bladder pressure due to the externally applied abdominal pressure (P_{abd}) for each LPP test as baseline bladder pressure subtracted from LPP. In addition, the rate of pressure increase (rate of Crede) was calculated to confirm that the experimental technique was consistent between groups. A mean of each outcome variable was calculated for each rat and each group.

Data Analysis

Data are presented as mean ± standard error of the mean (SEM) for each experimental group. We compared LPP, P_{abd}, and rate of Crede between all 5 groups using a one-way ANOVA followed by a Student-Newman-Keuls post hoc test (SigmaStat, SPSS, Chicago, IL). A value of $p < 0.05$ indicated a significant difference between groups.

RESULTS

LPP measurements demonstrated the characteristic shape observed previously, with a slow rise to peak and a rapid decrease in bladder pressure once the externally applied abdominal pressure was removed (**Figure 1**). LPP was significantly decreased 2 days (36.3 ± 3.1 cm H_2O), 4 days (32.2 ± 2.7 cm H_2O), 7 days (34.0 ± 2.8 cm H_2O), and 14 days (39.8 ± 2.6 cm H_2O) after nerve injury compared to controls (49.2 ± 4.4 cm H_2O) (**Figure 2**). P_{abd} was significantly decreased 4 days (22.4 ± 2.3 cm H_2O) and 7 days (23.8 ± 2.1 cm H_2O) after nerve injury compared to controls (32.6 ± 3.2 cm H_2O). Although P_{abd} was decreased 2 days (23.8 ± 3.0 cm H_2O) and 14 days (29.0 ± 2.3 cm H_2O) after nerve injury, these differences were not significantly different from control values (**Figure 2**).

No significant differences were found in any of the outcome variables between the 2-, 4-, 7-, and 14-day postinjury groups, although both LPP and P_{abd} appear to trend upward after their minimum value 4 days after injury. No significant difference was found in rate of Crede between any of the experimental groups (control: 6.7 ± 0.6 cm H_2O/s; 2 days: 5.9 ± 0.5 cm H_2O/s; 4 days: 7.0 ± 0.9 cm H_2O/s; 7 days: 6.3 ± 0.5 cm H_2O/s; 14 days: 7.3 ± 0.5 cm H_2O/s), indicating that the experimental technique was the same for all experimental groups (**Figure 3**).

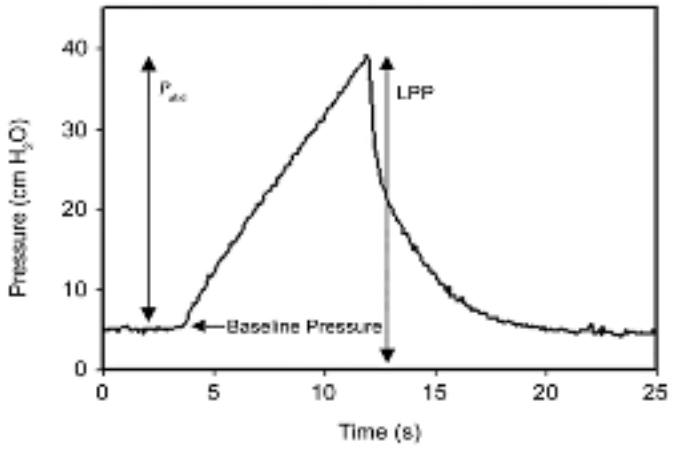

Figure 1.
Example of leak-point pressure (LPP) data from control rat. LPP and increase in externally applied abdominal pressure (P_{abd}) are indicated.

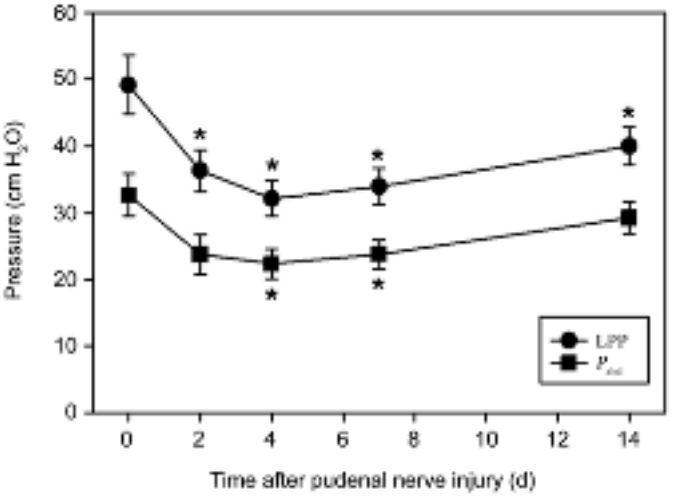

Figure 2.
Leak-point pressure (LPP) (circles) and increase in externally applied abdominal pressure (P_{abd}) (squares) of control rats and rats 2, 4, 7, or 14 days after bilateral pudendal nerve injury. Each symbol represents mean ± standard error of data from 12 to 16 rats. *Significant difference compared to sham-operated control group, shown as 0 days after pudendal nerve injury.

DISCUSSION

Recent clinical research has shown that the pudendal nerve is damaged during childbirth and that women with SUI have greater nerve damage [4,16]. The perineal branch of the pudendal nerve courses in Alcock's canal, lateral and anterior to the vagina [17], making it vulnerable

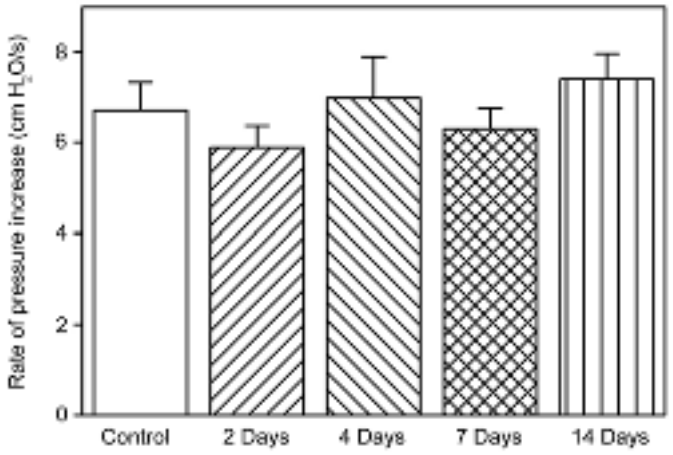

Figure 3.
Rate of Crede of control rats and rats 2, 4, 7, or 14 days after bilateral pudendal nerve injury. Each bar represents mean ± standard error of mean of data from 12 to 16 rats.

to crush and stretch injury during childbirth. Pudendal nerve damage has been observed during vaginal delivery [4] and is correlated with both vaginal delivery [18–19] and SUI [20]. Similarly, women who are incontinent postpartum have significantly more pudendal nerve damage than those who are continent [16].

Current treatments for SUI include sling surgeries, which are aimed at stabilizing the bladder neck or urethra during cough or other moments of stress [7], and injections of bulking agents, which are aimed at increasing coaptation of the urethral lumen [21]. Sling surgeries have a significant associated morbidity rate [22–23] and injections of bulking agents must be repeated to be effective [21,24]. Although pudendal nerve damage can be diagnosed in patients with SUI [20], no active treatment option is currently available to address this problem because of its late effect.

Since SUI symptoms often do not appear until years after the initial injury, an opportunity exists for rehabilitation protocols aimed at preventing SUI. Urethral or vaginal plugs, often supplemented with electrical stimulation [25–27], and/or behavior modification [28] and pelvic floor rehabilitation exercises [29–30] are used to strengthen the muscles of the pelvic floor and prevent SUI [2]. Six months of intensive rehabilitative exercise intervention can lead to a significant improvement in symptoms [31]. However, as with any rehabilitation protocol, motivation of the patient is paramount. One half of patients do not achieve success with pelvic floor exercises, likely because of lack of motivation, severity of incontinence, and/or deterioration of tissues [32].

Animal studies could be useful for preclinical testing of adjuvant agents to facilitate the effects of rehabilitation exercises. Despite the differences between animals and humans, rats have been used to demonstrate that nerve injury occurs during vaginal distension [10,33] and that the pudendal nerve is particularly vulnerable to damage during vaginal delivery [10], similar to the clinical situation. A variety of behavioral and functional outcomes have been used to demonstrate urethral dysfunction after either vaginal distension or pudendal nerve injury, since animals will not sneeze or cough on command. Voiding behavior studies [8], a sneeze test [33–34], LPP testing [10–11,35], modified LPP testing [36–38], maximum urethral closure pressure [38], and a vertical tilt table test [39] all have been used to demonstrate urinary function or behavior after vaginal distension or pudendal nerve injury. The results of these studies are consistent [10,40] and demonstrate symptoms consistent with development of SUI after either pudendal nerve injury or vaginal distension.

Rats are a useful model to use because many animals can be studied in a short period of time, the urologic anatomy and physiology of the rat are well described (reviewed by Steers [41]), and techniques can be easily transferred to mice for use in functional genomics studies. Nonetheless, no animal is a perfect representation of a clinical situation, particularly one such as SUI that is a function of injury, endocrine influences, neural activity, interaction of multiple muscle groups, and the effects of gravity. In addition, no animal has the baby's head-to-birth canal ratio of the human [42] that is thought to cause the traumatic injuries that lead to eventual development of SUI. Rat neural control of voiding is somewhat different than that of humans: unlike in humans, the urethra contracts during voiding in rats [43]. This effect is minimized by the use of LPP as a urethral functional measure, since it is measured in the absence of voiding. Therefore, while not a perfect representation of human voiding, female rats are a reasonable choice as one of several animal models in which to conduct preclinical testing of potential interventions.

This study determined the time course and extent of incontinence symptoms and recovery after bilateral pudendal nerve injury using LPP testing. LPP was significantly decreased at all four time points studied after pudendal nerve injury. Although we found no statistically significant differences between LPPs at any time point

after nerve crush, we did find an increasing trend after a minimum of 4 days after nerve crush. P_{abd} was significantly decreased compared to controls only 4 and 7 days after nerve crush, suggesting that nerve function may begin recovering by 2 weeks after injury or that compensatory events might occur in the urethra. These compensatory events could decrease urine leakage while the pudendal nerve recovers and may include an adaptive increase in urethral smooth muscle contraction and/or an increase in contraction of other pelvic floor muscles that are not innervated by the pudendal nerve.

We have previously shown that voiding behavior returns to normal 2 weeks and 3 months after pudendal nerve injury [8–9]. In contrast to these previous results, data from this study demonstrate that LPP remains decreased 2 weeks after nerve injury. Two weeks is probably not enough time for the regenerating pudendal nerve to complete functional connections with the EUS. Since LPP is a more sensitive measure than voiding behavior, it probably better represents the state of urethral functional recovery. Normalization of voiding behavior by 2 weeks after injury may be due to compensatory effects at the urethra other than those due to pudendal nerve regeneration.

The trend to increasing LPP and P_{abd} after the minimum 4 days after nerve crush suggests that the pudendal nerve will reinnervate the EUS and urethral resistance will recover. We have previously demonstrated a return to normal voiding behavior 3 months after pudendal nerve crush, even though only approximately 50 percent of the pudendal motoneurons had regenerated to the EUS [8]. Future experiments will be aimed at documenting functional changes and examining the natural recovery after pudendal nerve injury over a longer time period, as well as studying the effects of interventions.

CONCLUSIONS

This study characterizes the short-term time course of urinary functional outcomes after pudendal nerve injury in the female rat. This animal model could be useful for preclinical testing of pharmacologic agents and other protocols aimed at rehabilitation of the injuries incurred in vaginal delivery and prevention of SUI onset. Further functional, anatomical, and molecular characterization of the animal model is necessary prior to its use for preclinical testing.

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