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THE EFFECT OF NITRIC OXIDE SYNTHASE INHIBITION ON CHANGES INDUCED BY ESTRADIOL IN OVARIECTOMIZED RABBIT BLADDER

Hypothesis / aims of study

Our previous report demonstrated that estradiol has a significant hypertrophic effect on detrusor smooth muscle resulting in increased contractile function. There is circumstantial evidence that estradiol-stimulated increases in blood flow may be involved. The present study was designed to determine whether estradiol-induced increases in bladder blood flow could be inhibited by N^{ω}-nitro-L-arginine methyl ester (L-NAME), and whether a reduction of bladder blood flow can alter the increased contractile function and bladder hypertrophy that are observed following estradiol administration.

Study design, materials and methods

Sixteen female New Zealand White rabbits weighing 3.5 to 4.0 Kg. were separated into 4 groups of 4 rabbits each. 1) **Sham group** received sham operation and injections of vehicle (peanut oil). 2) **Ovariectomy (OVX) group** received ovariectomies and injections of vehicle. 3) **Ovariectomy + Estrogen (OVX+E) group** received ovariectomy and injections of 17ß-estradiol (1mg / kg) dissolved in peanut oil. 4) **Ovariectomy + estradiol + L-NAME (OVX+E+L-NAME) group** received ovariectomies and injections of 17ß-estradiol and L-NAME. Estradiol and vehicle were injected subcutaneously once a week. Using osmotic pumps (Alzet model 2ML4), L-NAME was administered. The pumps were loaded with L-NAME (12mg / kg / day) dissolved in saline and surgically implanted subcutaneously in the scapular region. After 1 week recovery period following ovariectomy or sham operation, all treatments were started and continued for 4 weeks.

At 4 weeks after treatment, each rabbit was anesthetized and cystometries were performed**.** After cystometry, blood flow to the detrusor muscle and mucosa was determined by standard fluorescent microsphere infusion technique. 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), adenosine triphosphate (2 mM), carbachol (20 μM), 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 and embedded in paraffin for α-actin immunostaining. In the immunostained section, the volume fraction of smooth muscle was calculated with an image analysis program. All values are reported as the mean ± SEM.

Results

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

Interpretation of results

These findings support the hypothesis that increased bladder blood flow plays an important role in both the estradiol-induced hypertrophic effect and the increased contractile function on detrusor smooth muscle, and that nitric oxide is involved in this increased blood flow.

Concluding message

Alteration in the concentration of circulating estrogen levels have pronounced effects on blood flow and tissue oxygenation, some of which are modulated through the nitric oxide pathway. Increasing estrogen levels can restore blood flow and oxygenation to the bladder and result in improved bladder functioning.

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