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EFFECTS OF ESTROGEN DEFICIENCY AND ITS REPLACEMENT ON VOIDING BEHAVIOUR AND PRIMARY BLADDER AFFERENT ACTIVITY IN THE RAT

Hypothesis / aims of study

The lower urinary tract (LUT) of both animals and humans has been shown to express estrogen receptors, and estrogen exerts a number of effects on the LUT that are important for its normal functioning. Thus, a decline in circulating estrogen levels can lead to various urogenital disorders. We investigated the effects of estrogen deficiency by ovariectomy (OVX) on voiding behaviour and single fiber activities of the primary bladder afferent nerves in the rat, and determined whether estrogen replacement can normalize the possible effect of estrogen-deficiency.

Study design, materials and methods

Forty-six female Sprague-Dawley rats were used and divided into 3 different groups:

- OVX+estrogen (OVX+E, N=17) – β-estradiol 17-cypionate (estrogen) replacement.
- OVX+vehicle (OVX+V, N=17) – vehicle replacement.
- SHAM+vehicle (SHAM+V, N=22) – sham surgery, vehicle replacement.

All rats were housed for 4 weeks after surgery at 8-9 months. Then, estrogen (250 μg/kg) or vehicle (cottonseed oil) was given subcutaneously once a week for 8 weeks. Before bladder afferent measurements, frequency volume chart measurements were performed with metabolic cage during 24 hours in a subset of rats of each group. Under urethane anesthesia, for monitoring single unit nerve activity of the primary bladder afferents, fine filaments were dissected from the left L6 dorsal roots and placed across a bipolar electrode. Nerve fibers primarily originating from the bladder were identified by electrical stimulation of the left pelvic nerve and by bladder distention. Nerves were classified as being an Aδ- or C-fiber if the conduction velocity was > or < 2.5 m/sec, respectively (1). The recording was repeated three times at an interval of 5 minutes to evaluate the reproducibility of the intravesical pressure and unitary afferent activity in response to intravesical saline instillation at 0.08 ml/min. The third recording served for analysis of afferent activity integrated for the whole filling phase, which is based on pressure and volume. After bladder afferent measurements, the blood serum was collected and the estradiol (E2) level was measured in all rats. The values are expressed as mean ± S.E. One-way ANOVA followed by Tukey's test and Kruskal-Wallis followed by Dunn's test were used for comparisons between all groups, and $p<0.05$ is considered as statistically significant.

Results

In the frequency volume chart measurements, mean voided volume of the OVX+V was significantly less than that of the SHAM+V. However, there were no significant differences in the total voided volume or voiding frequency among the 3 groups (Table 1).
In the afferent activity measurements, there was no significant difference in bladder compliance among the three groups (data not shown). No significant differences in the activities of Aδ-fibers (CV: 10.541±2.353 m/sec, n=17) were found among the three groups (Figure 1A). On the other hand, in term of C-fibers (CV: 1.242±0.059 m/sec, n=52), the activities integrated during the whole the filling phase in the OVX+E and SHAM+V were significantly higher than that in the OVX+V (Figure 1B). The mean E2 serum level of OVX+V rats was significantly lower than those of the other groups (Figure 2).

Interpretation of results

Serum E2 level measurements demonstrate that the OVX (at 8-9 months) induced a significant decrease in the serum estrogen, and the estrogen replacement used increased serum E2 level up to more than the control level (SHAM+V). The results of the present functional study indicate that the estrogen decline induced by OVX can cause a decrease in mean voided volume without significant changes in urine output per day, and this decrease in mean voided volume can be at least partially recovered by the estrogen replacement. In parallel with these findings, in the mechanosensitive C-fiber activity, but not Aδ-fiber activity, the response to bladder filling significantly decreased with the OVX-induced estrogen decline, and this decrease was counteracted by the estrogen replacement.

Concluding message

Estrogen deficiency may cause storage dysfunction as demonstrated by reduced voided volumes in the OVX group. However, since the activity in C-fibres decreased rather than increased after OVX, its contribution to the voiding dysfunction may be questioned.

Table 1. Frequency volume chart parameters calculated during 24 hours

	OVX+E (N=5)	OVX+V (N=5)	SHAM+V (N=10)
total voided volume (mL/day)	12.32±1.22	7.98±1.14	11.73±1.39
voiding frequency (times/day)	18.60±2.44	14.00±1.76	14.60±1.30
mean voided volume (mL/1 time)	0.68±0.05	0.57±0.04**	0.79±0.04

The values are expressed as mean ± S.E. **$P<0.01$ vs. SHAM+V.

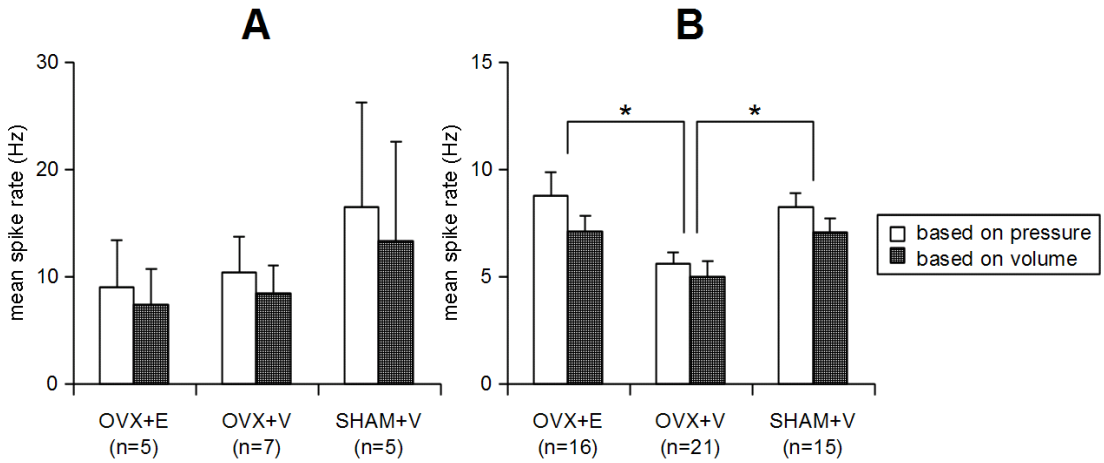

Figure 1. Responses of the Aδ-fibers (A) and C-fibers (B) integrated during the whole filling phase
*P<0.05: significant difference between all groups (one-way ANOVA followed by Tukey's test).

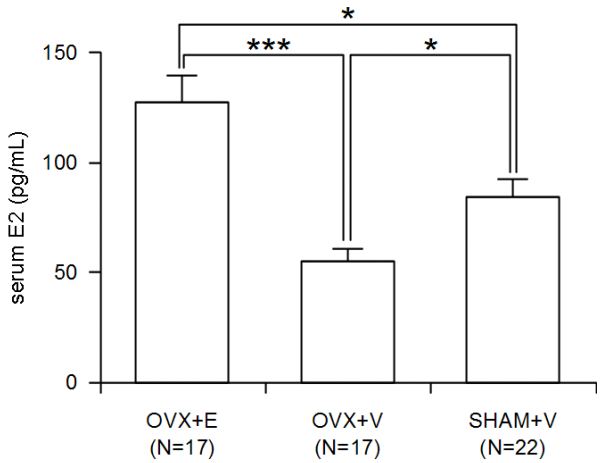

Figure 2. Estradiol (E2) levels in the rats with ovariectomy (OVX) or sham operation (SHAM) with estrogen (E)- or vehicle (V)-treatment.
*P<0.05, ***P<0.001: significant difference between all groups (Kruskal-Wallis followed by Dunn's test).

References

1. J Neurophysiol 1994; 72: 2420

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Ethics Committee, Antwerp University Faculty of Medicine**