

## Changes in the Micturition Profile and Bladder AQP3 after Ovariectomy in Rats

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*S u m m a r y.* The aim of the present study is to examine whether ovariectomy is associated with functional changes in the bladder function, as evidenced by the micturition profile, and whether this is related to changes in the bladder expression of AQP3. The results show that modulation of the concentration of the female sex hormones caused by ovariectomy is associated with changes in the expression levels of AQP3 in bladder wall, as well as in several parameters of the micturition profile in rats. It is possible that the changes in the micturition profile occur partly because of the change in AQP3 expression levels but further studies are needed to clarify the mechanisms involved and the role bladder AQP3 expression might have in the overall control of bladder function.

### INTRODUCTION

It has been demonstrated recently that ovariectomy is associated with increased AQP2 expression levels and plasma AVP levels, as well as increased body weight (1). Furthermore, it has been demonstrated that the modulation in the concentration of the female sex hormones caused by ovariectomy and subsequent administration of female sex hormones is also associated with changes in the renal expression of AQP2, phosphorylated AQP2, AQP3 and AQP1 and changes in renal expression of Na-K-ATPase, resulting in the modulation of body salt and water balance. Other recent studies (2) suggest that AQPs in the genitourinary tract urothelia play a role in the regulation of epithelial cell volume and osmolality and might play a role in the movement of water across urothelia. In principal, any water and solute transport through or across the bladder wall might influence the

structural characteristics of the bladder, possibly by changing the water content of the tissue. That might in turn result in changes in the function of the organ, thus interfering possibly with the normal micturition profile. Thus, the aim of the present study is to examine whether ovariectomy is associated with functional changes in the bladder function evidenced by the micturition profile and whether this is related to changes in the bladder expression of AQP3.

### METHODS

Studies were performed on female Munich-Wistar rats initially weighing 200±20 grams. Rats were anaesthetized with halothane and bilateral ovariectomy (OVX) or sham operation (SHAM) was performed. Rats were monitored for 7 and 14 days while maintained in the metabolic cages, allowing daily quantitative urine collections and measurements of water intake. Furthermore, while kept in the metabolic cages, the rats were monitored as to when, how often and what quantities of urine they excreted at days 0, 2 and 14, obtaining a detailed micturition profile. All rats were sacrificed under light halothane anaesthesia and the bladder was rapidly removed and processed for membrane fractionation and immunoblotting at the same day. For semiquantitative immunoblotting, a previously characterized affinity purified rabbit polyclonal antibody was used: AQP3 (LL 178 AP) (4), and the subsequent electrophoresis, immunoblotting and quantitation of AQP3 expression was performed as previously described (5,6). Values were presented in the text as means±SE. Comparison between groups

was made by the unpaired t-test. P values < 0.05 were considered significant.

### RESULTS

Densitometric analysis of all bladder samples from OVX and SHAM operated rats (corrected according to loading) revealed a marked increase in AQP3 expression in the bladder ( $132 \pm 5\%$  in OVX rats to  $100 \pm 11\%$  in SHAM operated rats,  $P < 0.05$ ). The micturition studies performed showed marked changes in the micturition profile 14 days after OVX, where a significant reduction in total urine output was observed ( $p < 0.05$ ). Furthermore, the minimum urine quantity that resulted in micturition was significantly lower in OVX rats 14 days after ovariectomy ( $p < 0.05$ ). The same was observed regarding the maximal urine quantity that resulted in micturition ( $p < 0.05$ ) (please see Table 1 for a summary of the results)

### CONCLUSIONS

Modulation of the concentration of female sex hormones caused by ovariectomy is associated with changes in the expression levels of AQP3 in bladder wall, as well as in several parameters of the micturition profile in rats. It is possible that the changes in the micturition profile occur partly

because of the change in AQP3 expression levels. Further studies are needed to clarify the mechanisms involved and the role bladder AQP3 expression might have in the control of bladder function.

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Table 1  
Micturition profile changes (during 24-hour periods)

Day	Group	Total U quantity (g)	Min. U quantity (g)	Max. U quantity (g)	Mict. frequency
Day 0	OVX	12±4	0.02±0.001	1.13±0.33	30±6
	SHAM	9.9±3.8	0.02±0.005	0.98±0.30	30±1
Day 2	OVX	8.5±3.03	0.02±0.005	1.12±0.27	28±7
	SHAM	10.2±4.7	0.02±0.009	1.04±0.27	29±3
Day 14	OVX	7.6±2.6*	0.02±0.004*	0.67±0.42*	24±8
	SHAM	11.4±4.4	0.04±0.003	1.53±0.74	27±9

Total U quantity: total urine production in g; Min. U quantity: minimum urine quantity in g; Max. U quantity: maximum urine quantity in g; Mict. Frequency: number of micturition episodes per observational period

\*:  $p < 0.05$  when OVX is compared to SHAM