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RELEASE OF ADENOSINE TRIPHOSPHATE FROM THE MUCOSAL LAYER AND CHANGES IN THE CONTRACTILE RESPONSE OF THE BLADDER IN OVARIECTOMISED GUINEA PIGS

Hypothesis / aims of study

The urothelium is more than a just a passive barrier to urine and bacteria; it actively produces bioactive substances in response to external stimuli. Adenosine triphosphate (ATP) released from the urothelium is considered to be a co-transmitter that innervates the urinary bladder and transduces information to interstitial cells in the mucosal/submucosal and detrusor muscle layers. Lower urinary tract symptoms such as urgency and urge incontinence are common in postmenopausal women, and ovariectomy is associated with increased urination frequency, decreased bladder volume, and increased acetylcholine release from the bladder wall, suggesting that decreased estrogen levels may be associated with lower urinary tract symptoms in females (1). The purpose of this study was to assess the effects of estrogen on the guinea pig bladder by investigating whether ovariectomy affects ATP release from the mucosal layer.

Study design, materials and methods

Twenty female Dunkin Hartley guinea pigs (weight: 400–600 g) were anesthetised with intraperitoneal injections of ketamine (80 mg/g) and xylazine (10 mg/g). Half of the guinea pigs underwent bilateral ovariectomy performed from a dorsal approach to induce surgical menopause (OVX; n=10), and the other half underwent sham operations (Sham; n=10). All animals were housed in metabolic cages, and at 4 weeks after surgery, urine output was recorded every 10 seconds for 24 hours. Water consumption was also recorded for 24 hours. The urinary bladders and uteri were then removed and weighed. The ventral wall of each bladder was opened longitudinally from the bladder neck to the top of the dome. Next, intact strips (length: 6–8 mm; width: 1–2 mm) of smooth muscle from the urinary bladders were then prepared. Mucosal strips composed of urothelial and suburothelial layers were then prepared by microscope-guided dissection of the mucosal and detrusor smooth muscle layers using iris scissors. Next, the mucosal strips were affixed to an isometric force transducer mounted on a micromanipulator to adjust resting length and then superfused with Tyrode's solution at 36°C. After superfusion for 60 minutes, the mucosal strips were stretched to 10 mN of passive tension using the micromanipulator. Some mucosal strips were also superfused in Tyrode's solution with the addition of carbachol (0.3 μM) for 30 minutes. ATP release from the prepared strips was measured in a 20 μL sample aspirated from a fixed point 2 mm above the centre of the preparation surface. ATP was assayed using the luciferin–luciferase assay (FL-AAM; Sigma, GloMax 20/20; Promega) at a 5-fold dilution. A total of 10 readings were taken for each sample and then averaged. Luminescence was calibrated against ATP standards (10^{-12} M to 10^{-8} M), and appeared linear on a log–log plot, with Tyrode's solution used as a blank solution where appropriate. Intact strips were also affixed to an isometric force transducer, after which tension was applied and recorded. After establishing passive tension, the intact strips were cumulatively administered carbachol (10^{-8} M to 10^{-5} M) and exposed to electrical field stimulation (EFS; 1–40 Hz, duration 0.5 mSec). Contractile responses to maximum concentrations of carbachol (T_{max}carb) and high stimulation frequencies (T_{max}EFS), as well as responses to half-maximal concentrations of carbachol (EC_{50}; pEC_{50} = -logEC_{50}) and $f_{1/2}$ (EFS frequency for 0.5*T_{max}EFS), were used to assess muscle contractility.

Results

Although body and bladder weights were similar between the Sham and OVX groups (Table 1), uterus weight was significantly smaller in the OVX than in the Sham group (OVX; 227.4 ± 38.0 mg, Sham; 1475 ± 89.9 mg, $p<0.001$), which was considered a result of decreased estrogen levels in the OVX group. Although 24-hour water consumption and urine production were similar in both groups, 24-hour urination frequency was increased and mean voided urine volume were significantly decreased in the OVX group ($p<0.05$) (Table 1). ATP release from mucosal strips stretched and superfused in Tyrode's solution is shown in Table 2. No differences were seen between groups in basal ATP levels. However, after stretching to 10 mN of passive tension, ATP levels significantly increased in both groups, and the rate of increase was significantly higher in the OVX (226.5%) than in the Sham group (127.6%) ($p<0.05$). Carbachol is known to stimulate ATP release from the mucosal layer (2). In this experiment, 0.3 μM of carbachol also stimulated ATP release from the mucosal layer in both groups; however, no difference was observed in the rate of increase between groups. Regarding the contraction of intact strips (Table 3), responses to the muscarinic agonist carbachol were similar at high concentrations in both groups, but the pEC_{50} values were significantly smaller in the OVX (6.21) than in the Sham group (6.36) ($p<0.05$). In addition, contractile responses to high stimulation frequencies (T_{max}EFS) were significantly lower in the OVX (1720 mN) than in the Sham group (2378 mN) ($p<0.05$).

Table 1. Comparison of characteristics between the Sham and OVX groups

	Sham (n=10)	OVX (n=10)
Guinea pig weight (g)	511.1 ± 4.55	483.3 ± 6.87
Uterus weight (mg)	1475 ± 89.9	227.4 ± 38.0****
Bladder weight (mg)	610.4 ± 53.7	479.5 ± 47.1
24-hour water consumption (mL)	29.3 ± 6.48	31.3 ± 6.74
24-hour urine production (mL)	27.2 ± 4.94	26.0 ± 3.45
24-hour frequency	5.83 ± 0.79	8.83 ± 1.33*

Mean voided urine volume (mL)	5.02± 0.47	3.57± 0.58*

**** p<0.001, *p<0.05 Sham vs. OVX. Data are expressed as mean ± standard error of the mean.0

Table 2. ATP release from mucosal strips stretched and superfused in carbachol

	Stretch			Carbachol		
	Control (pmol/g)	10 mN (pmol/g)	Increase (%)	Control V\(pmol/g)	0.3 µM (pmol/g)	Increase (%)
Sham (n=10)	54.9 [*27.0,137.9*]	75.4* [*30.4,233.7*]	127.6† [*107.1,163.4*]	32.45 [*24.4,80.8*]	119.4* [*42.0,183.9*]	250.4 [*158.7,394.1*]
OVX (n=10)	34.5 [*7.10,80.73*]	97.5* [*36.5,219.7*]	226.5 [*170.2,385.9*]	58 [*11.7,116.6*]	97.5* [*24.5,314.6*]	255.1 [*131.9,340.9*]

* p<0.05 control vs. 10 mN stretch; 0.3 µM carbachol; † p<0.05 Sham vs. OVX. Data are expressed as medians [25, 75% interquartiles].

Table 3. Contractile characteristics of isolated intact strips

	T_{max}carb (mN/g)	pEC_{50}	T_{max}EFS (mN/g)	$f_{1/2}$ (Hz)
Sham (n=10)	1471 [*1190,1828*]	6.36* [*6.27,6.45*]	2378* [*1666,2850*]	6.05 [*3.16,8.01*]
OVX (n=10)	1317 [*1108,1829*]	6.21 [*5.89,6.36*]	1720 [*985,1939*]	7.69 [*4.15,11.38*]

* p<0.05 Sham vs. OVX. Data are expressed as medians [25, 75% interquartiles]

Interpretation of results

Uterus weights were lower in the OVX group, supporting the notion that ovariectomy is associated with decreased estrogen levels. Bladders in the OVX group appeared be overactive based on reduced urine volume and increased urination frequency. Although the amount of ATP released from the mucosal layer varied widely between groups, no significant differences were found. ATP release from the mucosal layer was increased after stretching and superfusion in both groups; however, the rate of increase after stretching was significantly higher in the OVX group, which suggests that decreased estrogen levels contribute to bladder overactivity. The pEC_{50} value decreased in the OVX group, showing a dose-response curve that was shifted to the right, which suggests that muscle contractility was reduced in response to carbachol in the OVX group. Furthermore, T_{max}EFS was also decreased in the OVX group, which suggests that nerve-mediated contractions are also affected by decreased estrogen levels.

Concluding message

The rate of ATP release from mucosal strips prepared from ovariectomised guinea pigs was significantly increased after stretching. The results may be one of the causes that ovariectomy induced decreased bladder volume and increased urination frequency. Furthermore, decreased contractile responses to carbachol and EFS were observed in intact strips prepared from ovariectomised guinea pigs. The results may be one of the reasons that postmenopausal women develop underactive bladder.

References

1. Yoshida J. et al. Neurourol Urodyn. 2007; 26 (7): 1050-5.
2. Kushida N. et al. BJUI 2015 doi: 10.1111/bju.13240

Disclosures

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