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Upregulation of Renal Water Channels and Sodium Transporters in Response to Ovariectomy in Rats

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S u m m a r y. We report a highly significant increase in the expression levels of AQP2, -3, -1 and Na,K-ATPase in response to ovariectomy in rats. These findings were associated with increased levels of vasopressin 14 days after ovariectomy. Moreover, ovariectomy resulted in significant decrease in urine output and significant increase in body weight, suggesting that the functional impact of the AQP2, -3, -1 and Na,K-ATPase upregulation is water retention. Overall, the results of the present study support the view that female sex hormones play an important role in regulating renal water and sodium handling, through the regulation of AQPs.

INTRODUCTION

Body water balance is known to be modulated by the ovarian steroid hormones through a complex series of mechanisms involving both osmoregulatory components and changes in renal solute excretion. Conditions with high circulating levels of estrogens and progesterone, as that seen in pregnancy, are characterized by sodium and water retention, plasma volume expansion and arterial vasodilation (1). Likewise, states with high circulating levels of estrogens alone, as seen immediately preceding ovulation and while taking estrogens (2) or estrogen-dominant oral contraceptives are associated with sodium and water retention. In contrast, menopause is characterized by very low levels of estrogens or progesterone and a significant weight gain attributed partly to water retention[3]. This water retention may be caused by dysregulation of renal water and sodium metabolism, potentially modulated by altered levels of the antidiuretic hormone vasopressin (3-5). The water permeability of the collecting duct is regulated by vasopressin, which stimulates and increases water reabsorption down an osmotic gradient. It has previously been demonstrated that aquaporin-2 (AQP2) (6), located to the collecting duct principal cells is tightly regulated by vasopressin (7). Importantly, water reabsorption in the collecting duct is regulated both by short-term and long-term mechanisms, both of which have been shown to depend critically on AQP2. Recently it has been demonstrated that AQP2 expression is increased in pregnant rats (8), providing evidence that altered levels of female sex hormones might play an important role for AQP2 regulation. Additionally, an increased expression of the thiazide-sensitive NaCl cotransporter (TSC) has been demonstrated in ovariectomized rats after estradiol treatment while the same cotransporter expression levels were decreased in OVX rats (9), suggesting that sodium transporters may also be altered in response to changes in the circulating levels of female sex hormones. In order to understand the molecular mechanisms potentially involved in the changes in renal water handling after ovariectomy we examined the expression levels of AQP2, AQP3, AQP1 and Na,K-ATPase in kidneys from OVX and sham operated control rats together with day-by-day changes in renal water and solute handling 7 and 14 days after ovariectomy.

METHODS

Studies were performed on female Munich-Wistar rats initially weighing 200±20 g. Rats were anaesthetized with halothane and submitted to bilateral ovariectomy (OVX) or sham operation (SHAM). Rats were monitored for 7 and 14 days while maintained the metabolic cages, allowing daily quantitative urine collections and measurements of water intake. Urine volume, body weight, urine osmolality, creatinine (by application of dry matter chemistry), sodium and potassium concentration (using a flame photometer) were measured. Plasma was collected at the time of ovariectomy and sacrifice for measurement of sodium and potassium concentration (by application of dry matter chemistry), creatinine (by application of dry matter chemistry) and osmolality. Plasma vasopressin and aldosterone levels were measured using RIA. Serum was collected for the measurement of 17b-estradiol and progesterone levels (by competitive immunoassay using direct chemiluminescent technology). Urine and plasma osmolality values were determined by freezing point depression. All rats were sacrificed under light halothane anaesthesia and kidneys were rapidly removed and processed for membrane fractionation and immunoblotting at the same day. For semiquantitative immunoblotting, previously characterized mouse monoclonal and affinity purified rabbit polyclonal antibodies were used: AQP2 (LL 127 AP) (10), phosphorylated AQP2 (AN 244pp AP) (11), AQP3 (LL 178 AP) (12), AQP1 (LL 266) (13) and Na,K-ATPase, and the subsequent electrophoresis, immunoblotting and quantitation of AQP expression were performed as previously described (14,15). Values are 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

Semiquantitative immunoblotting revealed a persistent and significant increase in AQP2 levels in IM of kidneys from OVX rats to 202±4% (n=12) of sham levels at day 7 and 275±43% (n=8) of sham levels at day 14 ($p<0.05$). Phosphorylated AQP2 levels also increased significantly 14 days after ovariectomy (754±218% (n=8) of sham levels, $p<0.05$) demonstrating an increase in the level of PKA-induced phosphorylated AQP2. Plasma vasopressin levels were significantly increased 14 days after ovariectomy (OVX: 1.5±0.3pg/ml, n=6 vs. SHAM: 0.7±0.1pg/ml, n=6, $p<0.05$). Urine output was significantly reduced seven (OVX: 42±3 $\mu l \cdot min^{-1} \cdot kg^{-1}$ (n=18) vs. SHAM: 56±6 $\mu l \cdot min^{-1} \cdot kg^{-1}$ (n=20), $p<0.05$) and 14 days after ovariectomy (OVX: 77±2 $\mu l \cdot min^{-1} \cdot kg^{-1}$ (n=18) vs. SHAM: 89±4 $\mu l \cdot min^{-1} \cdot kg^{-1}$ (n=20), $p<0.05$). Consistent with the above results, ovariectomy was associated with a significant increase in body weight at day 7 (234±2 g (n=18) vs. 222±3 g (n=18), $p<0.05$) and day 14 (249±3 g (n=18) vs. 222±2 g (n=20), $p<0.05$). Moreover, AQP3 levels were increased in IM 14 days after OVX (265±41%, n=8 vs. 100±47%, n=7, $p<0.05$). AQP1 and Na,K-ATPase levels were significantly increased in WK 14 days after ovariectomy (142±12% (n=13, $p<0.05$) of sham levels and 140±15% (n=13, $p<0.05$) of sham levels respectively).

CONCLUSIONS

The data presented in this study show that OVX is associated with a significant increase of AQP2 expression levels, a reduction in urine output and a significant increase in body weight and plasma vasopressin levels. There is, therefore, support for our initial hypothesis that one could explain the increase in body weight after ovariectomy reported by previous studies and reproduced in this study, through water retention owed to the increase of the expression levels of aquaporin water channels. The results suggest that AQP2 dysregulation may play an important role in renal water handling after OVX and is at least at part, vasopressin mediated.

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