

Effect of Long-Term Estrogen Replacement on Bladder Function in Old Female Rats

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The effects of estradiol (E2) on urodynamic parameters were studied with twenty 16-month-old female Wistar rats. They were divided into 4 groups, i.e., Group I: untreated; Groups II and III: treated with E2 for 4 and 8 weeks, respectively; Group IV: treated with a placebo for 8 weeks. After treatment, we measured their plasma E2 levels, and recorded their voiding behavior for 24 h. Cystometry was performed and urodynamic parameters were analyzed. Particularly, bladder capacity as well as voided volume and frequency were surveyed. The results obtained were compared among groups. Levels of bladder capacity in the E2-replaced groups (Groups II, 0.52 ± 0.14 mL and Groups III, 0.58 ± 0.09 mL) were significantly ($P < 0.05$) higher than in the other groups (Group I, 0.38 ± 0.09 mL and Group IV, 0.40 ± 0.11 mL) respectively. The average voided volume was significantly ($P < 0.05$) higher in the E2-replaced groups (Groups II, 1.06 ± 0.22 mL and Groups III, 1.01 ± 0.16 mL) than in Group IV (0.79 ± 0.15 mL), respectively. Concerning the number of daily micturition per day, a significant difference ($P < 0.05$) was observed only between Group III (14.2 ± 2.7) and Group IV (18.8 ± 3.7). This suggests that E2-replacement therapy positively affects bladder function.

Key words: bladder function; estrogen; rat

Urinary incontinence is a debilitating problem particularly prevalent in elderly women. Changes in bladder function, associated with such postmenopausal states as detrusor instability, stress urinary incontinence, and a propensity toward urinary tract infection, are believed in part to be due to estrogen deficiency (Stenberg et al., 1995; Thom and Brown, 1998). Lower urinary tract and pelvic floor tissues are known to be estrogen sensitive because estrogen receptors are located throughout the bladder and urethra (Batra and Iosif, 1983; Klutke and Bergman, 1995). For several reasons, it is hypothesized that estrogen replacement therapy may be useful in treating urinary incontinence. In women suffering from stress incontinence, estrogen is ex-

pected to reverse the effects of urethral atrophy, improving coaptation, and therefore increasing urethral closure pressure (Fantl et al., 1996). Furthermore, because estrogen has also been implicated in raising the sensory threshold of the bladder to cholinergic stimulation, estrogen replacement therapy may be equally useful for detrusor instability (Ekstrom et al., 1993; Fantl et al., 1996).

To date, research on the success of estrogen replacement therapy for postmenopausal bladder dysfunction has been saddled with controversy because many studies are based on subjective reports. As early as 1941, Salmon et al. (1941) reported successful outcomes in postmenopausal women treated with intramuscular estrogen replacement therapy.

Abbreviation: E2, estradiol.

Other investigators reported similar results (Rud, 1980).

Animal studies have shed light on the role of estrogen in bladder function. Longhurst et al. (1992) found that estrogen treatment actually increases detrusor sensitivity and improves bladder construction. We studied the urodynamic changes associated with estrogen depletion and replacement therapy. In the current study we measured voiding behavior and cystometry and assessed estrogen treatment.

Materials and Methods

Animals

All animal experiments were performed in accordance with the Tottori University Committee Guidelines for Animal Experimentation. We divided 20 female Wistar rats aged 16 months into 4 groups of 5 animals each. A silastic tube (inside diameter = 2.5 mm, length = 30 mm, Kaneda Medix, Osaka, Japan, catalog number 100-2N) containing sesame oil (Sigma-Aldrich, St.Louis, MO, catalog number S3547) was subcutaneously planted in 3 groups. Ovariectomies were not performed in any group. Group I rats received no treatment. Groups II and III had implantation done with tubes containing 2.50 mg of estradiol (E2) for 4 weeks and 8 weeks, respectively. Group IV rats were implanted only with sesame oil without E2 for 8 weeks by the same methods as Groups II and III (Ohata, 2002). All animals were kept in an air-conditioned room lighted 12 h a day and allowed access to food and water ad libitum. At the end of experiment, micturition behavior in a metabolic cage was monitored and a cystometry under urethane anesthesia was performed. After that, blood was taken to measure plasma E2.

Measurement of plasma E2

Plasma E2 concentrations were assessed by radioimmunoassay with a Tokyo SRL kit. After the rats were anesthetized with a subcutaneous injection of 1.0 g/kg urethane, a 2.5 mL amount of blood was

taken from the heart and was placed in a cyclone separator to measure plasma E2 concentration levels.

Voiding behavior study

Each rat was placed in a metabolic cage containing a urine collection funnel to record their voiding behavior. We designed these so that the urine moved through a duct downward to a 250-mL plastic beaker, placed on an electronic balance (HF200, A.N.D., Tokyo, Japan) connected to a personal computer (Macintosh iBook G3, Apple Computer, Inc., Cupertino, CA) via a multiport controller (Maclab/400, AD Instruments, Castle Hill, Australia). The cumulative weight of the collected urine was then monitored. Voided volume and frequency were sampled by the computer every 150 s for 24 h, and the data were stored on hard disc. Monitoring per each group started at 1000. During the 24-h observation period, the rats were given water but no food.

Cystometry

Cystometry was performed under urethane anesthesia (1.0 g/kg subcutaneously using a 24-G catheter (Terumo, Tokyo, catalog number SR-OT2419C) inserted into the apex of the bladder dome to record pressure and fill the bladder with physiological saline. External bladder filling was carried out using an infusion pump (5200, TOP, Tokyo) at a constant rate of 0.4 mL/min until voiding was detected. A cystostomy catheter was connected to an external pressure transducer (P2310, Gould, Eastlake, OH) to measure intravesical pressure, which was recorded on a Macintosh Powerbook G3 via a bridge amplifier (ML112, AD Instruments) and a multiport controller Maclab/400. During voiding, filter paper was carefully placed at the meatus to absorb the voided urine without spillage, and the voided volume was weighed. The following parameters were evaluated: bladder capacity, bladder compliance, maximum detrusor pressure, voided volume and residual urine volume. Maximum detrusor pressure was defined as instantaneous pressure minus post-contraction resting pressure.

Table 1. Plasma E2 levels

Group	Plasma E2 level (pg/mL)
I	13.94 ± 3.85
II	38.58 ± 6.08
III	48.86 ± 9.19
IV	19.32 ± 8.46

Shown are mean ± SD.

* Significant difference: $P < 0.05$.

Statistical analysis

Statistical comparison of differences between groups was performed using analysis of variance and the multiple comparison Fisher's test; $P < 0.05$ was considered significant.

Results

Plasma E2 levels

Plasma E2 levels in the replaced groups were significantly higher than in the untreated and placebo groups, respectively. Between the replaced groups, Group III showed significantly higher levels in plasma E2 than Group II. There was no significant difference between Groups I and IV (Table 1).

Voiding behavior study

The number of micturitions per day significantly decreased in Group III compared with Group IV. However, there were no differences in volume excreted per day between those groups. The average voided volume in Groups II and III significantly increased compared with that in Group IV (Table 2).

Cystometry

Bladder capacity significantly increased in the E2-replaced groups compared with the other groups, respectively. Voided volume significantly increased in the E2-replaced groups compared with that in Group IV. However, bladder compliance showed no significant differences in the placebo or untreated groups (Table 3).

Discussion

Wistar rats have a fertility period that lasts until approximately 15 months, at which time the estrous cycle ceases (Handa et al., 1987; Shulman et al., 1987; Albert et al., 1991). Albert et al. (1991) found that a plasma E2 level of 15 pg/mL was slightly below the mean level of E2 present throughout the estrous cycle in Wistar rats that maintained normal

Table 2. Comparison of voiding behavior

Group	Volume excreted per day (mL)	Number of micturitions per day	Average voided volume (mL)	Maximum voided volume (mL)
I	15.83 ± 8.42	17.6 ± 3.1	0.87 ± 0.35	1.93 ± 0.66
II	17.87 ± 6.18	16.4 ± 2.9	1.06 ± 0.22	2.44 ± 0.49
III	14.70 ± 3.50	14.2 ± 2.7	1.01 ± 0.16	1.69 ± 0.36
IV	14.11 ± 2.56	18.8 ± 3.7	0.79 ± 0.15	2.26 ± 0.41

Shown are mean ± SD.

* Significant difference: $P < 0.05$.

Average voided volume = (volume excreted per day) divided by (number of micturitions per day)

Table 3. Comparison of urodynamic parameters

Group	Bladder capacity (mL)	Maximum detrusor pressure (kPa)	Voided volume (mL)	Residual urine volume (mL)	Bladder compliance (mL/kPa)
I	0.38 ± 0.09	25.4 ± 3.4	0.32 ± 0.09	0.13 ± 0.02	0.05 ± 0.02
II	0.52 ± 0.14	28.3 ± 2.4	0.39 ± 0.75	0.09 ± 0.07	0.08 ± 0.02
III	0.58 ± 0.09	30.9 ± 4.9	0.36 ± 0.10	0.15 ± 0.04	0.07 ± 0.01
IV	0.40 ± 0.11	30.7 ± 1.5	0.26 ± 0.02	0.22 ± 0.09	0.05 ± 0.03

Shown are mean ± SD.

* Significant difference; $P < 0.05$.

body weight. In the present study, plasma E2 levels in Group I were slightly lower than 15 pg/mL, as expected; in Group IV, the levels just exceeded 15 pg/mL, which were slightly higher than expected at such an age. This may have been caused by the tube plantation, but the difference between groups was not significant. Groups II and III, which received estrogen replacement, showed significant increases in plasma E2 levels, achieving levels of over 30 and 40 pg/mL, respectively. Therefore, the E2 replacement method in this experiment provided adequate levels for studying the effects of E2 on rat bladders, as reported (Shulman et al., 1987; Albert et al., 1991). In consideration of the significant difference observed between Groups III and IV, the increase in plasma E2 levels seemed to be caused by E2 replacement rather than tube plantation.

Voiding behavior changes with age, as demonstrated by increases in voiding frequency (Chun et al., 1988). The present study on voiding behavior proved no differences in volume excreted per day. The number of daily micturitions significantly decreased, and average voided volume increased in Group III compared with that in Group IV. It stands to reason that frequent urination improved due to an increase in average micturition. In the present Wistar rats, E2 replacement affected the improvement of bladder function. The difference in maximum voided volume was significant between Groups II and III. The underlying cause of this

difference remains to unknown, but the parameters of maximum micturition had little reference to the change of voiding behavior for E2 replacement. Along with that, cystometry demonstrated that E2-replaced groups experienced significantly increased bladder capacity and voided volume compared with Group IV. E2-replaced groups also showed an increased tendency toward bladder compliance, but this was not significant in our study. Furthermore, we observed a significant difference in maximum detrusor pressure between Groups I and III, and in residuals urine volume between Groups II and IV, which might be caused by E2 replacement. Because there was an age-based bias in sampling the above groups, intra-group examination of E2 effects was statistically inappropriate. The results of voiding behavior and cystometry showed an improvement in bladder function, correlatively. There was no significant difference in urodynamic parameters between Groups II and III. The term of E2 replacement affected the plasma E2 levels; however, the plasma E2 levels after 4 weeks of E2 replacement showed equivalent improvement of bladder function to those after 8 weeks of replacement.

According to previous reports, ovariectomized animals had a decrease in the smooth muscle density in the bladders (Hashimoto et al., 1999), and E2-replaced groups showed increases in the smooth muscle content of the bladder (Rocha et al., 2002;

Hikita et al., 2004). The increase in smooth muscles of the bladder may improve bladder contraction. These researchers supposed that E2 replacement improved voiding behavior and urodynamic parameters.

E2 replacement therapy might prevent apoptosis in the bladder and would influence voiding behavior (Cockayne et al., 2000; Wotherspoon and Winter, 2000; Ohata et al., 2002). Ohata et al. (2002) studied effects of E2 on apoptosis, but they observed no significant difference in the dose or replacement term of E2. In the present study, we observed no difference in the term of E2 replacement. Effects on the dose of E2 need to be studied further.

The results obtained suggest that hormone replacement therapy may improve the problem of urgent and frequent urination in older women.

References

- 1 Albert DJ, Jonik RH, Gorzalka BB, Newlove T, Webb B, Walsh ML. Serum estradiol concentration required to maintain body weight, attractiveness, proceptivity, and receptivity in the ovariectomized female rat. *Physiol Behav* 1991;49:225–231.
- 2 Batra S, Iosif C. Female urethra: a target for estrogen action. *J Urol* 1983;129:418–420.
- 3 Cockayne AD, Hamilton GS, Zhu MQ, Dunn MP, Zhong Y, Novakovic S. Urinary bladder hyporeflexia and reduced pain-related behavior in P2X3-deficient mice. *Nature* 2000;407:1011–1015.
- 4 Chun AL, Wallace LW, Gerald MC, Levine RM, Wein AJ. Effect of age on in vivo urinary bladder function in the rat. *J Urol* 1988;139:625–627.
- 5 Ekstrom J, Iosif CS, Malmberg L. Effects of long-term treatment with estrogen and progesterone on in vitro muscle responses of the female rabbit urinary bladder and urethra to autonomic drugs and nerve stimulation. *J Urol* 1993;150:1284–1288.
- 6 Fantl J, Bump RC, Robinson D, McClish DK, Wyman JF. Efficacy of estrogen supplementation in the target of urinary incontinence. *Obstet Gynecol* 1996;88:745–749.
- 7 Handa RJ, Stadelman HL, Resko JA. Effect of estrogen on androgen receptor dynamic in female rat pituitary. *Endocrinology* 1987;121:84–89.
- 8 Hashimoto T, Ishigooka M, Zermann DH, Sasagawa I, Nakada T. Effects of estrogen and progesterone on urinary bladder in the female rabbit: evaluation by quantitative morphometric analysis. *Urology* 1999;53:642–646.
- 9 Hikita K, Kobayashi N, Miygawa I. Effect of estrogen replacement therapy on bladder circulation in old female rats. *Yonago Acta Med* 2004;47:81–89.
- 10 Klutke JJ, Bergman A. Hormonal influence on the urinary tract. *Urol Clin North Am* 1995;22:629–639.
- 11 Longhurst P, Kauer J, Leggett RE, Levin RM. The influence of ovariectomy and estradiol on urinary bladder function in rats. *J Urol* 1992;148:915–919.
- 12 Ohata R. Effect of estrogen replacement therapy on the expression of apoptosis-related genes in old female rats. *Yonago Acta Med* 2002;45:95–102.
- 13 Rocha MA, Sartori MGF, Girao MJBC, De Jesus Simoes M, Baracat EC, Rodorius of Lima G. Histomorphometric study of the inferior urinary tract of adult female rats during the interval between castration and hormonal replacement. *Clin Exp Obstet Gynecol* 2003;30:111–116.
- 14 Rud T. The effect of estrogens and gestagens on the urethral pressure profile in urinary continent and stress incontinent women. *Acta Obst Gynecol Scand* 1980;59:265–270.
- 15 Salmon UJ, Walter RI, Geist SH. The use of estrogen in the treatment of dysuria and incontinence in postmenopausal women. *Am J Obstet Gynecol* 1941;42:845–851.
- 16 Shulman DI, Sweetland M, Duckett G, Root AW. Effect of estrogen on the growth hormone (GH) secretory response to GH-releasing factor in the castrated adult female rat in vivo. *Endocrinology* 1987;120:1047–1051.
- 17 Stenberg A, Heimer G, Ulmsten U. The prevalence of urogenital symptoms in postmenopausal women. *Maritatas* 1995;22:S17–20.
- 18 Thom DH, Brown JS. Reproductive and hormonal risk factors for urinary incontinence in later life: a review of clinical and epidemiologic literature. *J Am Geriatr Soc* 1998;46:1411–1417.
- 19 Wotherspoon G, Winter J. Blydykinin B1 receptor is constitutively expressed in the rat sensory nervous system. *Neurosci Lett* 2000;294:175–178.

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