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Effect of estrogen on nitric oxide-induced relaxation of the rabbit urethra

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Abstract

We evaluated the effect of estrogen on nitric oxide (NO)-mediated urethral relaxation in rabbits. Female New Zealand white rabbits, 4–5 weeks old, were treated with 5 mg/kg estradiol dipropionate (estrogen group) or saline (control group) injected intramuscularly weekly for 2 weeks. Electrical field stimulation (supramaximum voltage, 2 ms pulse duration, 0.3–15 Hz and 3 s train) caused frequency-dependent relaxation of urethral strips in both groups, which was inhibited by N^{ω}-nitro-L-arginine (L-NNA). This inhibition was overcome by addition of L-arginine. The relaxation induced by nitrergic nerve stimulation was significantly lower in the estrogen group than in the control group. There was no significant difference in sodium nitroprusside-induced urethral relaxation between the two groups. The production of NO in urethral strips during nitrergic nerve stimulation was evaluated by measuring nitrite/nitrate (NO_2^-/NO_3^-) levels in both groups, using microdialysis. The NO_2^-/NO_3^- production during electrical field stimulation in the estrogen group was significantly less than that in the control group. The NADPH diaphorase-positive reaction in the control group was greater than that in the estrogen group. The results suggest that estrogen treatment may reduce NO synthase activity, and inhibit the relaxation induced by nitrergic nerve stimulation in rabbit urethral smooth muscle. © 1997 Elsevier Science B.V.

Keywords: Estrogen; Urethra; Nitric oxide (NO); Nitric oxide (NO) synthase; (Rabbit)

1. Introduction

It has been reported that estrogen replacement therapy increases urethral pressure and improves stress incontinence in postmenopausal women (Salmon et al., 1941; Faber and Heidenreich, 1977; Walter et al., 1978; Elia and Bergman, 1993). Furthermore, estrogen influences the functioning of the lower urinary tract (Kuroda et al., 1985; Batra et al., 1986; Bhatia et al., 1989; Van der Linden et al., 1993). Several studies have shown that estrogen treatment decreases muscarinic acetylcholine receptor density of the rabbit urinary tract (Shapiro, 1986), and that estrogen causes an increase in number of post-junctional α_2-adrenoceptors, which contributes to the increased contractile response of α_2-adrenergic-nerve mediated stimulation in the rabbit urethra (Larsson et al., 1984).

Recently, relaxation mediated by non-adrenergic and non-cholinergic (NANC) nerves has been demonstrated in the urethra. NO has been identified as a nitrergic neurotransmitter released by NANC nerves in various mammalian urethra in vitro (Dokita et al., 1991; Andersson et al., 1991, 1992; Hashimoto et al., 1993; Ehrén et al., 1994; García-Pascual and Triguero, 1994; Leone et al., 1994) and in vivo (Bennett et al., 1995). In addition, it has been demonstrated that estrogen influences NO synthase activity in several tissues (Yallampalli et al., 1994; Figueroa and Massmann, 1995). However, there is no information about the effect of estrogen on the NO pathway in the urethra. Therefore, in the present study, we evaluated the effect of estrogen on NO-mediated urethral relaxations in rabbits by means of a muscle bath technique, NADPH diaphorase histochemistry and measurement of NO release using a microdialysis method.

2. Materials and methods

Forty female New Zealand white rabbits weighing 1.0–1.2 kg were separated into two groups. The estrogen group ($n = 20$) was treated weekly with an intramuscular injection of 5 mg/kg estradiol dipropionate, which was dissolved in 1 ml saline solution, and the control group ($n = 20$) was treated weekly with an intramuscular injection of 1 ml normal saline solution.

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2.1. Tissue preparation

Two weeks after the treatment, all rabbits were killed by exsanguination after intravenous administration of 50 mg/kg sodium pentobarbital. A blood sample was collected from each rabbit and centrifuged, then the serum was stored at −20°C until assay. In 12 rabbits in each group, the urethra was dissected free down to the entrance of the vaginal wall and was placed in Krebs–Henseleit (K–H) solution. The urethra was cut into transverse muscle strips, approximately 2 × 10 mm for pharmacological experiments. For histochemical studies, the urethra of 8 rabbits in each group was fixed for 2 h in 4°C freshly prepared solution of 2% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) and then incubated in 20% sucrose in PBS for 48 h. The tissues were then frozen and embedded in optimal cutting temperature compound, and stored at −70°C before starting the experiments.

Serum estradiol was measured by direct radioimmunoassay as previously described (Shay et al., 1994) in order to confirm the efficacy of our hormonal manipulations. Serum triglyceride and cholesterol were measured by enzymatic assay as previously described (Crook et al., 1992).

2.2. Functional experiments

The muscle strips were put in 20 ml organ baths filled with modified K–H solution at 37°C bubbled with 95% O_2 and 5% CO_2 resulting in a pH of 7.4, and were attached to two L-shaped metal specimen holders by tying both ends of the preparations with silk ligatures. One end of each strip was connected to a force-displacement transducer (TB-611T, Nihon Kohden) and isometric forces were recorded and monitored on a pen-writing recorder (R-02A, RIKADENKI). During a 1 h equilibration period, the muscle strip was stretched to the length of optimal force development, and the bath solution was changed every 15 min. The resting tensions at the length of optimal force development in both groups were 600–800 mg.

For evaluation of muscle contractility in both groups, concentration–response curves were made for phenylephrine and 80 mM KCl-induced contractions. Electrical field stimulation was applied to the strips precontracted with 3 μM phenylephrine through two parallel platinum electrodes (10 mm wide and 8 mm apart). Electrical impulses for field stimulation of intrinsic nerves were delivered with a stimulator (SEN-3301, Nihon Kohden) and boosted by an amplifier (SEG-3104, Nihon Kohden). The electrodes delivered square wave pulses, voltage; supramaximum, pulse duration; 2 ms, frequency; 0.3–15 Hz and train duration; 3 s at 2 min intervals. After the experiment, K–H solution was changed to Ca^{2+}-free K–H solution to obtain 100% relaxation. Subsequently, the strips were washed out several times with K–H solution, and were equilibrated for 60 min, then precontracted with 3 μM phenylephrine and a second series of electrical field stimulation was applied. L-NNA (100 μM) was added for 30 min before the second series of electrical field stimulation. When used, L-arginine (1 mM) was added to the organ chamber 5 min after the L-NNA was added. Atropine (1 μM) and propranolol (1 μM) were present throughout the experiment unless otherwise noted.

Sodium nitroprusside (1 nM–100 μM) was cumulatively added to the preparations precontracted with 3 μM phenylephrine for evaluation of direct drug effects on urethral relaxation.

2.3. NADPH diaphorase histochemistry

NADPH diaphorase staining was performed as previously described (Sobey et al., 1995). The frozen tissue was cut at a thickness of 10 μm with a cryostat, mounted on a glass slide and air dried. The slide-mounted tissues were incubated at 37°C in 0.1 mM Triton X-100, 1 mg/ml β-NADPH and 0.5 mg/ml nitro blue terazolium in 0.1 M PBS (pH 7.4) for 4 h in darkness. Slides were dehydrated in a series of ethanol solutions (70%, 95% and 100%), cleaned in xylene and a coverslip was applied. In control experiments, tissue sections were incubated in the absence of β-NADPH. Parallel slide-mounted sections were stained with hematoxylin and eosin. All preparations were examined and pictures taken with an Olympus microscope (VANOS-S Olympus, Tokyo).

2.4. Measurement of NO release

NO release from urethral muscle was measured using the microdialysis method (Shintani et al., 1994). A microdialysis probe (Kurata et al., 1993) (outer diameter; 220 μm, inner diameter; 200 μm, length; 10 mm, cellulose membrane, molecular cut-off; 50 kDa, A-I-8-03, Eicom, Kyoto) was inserted into the urethral strip. The probe was perfused with K–H solution at a constant flow rate of 2 μl/min using a microsyringe pump (EP-60, Eicom). The strip with the microdialysis probe was set up as in the functional experiment. Following a 2 h equilibration period, dialysate was collected for 20 min (40 μl) for evaluation of basal NO production in each group. Then, electrical field stimulation (supramaximum voltage, pulse duration; 2 ms, frequency; 7 Hz, train duration; 3 s at 1 min interval) was applied to the strip precontracted with 3 μM phenylephrine for 20 min, and dialysate obtained during electrical field stimulation was collected in polyethylene tubes at room temperature. All dialysate fractions were stored at −20°C for measurement of NO_2^-/NO_3^-.

To quantitate the NO_2^-/NO_3^- in the dialysate fraction, 10 μl dialysate was directly infused into an automated NO_2^-/NO_3^--analyzing system based on the Griess reaction (Ohta et al., 1994b). The levels of NO_2^-/NO_3^- in the standard solution and in the samples were determined as a mixture of NO_2^-/NO_3^-.

2.5. Solutions and drugs

K–H solution was composed as follows (mM): NaCl, 117.7; KCl, 4.69; $CaCl_2$, 2.16; $MgSO_4$, 1.20; $NaHCO_3$, 24.39; KH_2PO_4, 1.20 and glucose, 9.99. Ca^{2+}-free solution was made by omitting $CaCl_2$ from K–H solution and adding 0.1 mM EGTA. KCl solution (80 mM) was prepared by replacing Na^+ with equimolar amounts of K^+ in K–H solution.

The following drugs were used: L-phenylephrine hydrochloride, atropine sulfate, DL-propranolol hydrochloride, sodium nitroprusside, N^{ω}-nitro-L-arginine, L-arginine hydrochloride, tetrodotoxin, EGTA and hexamethonium chloride were obtained from Sigma Chemical. Indomethacin and estradiol dipropionate were obtained from Nacalai tesque, Japan and Teikokuzouki Pharmaceutical, Japan, respectively. Indomethacin was dissolved in 1% w/v sodium bicarbonate. All other drugs were dissolved in distilled deionized water.

2.6. Data analysis

For functional experiment, the relaxation induced by Ca^{2+}-free K–H solution was taken as 100% and the percent relaxation was calculated. For evaluation of NO production during electrical field stimulation, the amount of NO_2^-/NO_3^- released in the 10 μl dialysate fraction was calculated for each group. The data are expressed as means ± S.E.M. Statistical analysis of differences between groups was performed using the analysis of variance (ANOVA) and the multiple comparison Fisher's test. P values of 0.05 or less were taken as statistically significant.

3. Results

3.1. Body weights and blood parameters

At the end of the experimental period the body weight of the two groups was not significantly different (control group, $n = 20$; 1.47 ± 0.05 kg, and estrogen group, $n = 20$; 1.47 ± 0.03 kg). The wet weight of the urethra was significantly greater in the estrogen group (246.3 ± 9.2 mg, $n = 20$) than in the control group (85.5 ± 7.0 mg, $n = 20$). In the estrogen group, the level of estradiol (101.3 ± 25.1 pg/ml, $n = 20$) was significantly higher and the level of triglyceride (36.2 ± 4.1 mg/dl, $n = 20$) and total cholesterol (41.9 ± 3.5 mg/dl, $n = 20$) were significantly lower than those in the control group (< 10 pg/ml, 97.7 ± 13.4 mg/dl and 56.2 ± 4.0 mg/dl, respectively, $n = 20$). Urine volume was not significantly affected by estrogen treatment.

3.2. Functional experiments

The contractile responses for phenylephrine and KCl (80 mM) were similar in both groups. The contractile responses induced by 3 μM phenylephrine in the control and estrogen groups were 1.02 ± 0.12 g and 1.06 ± 0.10 g, respectively, which was about 70% of the maximum phenylephrine-induced contraction in each group. Electrical field stimulation caused a frequency-dependent relaxation of urethral strips precontracted by 3 μM phenylephrine in both control and estrogen groups (Fig. 1). Fig. 2 shows the frequency–response curves for the urethral relaxation induced by nitrergic nerve stimulation in both groups. The maximal relaxation in the estrogen group (33.1 ± 3.4%, $n = 12$) was significantly less than that in the control group (56.0 ± 4.4%, $n = 12$). All responses were completely blocked by tetrodotoxin (1 μM), but not by atropine (1 μM), propranolol (1 μM), hexamethonium (100 μM) or indomethacin (10 μM). In both groups, addition of L-NNA (100 μM) completely inhibited the relaxation induced by electrical field stimulation. These

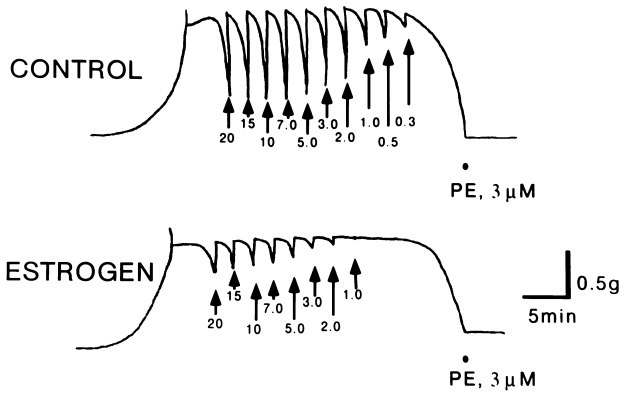

Fig. 1. Representative tracing showing relaxation in response to electrical field stimulation in rabbit urethral smooth muscle of control group (upper panel) and estrogen group (bottom). Electrical field stimulation was delivered to muscle strips precontracted with phenylephrine (PE; 3 μM) in the presence of atropine (1 μM) and propranolol (1 μM).

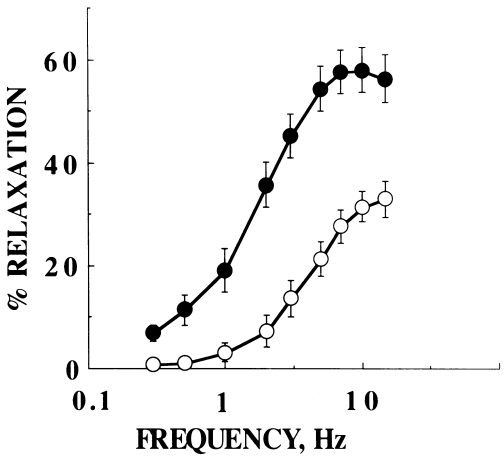

Fig. 2. Effects of estrogen treatment on electrical field stimulation-induced relaxation in the female rabbit urethra of control group (●) and estrogen group (○). Muscle strips were precontracted with 3 μM phenylephrine in the presence of 1 μM propranolol and atropine. Each point shows mean ± S.E.M. of 12 experiments.

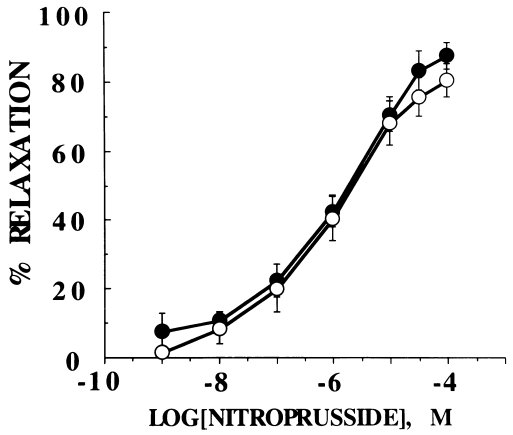

Fig. 3. Effects of estrogen treatment on the concentration–response curve for relaxation effects of sodium nitroprusside in the female rabbit urethra of control group (●) and estrogen group (○). Muscle strips were precontracted with phenylephrine (3 μM). Effect of sodium nitroprusside is expressed as percent relaxation of phenylephrine-induced tone. Data are shown as means ± S.E.M. of 12 experiments.

inhibitory effects of L-NNA were overcome by L-arginine (1 mM).

Sodium nitroprusside (1 nM–100 μM) produced a concentration-dependent relaxation in the urethral smooth muscles precontracted with 3 μM phenylephrine in both groups (Fig. 3). The maximum relaxation and ED_{50} values

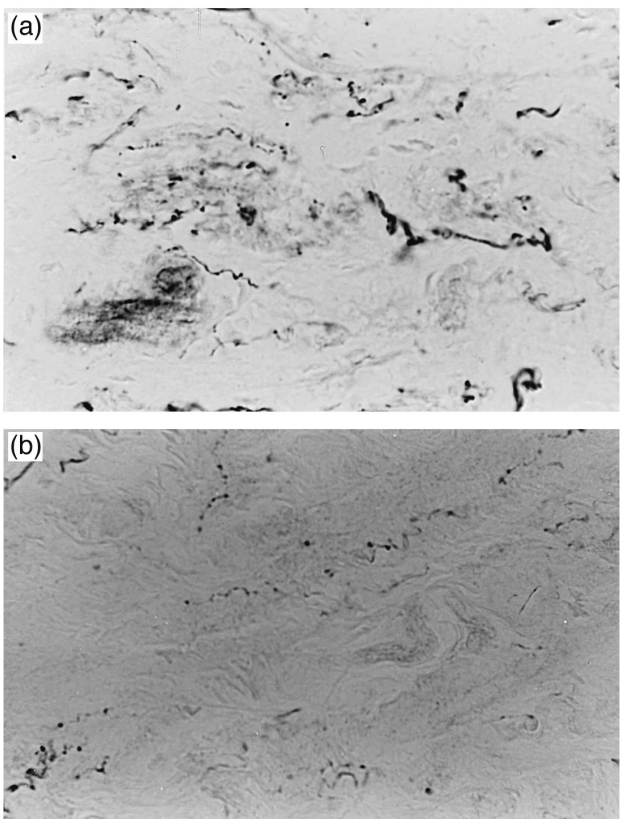

Fig. 4. NADPH diaphorase activity in the female rabbit urethra (a) control group; (b) estrogen group). Reduced from ×400.

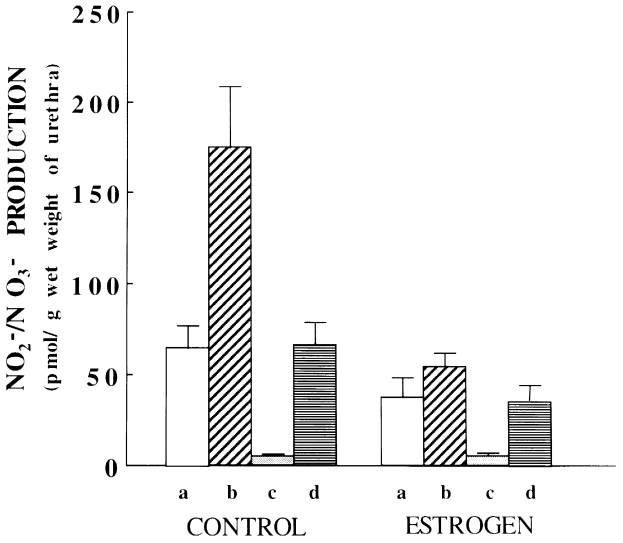

Fig. 5. Effects of estrogen treatment on production of NO_2^-/NO_3^- during electrical field stimulation in the female rabbit urethra of control group and estrogen group. The production of NO_2^-/NO_3^- was measured by means of the Griess reaction combined with the microdialysis technique. Each bar shows the mean ± S.E.M. of 12 experiments. (a) Basal value of NO_2^-/NO_3^- production, (b) NO_2^-/NO_3^- production during electrical field stimulation, (c) NO_2^-/NO_3^- production during electrical field stimulation after treatment with L-NNA (10^{-4} M), (d) NO_2^-/NO_3^- production during electrical field stimulation after treatment with tetrodotoxin (10^{-6} M).

were 80.8 ± 4.8% and 1.28 ± 0.30 μM ($n = 12$) in the estrogen group, and 87.8 ± 4.0% and 1.80 ± 0.32 μM ($n = 12$) in the control group, respectively. There were no significant differences between the values for the two groups.

3.3. NADPH diaphorase histochemistry

In both groups NADPH diaphorase-positive nerve structures were found in the lamina propria, the outer parts of the smooth musculature and around arteries. The urothelium and the endothelium of arteries also showed a positive NADPH diaphorase reaction. The NADPH diaphorase-positive reaction in the estrogen group was weaker than that in the control group (Fig. 4).

3.4. Measurement of NO release

Fig. 5 shows the values measured for NO release from the urethral strip in the control and estrogen groups. The basal levels of NO_2^-/NO_3^- in the dialysate fraction in the estrogen and control groups were 37.1 ± 11.3 and 70.2 ± 13.4 pmol/g wet weight of urethra ($n = 12$), respectively. The values were not significantly different between two groups. During electrical field stimulation, the amount of NO_2^-/NO_3^- production in the control group was 175.0 ± 34.3 pmol/g wet weight of urethra, which was significantly higher than the basal value. In the estrogen group,

however, NO_2^-/NO_3^- production during stimulation was 51.3 ± 7.1 pmol/g wet weight of urethra, which was not significantly different from the basal value. Pretreatment with L-NNA (100 μM) significantly inhibited the production of NO_2^-/NO_3^- in both groups. After treatment with tetrodotoxin (1 μM), the amounts of NO_2^-/NO_3^- released by nitrergic nerve stimulation in the estrogen and control groups were 38.5 ± 8.9 and 68.8 ± 11.4 pmol/g wet weight of urethra, respectively, thus similar to the basal values.

4. Discussion

Several reports demonstrate that NO is a neurotransmitter released from the nitrergic component of NANC nerves (Rand and Li, 1995a,b). There are many reports showing that NO produces urethral relaxation in rats (Bennett et al., 1995), dogs (Hashimoto et al., 1993), pigs (Bridgewater et al., 1993), sheep (García-Pascual and Triguero, 1994), and humans (Leone et al., 1994). In the present study, electrical field stimulation-induced urethral relaxations were completely inhibited by pretreatment with L-NNA, an inhibitor of NO synthase, and the inhibition was overcome by the addition of L-arginine, a precursor of NO, in both estrogen and control groups. This suggested that the L-arginine/NO pathway contributes to the relaxation induced by nitrergic nerve stimulation in rabbit urethral smooth muscles, which is consistent with previous reports (Dokita et al., 1991; Andersson et al., 1991, 1992).

It is known that sodium nitroprusside releases NO, which activates soluble guanylate cyclase, elevates the tissue cyclic GMP level, and relaxes smooth muscles (Dokita et al., 1991; Bridgewater et al., 1993). In the present experiment, exogenous application of sodium nitroprusside also produced relaxation responses in both groups, although there was no significant difference in response between two groups. Estrogen treatment inhibited the relaxation induced by nitrergic nerve stimulation. Thus, our data indicate that estrogen treatment does not affect the L-arginine/NO pathway after the activation of soluble guanylate cyclase.

Various methods for measuring NO production in biological models have been described for several tissues (Shibuki, 1990; Malinski and Taha, 1992; Archer, 1993; Balcioglu and Maher, 1993; Ohta et al., 1994b). However, little information is as yet available on the measurement of NO production in the urethra. Recently, Ohta et al. (1994a) reported on an assay system for consecutive measurement of NO production in rat brain that is combined with microdialysis methods. In this method, NO production is measured based on Griess reaction as the amount of its stable metabolites, NO_2^- and NO_3^-. This method has the advantage of consecutive measurement, high precision, good reproducibility, technical simplicity and relatively short resolution time (Ohta et al., 1994a). In the present study, we used this method to measure NO production during electrical field stimulation in both estrogen and control groups. The basal levels of NO_2^-/NO_3^- in both groups were similar and the levels during electrical field stimulation increased in both groups. Pretreatment with L-NNA or tetrodotoxin significantly decreased the levels of NO_2^-/NO_3^- during electrical field stimulation in both groups. However, the values during electrical field stimulation were smaller in the estrogen group than in the control group. These results demonstrate that nitrergic nerve stimulation produced NO in the rabbit urethra in both groups, and that estrogen treatment decreased NO production from nitrergic nerve stimulation. On the basis of the above findings it is suggested that estrogen treatment may cause a decrease in NO production from nitrergic nerves, and inhibit the relaxation induced by nitrergic nerve stimulation in the rabbit urethra.

In the present study, we have evaluated NO synthase activity by NADPH histochemistry in urethral smooth muscle. Dawson et al. (1991) demonstrated that the enzyme responsible for NADPH diaphorase reaction in neurons was actually NO synthase. In addition, Bredt et al. (1991) have shown that all NADPH diaphorase-positive neurons exhibit NO synthase immunoreactivity and contain NO synthase mRNA. Based on these findings, NADPH diaphorase-positive neurons are believed to be identical to NO synthase neurons. It has been reported that both NO synthase and NADPH diaphorase-positive neurons were present in the pig (Persson et al., 1993) and dog (Takeda and Lepor, 1995) proximal urethra, and that both densities were well correlated with the functional urethral smooth muscle responses involving the L-arginine/NO pathway (Takeda and Lepor, 1995). In the present experiments, the NADPH diaphorase-positive reaction in the urethra was weaker in the estrogen group than in the control group. The data suggest that estrogen treatment may inhibit NO synthase activity in rabbit urethral smooth muscles. Thus, the decreased level of NO synthase activity may contribute to the decreased NO production and urethral relaxation responses induced by nitrergic nerve stimulation in the estrogen group.

It has been demonstrated that estrogen influences NO synthase activity in various tissues. Ekström et al. (1993) reported on the effects of long-term treatment with estrogen and progesterone on in vitro muscle responses of the female rabbit urinary bladder and urethra. In their study, estrogen treatment led to the disappearance of the relaxant response in urethral strips, which is in agreement with our results. Yallampalli et al. (1994) reported that estrogen inhibited the NO-cGMP system and increased uterine activity in rats. On the other hand, results of several studies suggested that estrogen treatment increased NO synthase activity in some tissues (Goetz et al., 1994; Figueroa and Massmann, 1995; Suburo et al., 1995; Gregg et al., 1995; Hayashi et al., 1995; Veille et al., 1996). It has been also suggested that the discrepancies may represent species

differences and/or tissue specificity (Figueroa and Massmann, 1995). Further investigations are needed to elucidate the mechanism for the effects of estrogen on the L-arginine/NO pathway.

Estrogen deficiency has been indicated as one of the factors involved in the increase in the incidence of stress incontinence during the menopause. The results of several animal experiments suggested that many factors may contribute to the beneficial effects of treatment with estrogen in stress incontinence. The increase in urethral tissue mass and blood flow following estrogen treatment is probably of importance for maintaining positive intraurethral pressure. In the present study, urethral volume in the estrogen group was significantly greater than that in the control group. These results were similar to those reported by Batra and Iosif (1983), Shapiro (1986) and Crook et al. (1992), in which the increase in urethral weight was not secondary to estrogen-induced water retention since, in their study, the total urethral DNA increased concomitantly, and the increase in cellular mass of the urethra may have been secondary to interstitial cell and mucosal hyperplasia. An increased adrenoceptor content and sensitivity to sympathomimetic drugs in urethral smooth muscles have been also reported to follow estrogen treatment (Larsson et al., 1984). However, in the present experiment, the phenylephrine-induced contractile responses, which were mediated through α_1-adrenoceptor, were similar in both groups. Thus, the decrease in NO-induced relaxation in estrogen treatment may be taken as additional rationale for the use of this hormone in patients with stress incontinence.

In conclusion, the present study demonstrated that estrogen treatment may cause a decrease in NO synthase activity in the female rabbit urethra, which contributes to the inhibition of NO production and reduces the relaxant responses induced by nitrergic nerve stimulation.

References

Andersson, K.-E., Garcia-Pascual, A., Forman, A., Tøttrup, A., 1991. Non-adrenergic, non-cholinergic nerve-mediated relaxation of rabbit urethra is caused by nitric oxide. Acta. Physiol. Scand. 141, 133–134.

Andersson, K.-E., Garcia-Pascual, A., Persson, K., Forman, A., Tøttrup, A., 1992. Electrically-induced, nerve-mediated relaxation of rabbit urethra involves nitric oxide. J. Urol. 147, 253–259.

Archer, S., 1993. Measurement of nitric oxide in biological models. FASEB J. 7, 349–360.

Balcioglu, A., Maher, T.J., 1993. Determination of kainic acid-induced release of nitric oxide using a novel hemoglobin trapping technique with microdialysis. J. Neurochem. 61, 2311–2313.

Batra, S.C., Iosif, C.S., 1983. Female urethra: A target for estrogen action. J. Urol. 129, 418–420.

Batra, S., Bjellin, L., Sjögren, C., Iosif, S., Widmark, E., 1986. Increases in blood flow of the female rabbit urethra following low dose estrogens. J. Urol. 136, 1360–1362.

Bennett, B.C., Kruse, M.N., Roppolo, J.R., Flood, H.D., Fraser, M., DeGroat, W.C., 1995. Neural control of urethral outlet activity in vivo: Role of nitric oxide. J. Urol. 153, 2004–2009.

Bhatia, N.N., Bergman, A., Karram, M., 1989. Effects of estrogen on urethral function in women with urinary incontinence. Am. J. Obstet. Gynecol. 160, 176–181.

Bredt, D.S., Hwang, P.M., Glatt, C.E., Lowenstein, C., Reed, R.R., Snyder, S.H., 1991. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351, 714–718.

Bridgewater, M., Macneil, H.F., Brading, A.F., 1993. Regulation of tone in pig urethral smooth muscle. J. Urol. 150, 223–228.

Crook, D., Cust, M.P., Gangar, K.F., Worthington, M., Hillard, T.C., Stevenson, J.C., Whitehead, M.I., Wynn, V., 1992. Comparison of transdermal and oral estrogen-progestin replacement therapy: Effects on serum lipids and lipoproteins. Am. J. Obstet. Gynecol. 166, 950–955.

Dawson, T.M., Bredt, D.S., Fotuhi, M., Hwang, P.M., Snyder, S.H., 1991. Nitric oxide synthase and neuronal NADPH diaphorase are identical in brain and peripheral tissues. Proc. Natl. Acad. Sci. USA 88, 7797–7801.

Dokita, S., Morgan, W.R., Wheeler, M.A., Yoshida, M., Latifpour, J., Weiss, R.M., 1991. N^G-nitro-L-arginine inhibits non-adrenergic, non-cholinergic relaxation in rabbit urethral smooth muscle. Life Sci. 48, 2429–2436.

Ehrén, I., Iversen, H., Jansson, O., Adolfsson, J., Wiklund, N.P., 1994. Localization of nitric oxide synthase activity in the human lower urinary tract and its correlation with neuroeffector responses. Urology 44, 683–687.

Ekström, J., Iosif, C.S., Malmberg, L., 1993. 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. 150, 1284–1288.

Elia, G., Bergman, A., 1993. Estrogen effects on the urethra: Beneficial effects in women with genuine stress incontinence. Obstet. Gynecol. Survey 48, 509–517.

Faber, P., Heidenreich, J., 1977. Treatment of stress incontinence with estrogen in postmenopausal women. Urol. Int. 32, 221–223.

Figueroa, J.P., Massmann, G.A., 1995. Estrogen increases nitric oxide synthase activity in the uterus of nonpregnant sheep. Am. J. Obstet. Gynecol. 173, 1539–1545.

García-Pascual, A., Triguero, D., 1994. Relaxation mechanisms induced by stimulation of nerves and by nitric oxide in sheep urethral muscle. J. Physiol. 476, 333–347.

Goetz, R.M., Morano, I., Calovini, T., Studer, R., Holtz, J., 1994. Increased expression of endothelial constitutive nitric oxide synthase in rat aorta during pregnancy. Biochem. Biophys. Res. Commun. 205, 905–910.

Gregg, A.R., Thompson, L.P., Herrig, J.E., Weiner, C.P., 1995. Regionalization of endothelium-dependent relaxation in the thoracic aorta of pregnant and nonpregnant guinea pigs. J. Vasc. Res. 32, 106–111.

Hashimoto, S., Kigoshi, S., Muramatsu, I., 1993. Nitric oxide-dependent and -independent neurogenic relaxation of isolated dog urethra. Eur. J. Pharmacol. 231, 209–214.

Hayashi, T., Yamada, K., Esaki, T., Kuzuya, M., Satake, S., Ishikawa, T., Hidaka, H., Iguchi, A., 1995. Estrogen increases endothelial nitric oxide by a receptor-mediated system. Biochem. Biophys. Res. Commun. 214, 847–855.

Kurata, N., Inagaki, M., Kobayashi, S., Nishimura, Y., Oguchi, K., Yasuhara, H., 1993. Antipyrine concentrations in liver and blood monitored by microdialysis of unrestrained conscious rats. Res. Com. Chemi. Patho. Pharmaco. 79, 363–369.

Kuroda, H., Kohrogi, T., Uchida, N., Imai, I., Terada, N., Matsumoto, K., Kitamura, Y., 1985. Urinary retention induced by estrogen injections in mice: An analytical model. J. Urol. 134, 12681270.

Larsson, B., Andersson, K.-E., Batra, S., Mattiasson, A., Sjögren, C., 1984. Effects of estradiol on norepinephrine-induced contraction, alpha adrenoceptor number and norepinephrine content in the female rabbit urethra. J. Pharmacol. Exp. Ther. 229, 557–563.

Leone, A.M., Wiklund, N.P., Hökfelt, T., Brundin, L., Moncada, S., 1994. Release of nitric oxide by nerve stimulation in the human urogenital tract. Neuroreport 5, 733–736.

Malinski, T., Taha, Z., 1992. Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. Nature 358, 676–678.

Ohta, K., Araki, N., Shibata, M., Hamada, J., Komatsumoto, S., Shimazu, K., Fukuuchi, Y., 1994a. A novel in vivo assay system for consecutive measurement of brain nitric oxide production combined with the microdialysis technique. Neurosci. Lett. 176, 165–168.

Ohta, K., Shimazu, K., Komatsumoto, S., Araki, N., Shibata, M., Fukuuchi, Y., 1994b. Modification of striatal arginine and citrulline metabolism by nitric oxide synthase inhibitors. NeuroReport 5, 766–768.

Persson, K., Alm, P., Johansson, K., Larsson, B., Andersson, K.-E., 1993. Nitric oxide synthase in pig lower urinary tract: Immunohistochemistry, NADPH diaphorase histochemistry and functional effects. Br. J. Pharmacol. 110, 521–530.

Rand, M.J., Li, C.G., 1995a. Nitric oxide as a neurotransmitter in peripheral nerves: Nature of transmitter and mechanism of transmission. Annu. Rev. Physiol. 57, 659–682.

Rand, M.J., Li, C.G., 1995b. Discrimination by the NO-trapping agent, carboxy-PTIO, between NO and the nitrergic transmitter but not between NO and EDRF. Br. J. Pharmacol. 116, 1906–1910.

Salmon, U.J., Walter, R.I., Geist, S.H., 1941. The use of estrogens in the treatment of dysuria and incontinence in postmenopausal women. Am. J. Obstet. Gynecol. 42, 845–851.

Shapiro, E., 1986. Effect of estrogens on the weight and muscarinic cholinergic receptor density of the rabbit bladder and urethra. J. Urol. 135, 1084–1087.

Shibuki, K., 1990. An electrochemical microprobe for detecting nitric oxide release in brain tissue. Neurosci. Res. 9, 69–76.

Shay, J., Futo, J., Badrov, N., Moss, J., 1994. Estrogen withdrawal selectively increases serotonin reactivity in rabbit basilar artery. Life Sci. 55, 1071–1081.

Shintani, F., Kanba, S., Nakaki, T., Sato, K., Yagi, G., Kato, R., Asai, M., 1994. Measurement by in vivo brain microdialysis of nitric oxide release in the rat cerebellum. J. Psychiatr. Neurosci. 19, 217–221.

Sobey, C.G., Brooks, R.M. 2nd, Heistad, D.D., 1995. Evidence that expression of inducible nitric oxide synthase in response to endotoxin is augmented in atherosclerotic rabbits. Circ. Res. 77, 536–543.

Suburo, A.M., Chaud, M., Franchi, A., Polak, J.M., Gimeno, M.A.F., 1995. Distribution of neuronal and non-neuronal NADPH diaphorases and nitric oxide synthases in rat uterine horns under different hormonal conditions. Biol. Reprod. 52, 631–637.

Takeda, M., Lepor, H., 1995. Nitric oxide synthase in dog urethra: A histochemical and pharmacological analysis. Br. J. Pharmacol. 116, 2517–2523.

Van der Linden, M.C.G.J., Gerretsen, G., Brandhorst, M.S., Ooms, E.C.M., Kremer, C.M.E., Doesburg, W.H., 1993. The effect of estriol on the cytology of urethra and vagina in postmenopausal women with genito–urinary symptoms. Eur. J. Obstet. Gynecol. Reprod. Biol. 51, 29–33.

Veille, J.-C., Li, P., Eisenach, J.C., Massmann, A.G., Figueroa, J.P., 1996. Effects of estrogen on nitric oxide biosynthesis and vasorelaxant activity in sheep uterine and renal arteries in vitro. Am. J. Obstet. Gynecol. 174, 1043–1049.

Walter, S., Wolf, H., Barlebo, H., Jensen, H.K., 1978. Urinary incontinence in postmenopausal women treated with estrogens. Urol. Int. 33, 135–143.

Yallampalli, C., Byam-Smith, M., Nelson, S.O., Garfield, R.E., 1994. Steroid hormones modulate the production of nitric oxide and cGMP in the rat uterus. Endocrinology 134, 1971–1974.