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EFFECT OF OVARIECTOMY AND VAGINAL DISTENSION ON URETHRAL FUNCTION AND NEUROREGENERATIVE RESPONSE OF PUDENDAL MOTONEURONS

Aims of Study

Stress urinary incontinence (SUI) is a significant medical problem, defined as the leakage of urine due to increased abdominal pressure, such as when laughing or coughing. In women, it is associated with damage to the nerves, muscles, and connective tissues responsible for continence during vaginal childbirth. The pudendal nerve is among those tissues that can be injured and results in denervation of the external urethral sphincter [1]. Symptoms of SUI often do not occur until menopause, suggesting that hormonal factors, particularly the decreased estrogen levels of menopause, also play an important role [2,3]. Estrogen has also been shown to improve regeneration of injured nerves [4,5]. The purpose of this study was to determine the effect of ovariectomy on urethral function and pudendal nerve regenerative pattern after vaginal distension in female rats.

Methods

Ovariectomy. Twenty female virgin Sprague-Dawley rats were ovariectomized (OVX). The rats were anesthetized, the Fallopian tubes were sutured, and the ovaries removed. Seventeen control rats did not receive ovariectomy (non-OVX).

Vaginal Distension. Three days later, 11 OVX rats and 9 non-OVX rats underwent vaginal distension for 1 hour. The rats were anesthetized and urethral dilators of increasing size (24fr to 32fr) were inserted and removed to accommodate the vagina to larger capacities. A 10fr Foley catheter was then inserted into the vagina, secured with a single stitch, and distended with 3ml water. After 1 hour the catheter was deflated and removed. 9 OVX rats and 8 non-OVX rats underwent sham-distension consisting of vaginal accommodation only.

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 after vaginal distension, the animals were anesthetized with urethane and placed supine for LPP testing [6]. The bladder was palpated to empty and filled with saline at 5 ml/hr. Gentle pressure was applied to the 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.

Tissue Harvest and Preparation for In Situ Hybridization. Immediately after the LPP study, 4 rats in each group underwent cardiac perfusion with saline. The spinal cord was exposed and frozen with liquid nitrogen in situ. The lumbar and sacral segments were dissected and sectioned transversely (15 μm) on a cryostat through both the dorsal motoneurons (DLM) of Onuf's nucleus, which innervate the external urethral sphincter, and the retrodorsolateral motoneurons (RDLM), which course in the sciatic nerve. RDLM cells are not affected by vaginal distension [7] and serve as an uninjured control.

In Situ Hybridization. Part of a neuroregenerative response to injury involves upregulation of β_{II} tubulin, a cytoskeletal protein involved in pudendal nerve regrowth [7]. We used in situ hybridization to determine β_{II} tubulin mRNA upregulation as a measure of the neuro-regenerative response of the pudendal nerve. A rat cDNA insert specific for β_{II} tubulin, was prepared and labeled with 33P-dATP using a nick translation kit. Hybridization of the spinal cord tissue with labeled probe was done using a standard procedure [7]. Slides were dipped in emulsion, exposed in light tight boxes at 4°C, and developed after determining proper exposure time through similarly treated test slides. Net grain density in 3 DLM and 3 RDLM cells from each animal were taken by subtracting background density from cellular grain density, and a mean for each animal was calculated. Mean DLM grain density for each animal

was normalized to mean RDLM grain density to account for different hybridization levels of different slides.

Data Analysis. Data is presented as mean ± standard error of the mean for each experimental group. Statistical comparisons were made using a t-test with $p<0.05$ indicating a significant difference.

Results

LPP. For non-OVX rats, both LPP and P_{diff} were significantly decreased in the vaginal distension group (LPP: 35.6 ± 10.7 cmH_2O; P_{diff}: 23.7 ±11.1 cmH_2O) compared to the sham distension group (LPP: 58.6 ± 10.3 cmH_2O; P_{diff}: 48.4 ± 15.5 cmH_2O). However, OVX had the effect of decreasing the difference in urodynamic variables between the vaginal distension (LPP: 42.3 ± 12.84 cmH_2O; P_{diff}: 29.65 ± 11.4 cmH_2O) and sham (LPP: 48.6 ±12.9 cmH_2O; P_{diff}: 36.5 ±13.7 cmH_2O) groups, and there was no significant difference between them. OVX increased both LPP and P_{diff} in VD rats, but not enough to represent a significant difference.

Neuroregenerative Response of Pudendal Motoneurons. Normalized DLM grain density in non-OVX rats that underwent vaginal distension (0.97 ± 0.04) was not significantly different from that of sham distension rats (1.08 ± 0.33). Similarly, normalized DLM grain density in OVX rats that underwent vaginal distension (0.96 ± 0.38) was not significantly different from that of sham distended rats (1.19 ± 0.94). All normalized DLM grain density values are near unity, indicating that little neuroregenerative response was generated in the pudendal nerve.

Conclusions

A one hour vaginal distension in rats injures urethral tissues and causes decreased urethral resistance, as measured by decreased LPP and P_{diff}. It has also been shown to cause distal nerve damage [8,9]. Estrogen has also been shown to improve neuro-regeneration after a nerve injury and reproductive hormones are implicated clinically in incontinence development. Therefore, the purpose of this project was to investigate the effect of ovariectomy on urethral function and the neuroregenerative response of pudendal motoneurons to vaginal distension.

Despite a small increase in LPP and P_{diff}, there was no significant difference in LPP or P_{diff} due to ovariectomy. Therefore, we conclude that ovariectomy has a minimal effect on urethral dysfunction as measured by LPP and P_{diff} at the time point investigated. Vaginal distension did not create a neuro-regenerative response in the pudendal nerve and ovariectomy did not affect this outcome. This suggests that, despite histological evidence of nerve injury from vaginal distension, the injury to pudendal motoneurons is not sufficient to generate a strong neuro-regenerative response. An animal model with increased nerve injury would likely better represent the clinical situation.

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