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2

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## Abstract Reproduction Form B-1

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Title (type in  
CAPITAL  
LETTERS)THE OCCURRENCE OF NITRIC OXIDE AND ESTROGEN  
RECEPTORS IN FEMALE URINOGENITARY TRACT IN  
RELATION TO INCONTINENCE DURING AGE ADVANCEMENT

**Aims of Study** Urinary incontinence occurs in 30 % of females above 50 years old (1). Hypothetically, abnormal micturition is associated with low estrogen level (2) that can lead to urogenital tract changes in particular neuromuscular function (1). Nitric oxide (NO) is a non-classical neurotransmitter of nociceptive information in the spinal cord and peripheral synapses in the urogenital tract (3). It is believed NO released from nerves in the detrusor is responsible for maintenance of bladder relaxation during micturition. Estrogen receptors can be located in bladder, urethra, and reproductive organs (4). Treatment of estrogen to aging females not only can prevent urinary incontinence and is proposed to stimulate nitric oxide synthase (NOS) found in outflow region nerve fibers of the lower urinary tract (5,6). The scope of our study is to investigate the distribution and changes of estrogen receptors and neuronal-NOS (n-NOS) in epithelium and smooth muscle of the urethra in aging rats.

**Methods** Sixty female Sprague-Dawley rats of similar body weight (<100g) were selected from Dae Duk Research Center. Urethra specimens were taken at 4, 16, 30 and 60 weeks old rats under anaesthetic. Microscopic observation and expressions of ER (ID5 clone) and n-NOS (neuronal NO) were investigated by immunohistochemistry of fixed urethra specimen. Western Blot was performed to confirm the expressions of urethral n-NOS (155 kDA) using gel-electrophoresis marking with 155 kDA.

**Results**

As presented in Table 1, the ER in the rat urethra epithelium and smooth muscle and n-NOS constantly increased up to 30 weeks of age. As it reached 60 weeks of age, both ER and n-NOS significantly decreased ( $p < 0.001$ ).

Table 1.

Age (weeks)	4	16	30	60
ER-Epithelium <sup>+</sup>	10.33±1.84	10.73±1.83	11.13±1.30	7.60±1.640***
ER-Smooth Muscle <sup>+</sup>	0.33±0.05	0.47±0.04	0.53±0.05	0.1±0.03***
n-NOS <sup>+</sup>	133.40±34.25	134.47±29.70	136.73±19.19	91.20±26.04***

Mean±SD (n=15)

Analysis of Variance (ANOVA) \*\*\* $p < 0.001$  vs 4, 16, 30 weeks

\* Ratio of positive stain to negative stain of urethral ER or n-NOS

By Western Blot, the strongest chromogen of n-NOS on was shown in urethra of 30 weeks age rats and the weakest at 60 weeks old rats. Furthermore positive correlation was found between ER-epithelium and n-NOS ( $r = 0.694$ ,  $p < 0.001$ ) and between ER-muscle and n-NOS ( $r = 0.761$ ,  $p < 0.001$ ).



Category No.

8

Video  
Demonstration

Ref. No. (Page 2)

232

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Conclusions

Our investigation clearly showed that n-NOS the enzyme that converts L-arginine to produce NO and L-citrulline in urethral muscles (7) is intimately correlated with ER such that both decreased in age advancement. The correlation suggests that estrogen therapy to females with urinary incontinence (6) stimulate the non-andrenergic non-cholinergic (NANC) parasympathetic neurotransmitter (8) NO in urethral epithelium, inducing muscle relaxation of the urethra (9) for normal micturition.

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