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Aikawa K[1], Chichester P[2], Levin R[2], Yamaguchi O[1]
1. Department of Urology, Fukushima Medical University, 2. Division of Basic and Pharmaceutical Sciences, Albany College of Pharmacy

EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON CHANGES INDUCED BY ESTRADIOL IN OVARIECTOMIZED RABBIT BLADDER SMOOTH MUSCLE.

Aims of Study

The decrease in circulating estrogen of post-menopausal women may be linked to urinary bladder dysfunctions including detrusor instability and impaired contractility. Our previous report has demonstrated that estradiol has a significant hypertrophic effect on detrusor smooth muscle resulting in increased contractile function (1). However, the mechanisms for these effects are unclear. The recent study has demonstrated that estradiol decreases vascular resistance of the bladder and increases bladder blood flow (2). In another study, estradiol induced increases in uterine blood flow, and this increase was antagonized by the nitric oxide synthase inhibitor L-NAME (3). The present study was designed to determine whether estradiol-induced increases in bladder blood flow could be inhibited by L-NAME, and whether a reduction of this blood flow alters the increased contractile function and a hypertrophic effect of estradiol on detrusor smooth muscle.

Methods

Sixteen female New Zealand White rabbits, weighing 3.5 to 4.0 Kg, were ovariectomized (12) or sham-operated (4). At 1 week following surgery, four of the ovariectomized rabbits were treated with vehicle and eight were treated with estradiol at 1mg / kg / week for 4 weeks. Using osmotic pumps, L-NAME (12mg / kg / day) was also given to four of the estradiol-treated rabbits for 4 weeks. Serum estradiol was measured using ELIZA assays at 3 and 5 weeks following surgery. At 4 weeks after treatment, each rabbit was anesthetized and cystometry was performed**.** After cystometry, blood flow to the detrusor muscle, mucosa, uterus and kidney was determined by standard fluorescent microsphere infusion technique. After the blood flow study, the bladder was excised and approximately one fourth of the bladder body was analyzed for blood flow (by IMT Inc.). Then four longitudinal detrusor strips and two rings of descending thoracic aorta were mounted in individual 15 ml baths containing oxygenated Tyrode's solution at 37°C. The contractile responses to EFS (80 V, 32Hz, 1msec), carbachol (20 µM), adenosine triphosphate (2 mM), and KCl (120 mM) were determined. To estimate endothelial dependent nitric oxide-mediated vasorelaxation, the relaxant responses to acetylcholine (1 nM to 10 µM) were measured in the aortic rings contracted with 1 µM phenylephrine. Full-thickness sections of detrusor were fixed in buffered formalin for 8 hours and embedded in paraffin for a-actin immunostaining. In this immunostained section, the volume fraction of smooth muscle was calculated with an image analysis program.

Results

In thoracic aortic rings, L-NAME resulted in significant reduction of the ACh-induced endothelial dependent relaxation, providing the evidence for inhibition of endothelial dependent nitric oxide production. In the bladder: **1)** Estradiol resulted in an increase in blood flow to the detrusor; L-NAME inhibited this increase (Table 1). **2)** Estradiol resulted in a significant increase in bladder weight which was inhibited by L-NAME (Table 1). **3)** Estradiol resulted in increased cystometric capacity and no change in micturition pressure. L-NAME treatment attenuated the increased capacity (Table 1). **4)** Estradiol resulted in increased contractile response to all forms of stimulation, whereas L-NAME inhibited these increased responses (Table 2). **5)** Estradiol resulted in a significant increase in smooth muscle from 37.5±0.6% to 56.3±1.6%, whereas L-NAME significantly inhibited this increased ratio to 49.4±1.2%.

Table 1 Bladder weight, Cystometric parameter and Bladder blood flow. Values are mean ± SE. OVX: Ovariectomy + vehicle. OVX + E: Ovariectomy + estradiol. OVX + E + L-NAME: Ovariectomy + estradiol + L-NAME. * $p<0.05$ vs. OVX and sham. ** $p<0.05$ vs. OVX + E.

	OVX	Sham	OVX+E	OVX+E+L-NAME
Bladder weight (g)	1.9±0.1	2.0±0.3	5.3±0.1*	3.8±1.1**
Cystometric capacity (ml)	4.2±0.8	7.5±1.6	21±2.5*	11±5.9**
Micturition pressure (cm H_2O)	8.1±1.4	14±2.2	12±2.8	14±1.3
Bladder muscle blood flow (ml/min/g)	0.1±0.05	0.3±0.07	1.8±1.0	0.3±0.1

Table 2. Maximal contractile responses (g tension / 100mg tissue). * $p<0.05$ vs. sham. † $p<0.05$ vs. OVX. ‡ $p<0.05$ vs. OVX + E

	OVX	Sham	OVX+E	OVX+E+L-NAME
32Hz	5.4 ± 0.6*	7.9 ± 0.3	11 ± 0.9*†	8.8 ± 0.5†‡
Carbachol	3.7 ± 0.4	5.3 ± 0.3	11 ± 1.0*†	8.2 ± 0.6†
ATP	0.4 ± 0.05*	1.7 ± 0.2	2.1 ± 0.3†	1.3 ± 0.2†
KCl	1.2 ± 0.2	1.7 ± 0.2	2.1 ± 0.2†	2.0 ± 0.2†

Conclusions

These findings support the conclusion that increased bladder blood flow plays an important role in both the estradiol-induced hypertrophic effect on bladder smooth muscle and the increased contractile function.

References

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