

## THE EFFECTS OF OVARIECTOMY AND ESTROGEN REPLACEMENT ON NERVE STIMULATION AND PASSIVE STRETCH INDUCED ACETYLCHOLINE RELEASE IN RAT BLADDER.

### Hypothesis / aims of study

Acetylcholine (ACh) released from activated cholinergic nerves plays an essential role in contraction of the bladder during the voiding phase. However, it has been suggested recently that during the storage phase, there is a release of ACh from nerves or from a non-neuronal source (possibly the urothelium), which may be increased by distension of the bladder. The ACh release mentioned above would be influenced by estrogens because estrogen receptors have been found in bladder autonomic neurons (pre- and post-ganglionic neurons) as well as in the bladder urothelium. Thus, in order to elucidate the functional roles of estrogens in lower urinary tract, the present study investigates whether ovariectomy alters the ACh release in isolated rat bladder, and determines whether estradiol replacement restores its changes. We also evaluate the effects of ovariectomy and estradiol replacement on micturition characteristics in female rats.

### Study design, materials and methods

Thirty adult (24 weeks-old) Sprague-Dawley rats were randomly received sham operation (n=10), or ovariectomy (n=10), or ovariectomy plus estrogen replacement (n=10).

In estrogen replacement group, the rats were immediately treated with one milligram per kilogram weekly injection with  $\beta$ -estradiol for four weeks. Using metabolic cages, urine output was recorded for 2 days at 4 weeks after surgery. Finally, all rats from each group were sacrificed, bladder were taken, and in vitro studies were performed. In vitro studies include the measurements of nerve-mediated detrusor muscle contraction, and the amount of ACh release. The intramural nerves of the strip was stimulated by supramaximum voltage, rectangular pulses of 500 $\mu$ s duration and at a stimulation frequency of 5, 10, 20, 30, and 40 Hz on this duration. Train of pulses lasted for 5 sec and an interval between stimulation was 3 min. ACh in the bladder tissue was sampled by the technique of microdialysis at the time of nerve-mediated contraction or when bladder strips were stretched passively (1 to 4 grams tension). The amount of ACh was measured by a High-Performance Liquid Chromatography with enzyme reaction and electrochemical detection (ECD-500; Eicom). The stretch-induced ACh release was measured in the presence of 1 $\mu$ M TTX.

### Results

There were no differences in the rat weights and the bladder wet weights. However, ovariectomized rats showed a significant decrease in uterus weight, compared to the other groups ( $p < 0.01$ ). Ovariectomy had no effects on 24-hour water consumption or 24-hour urine production. However, ovariectomized rats showed a significant increase in 24-hour frequency of voiding ( $p < 0.05$ ), and a significant decrease in voided volume ( $p < 0.05$ ) (Table 1).

The maximum contractile response gradually increased with frequency dependent in all groups. Ovariectomy caused the significant decrease in the contractile responses at each stimulation frequency, while estrogen replacement restored this decrease in contraction (Figure 1). The ACh release induced by nerve stimulation gradually increased with the increase in stimulation frequency in all groups. Ovariectomy significantly decreased acetylcholine release from activated cholinergic neurons, and estrogen replacement also restored this decrease (Figure 2). The TTX insensitive ACh release gradually increased as a stretch force was increased in all groups. Ovariectomy caused the significant increase in TTX insensitive ACh release at basal tension as well as by passive stretch. After estrogen replacement, the release of TTX insensitive ACh returned to control level (Figure 3).

### Interpretation of results

Our findings showed that as a result of estrogen deficiency, ACh released from activated cholinergic neurons decreased significantly, suggesting that this reduction of ACh release may cause the decrease in detrusor contractility. Furthermore, this study also demonstrated that when bladder strips were stretched, TTX insensitive ACh release was increased in ovariectomized rats. Considering that such stretch may occur during bladder filling, this increased ACh release may be a contributing factor to detrusor overactivity or indirectly to storage symptoms, such as urgency.

### Concluding message

Estrogen deficiency may reduce ACh release from excited cholinergic neurons during the voiding phase as well as may increase the stretch-induced ACh release during the storage phase. As a clinical implication from this study, these findings may explain the possible mechanisms underlying detrusor hyperactivity with impaired contractility in elderly women.

Figure 1

Figure 2

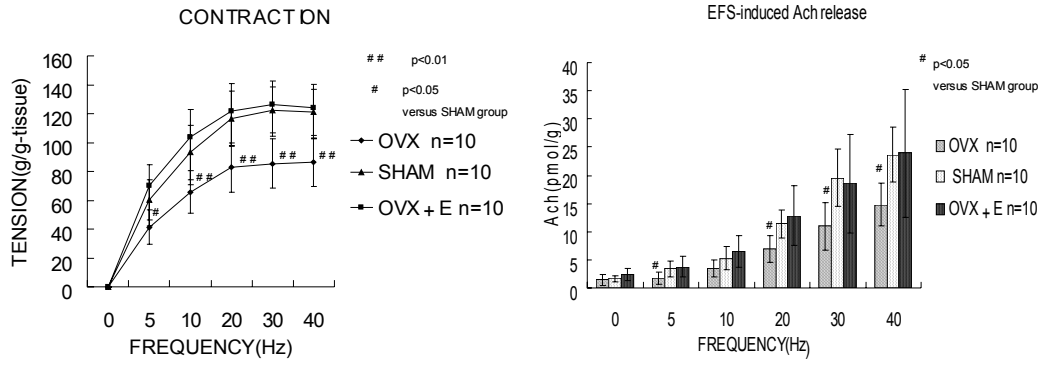


Figure3

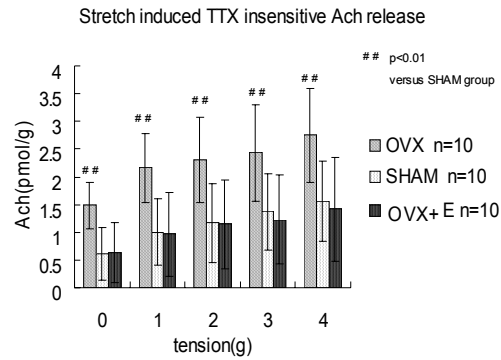


Table1

**FVC at 4weeks after ovariectomy and estrogen replacement**

	OVX	SHAM	OVX + E
24-hour consumption(ml)	15.3 ± 7.8	21.2 ± 11.1	22.4 ± 10.1
24-hour production(ml)	13.7 ± 5.6	15.9 ± 6.3	18.1 ± 8.0
24-hour frequency	22.6 ± 8.5 #	18 ± 4.5	14.6 ± 5.2
Mean voided volume(ml)	0.61 ± 0.15 #	0.85 ± 0.23	1.24 ± 0.43 ##
Maximum voided volume(ml)	1.51 ± 0.46 ##	2.02 ± 0.52	2.27 ± 0.59

## p<0.01 # p<0.05 versus SHAM group

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Fukushima Medical University