



# # 358 Mechanisms underlying bladder hypersensitivity in female mice with estrogen deficiency



Ei-ichiro Takaoka<sup>1</sup>, Takahisa Suzuki<sup>2</sup>, Shinsuke Mizoguchi<sup>2</sup>, Masahiro Kurobe<sup>2</sup>, Ni Jianshu<sup>2</sup>, Kwon Joonbeom<sup>2</sup>, Nobutaka Shimizu<sup>2</sup>, Mizuki Onozawa<sup>3</sup>, Jun Miyazaki<sup>3</sup>, Hiroyuki Nishiyama<sup>4</sup>, Naoki Yoshimura<sup>2</sup>

1. University of Pittsburgh / International university of health and welfare, 2. University of Pittsburgh, 3. International University of health and welfare, 4. University of Tsukuba

Figure 1: Study design.

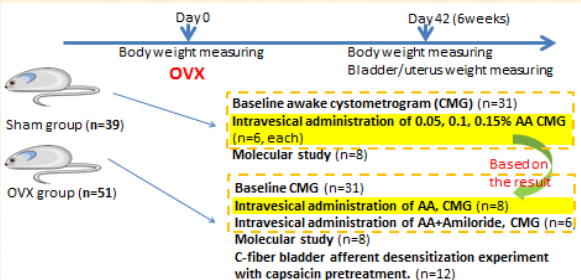


Figure 2: Representative CMG.

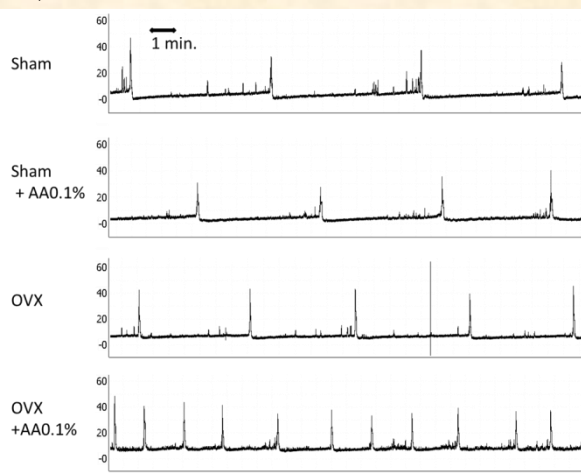


Table 1: CMG parameters of OVX mice with Intravesical 0.1 % Acetic Acid (AA) administration

	ICI (s)	Amplitude (cmH2O)	PB (cmH2O)	PT (cmH2O)	NVC rate (NVC/min)	VV (μl)	PVR (μl)	Capacity (μl)	Compliance (ml/cmH2O)	VE (%)
saline	700 ±100	45 ±1.9	4.0 ±0.61	6.7 ±0.62	0.14 ±0.030	110 ±16	0.5 ±0.5	110 ±16	0.046 ±0.0052	100 ±0.49
0.1% AA	270 ±60	44 ±3.4	5.3 ±0.72	7.0 ±0.75	0.13 ±0.065	50 ±11	0	50 ±11	0.038 ±0.012	100 ±0.12
p	0.0047	0.73	0.014	0.48	0.87	0.0041	0.35	0.0041	0.48	0.3506

Figure 3: Representative CMG of OVX mice with intravesical 1mM Amiloride administration with/without 0.1% AA.

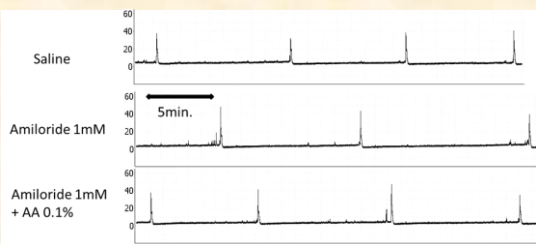
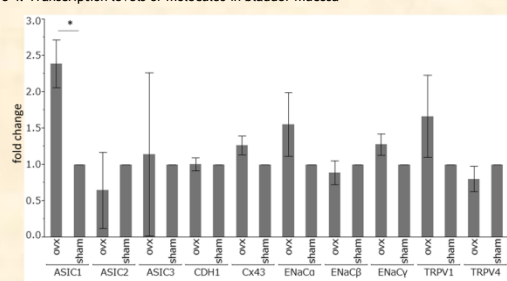


Figure 4: Transcription levels of molecules in bladder mucosa



## RESULTS

- ✓ Body weight was significantly increased ( $27 \pm 0.48$  vs.  $20 \pm 0.23$  g,  $p < 0.0001$ ) and the uterus weight was significantly decreased ( $0.016 \pm 0.0011$  vs.  $0.076 \pm 0.0045$  g,  $p < 0.0001$ ) in OVX vs. control mice.
- ✓ In baseline CMG, there were no significant differences in CMG parameters between sham and OVX. (Figure 2).
- ✓ OVX mice showed a significant decrease in ICI, voided volume, and bladder capacity after intravesical 0.1% AA administration (Figure 2, Table 1), whereas 0.1% AA did not affect any CMG parameters in control mice.
- ✓ Intravesical administration of 1mM amiloride blocked the effect of AA in OVX mice (Figure 3, Table 2).
- ✓ In RT-PCR, the expression of ASIC1 was significantly increased in bladder mucosa of OVX mice (Figure 4).
- ✓ OVX mice with C-fiber desensitization by capsaicin pretreatment, intravesical 0.1% AA administration had no effect on bladder function, although, in OVX mice with vehicle pretreatment, intravesical 0.1% AA irritation still significantly decreased ICI, voided volume, and bladder capacity (Figure 5, Table 3).

## ABSTRACT

**Objective:** In this study, we analysed bladder function, molecular changes in bladder mucosa, and the effect of intravesical chemical irritation in OVX mice to elucidate the pathophysiological mechanisms and explore the therapeutic targets of postmenopausal bladder dysfunction.

**Materials and Methods:** In female C57BL/6N mice (8-week-old), OVX was performed via a dorsolumbar approach without touching the bladder. Six weeks after the operation, awake cystometrograms (CMG) were recorded in sham and OVX mice. In both groups, intravesical acetic acid (AA) administration with or without intravesical administration of amiloride was performed, and changes in CMG parameters were evaluated. The transcript levels of some molecules in bladder mucosa were evaluated by RT-PCR. Furthermore, intravesical AA irritation was performed in OVX mice with C-fiber afferent desensitization induced by capsaicin pretreatment.

**Results:** In CMG, there were no significant differences in CMG parameters between sham and OVX at baseline. However, OVX mice showed a significant decrease in ICI, voided volume, and bladder capacity after intravesical 0.1% AA administration, whereas 0.1% AA did not affect any CMG parameters in control mice. Intravesical administration of 1mM amiloride blocked the effect of AA in OVX mice. In RT-PCR, the expression of ASIC1 was significantly increased in bladder mucosa of OVX mice. Furthermore, OVX mice with C-fiber desensitization by capsaicin pretreatment, intravesical 0.1% AA administration had no effect on bladder function, although, in OVX mice with vehicle pretreatment, intravesical 0.1% AA irritation still significantly decreased ICI, voided volume, and bladder capacity.

**Conclusion:** It seems likely that the estrogen-deficient condition makes the bladder more susceptible to intravesical stimuli to induce C-fiber-dependent bladder overactivity via activation of acid-sensing receptors such as ASIC1, which might be a mechanism of storage bladder dysfunction in postmenopausal women.

## METHODS

- ✓ Study design is shown in Figure 1.
- ✓ Female C57BL/6N mice (8-week-old), OVX was performed via a dorsolumbar approach without touching the bladder. Sham operated animals were used as controls. The evaluation s were performed 6 weeks after the operations.
- ✓ In both groups, intravesical acetic acid (AA) administration with or without intravesical administration of amiloride (an inhibitor of ASICs & ENaC) was performed, and changes in CMG parameters were evaluated.
- ✓ The transcript levels of junction molecules (CDH1, Cx43), acid-and/or mechano-sensitive receptors (TRPV4, ASICs, ENaCs) in bladder mucosa were evaluated by RT-PCR.
- ✓ Intravesical AA irritation was performed in OVX mice with C-fiber afferent desensitization induced by capsaicin pretreatment [2].

Table 2: CMG parameters of OVX mice with intravesical 1mM Amiloride administration with/without 0.1% AA.

	ICI (s)	Amplitude (cmH2O)	PB (cmH2O)	PT (cmH2O)	NVC rate (NVC/min)	VV (μl)	PVR (μl)	Capacity (μl)	Compliance (ml/cmH2O)	VE (%)
saline	510 ±46	42 ±3.5	2.3 ±0.15	5.0 ±0.16	0.078 ±0.036	85 ±8.0	1 ±0.63	86 ±8.0	0.033 ±0.0031	99 ±0.83
Amiloride 1mM	550 ±61	37 ±3.6	2.8 ±0.49	5.3 ±0.17	0.064 ±0.036	90 ±1.6	3 ±1.6	93 ±8.4	0.042 ±0.0088	96 ±1.9
p*	0.26	0.15	0.26	0.10	0.34	0.54	0.28	0.42	0.24	0.76
Amiloride 1mM + AA 0.1%	540 ±41	35 ±3.5	3.2 ±0.55	5.6 ±0.37	0.027 ±0.020	90 ±8.0	0.83 ±0.83	90 ±8.5	0.047 ±0.014	99 ±0.72
p**	0.46	0.19	0.093	0.095	0.29	0.48	0.66	0.14	0.27	0.57

Figure 5: Representative CMG of Intravesical 0.1 % AA administration in OVX mice with/ without capsaicin pretreatment.

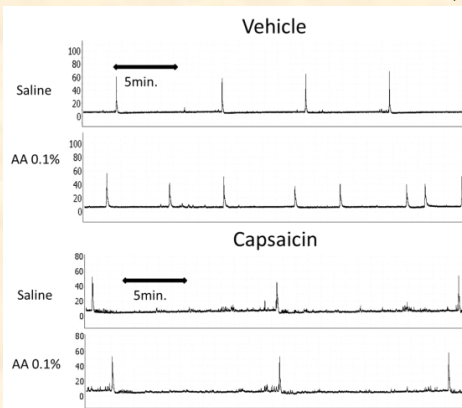


Table 3: CMG parameters of Intravesical 0.1 % AA administration in OVX mice with/ without capsaicin pretreatment.

	ICI (s)	Amplitude (cmH2O)	PB (cmH2O)	PT (cmH2O)	NVC rate (NVC/min)	VV (μl)	PVR (μl)	Capacity (μl)	Compliance (ml/cmH2O)	VE (%)
Vehicle 0.1% AA	330 ±68	41 ±5.6	4.7 ±0.77	6.4 ±0.63	0.099 ±0.052	53 ±13	0	53 ±13	0.032 ±0.0044	100 ±0.16
Vehicle saline	570 ±96	45 ±4.6	3.5 ±0.35	6.3 ±0.35	0.28 ±0.12	91 ±14	3.5 ±2.2	95 ±16	0.035 ±0.0048	98 ±1.6
p	0.0031	0.52	0.055	0.76	0.18	0.0018	0.18	0.0026	0.50	0.18
Capsaicin 0.1% AA	740 ±59	39 ±3.0	2.3 ±0.64	6.4 ±0.66	0.15 ±0.049	120 ±7.0	2.7 ±1.8	120 ±7.7	0.034 ±0.0055	82 ±16
Capsaicin saline	730 ±62	42 ±2.1	2.0 ±0.55	6.5 ±0.59	0.29 ±0.074	120 ±6.9	3.3 ±1.8	120 ±7.7	0.029 ±0.0036	97 ±1.3
p	0.78	0.31	0.24	0.68	0.084	0.43	0.73	1.0	0.051	0.37

## CONCLUSIONS

- ✓ It seems likely that the estrogen-deficient condition makes the bladder more susceptible to intravesical stimuli to induce C-fiber-dependent bladder overactivity via activation of acid-sensing receptors such as ASIC1, which might be a mechanism of storage bladder dysfunction in postmenopausal women.

## REFERENCES

1. Chen HY, Chen CJ, Chen WC, Wang SJ, Chen YH. A promising protein responsible for overactive bladder in ovariectomized mice. *Taiwan J Obstet Gynecol*. 56: 196-203, 2017.

2. Kadekawa K, Majima T, Shimizu T, Wada N, de Groat WC, Kanai AJ, Goto M, Yoshiyama M, Sugaya K, Yoshimura N. The role of capsaicin-sensitive C-fiber afferent pathways in the control of micturition in spinal-intact and spinal cord-injured mice. *Am J Physiol Renal Physiol*. 313: F796-F804, 2017.