Sex differences in vasopressin V2 receptor expression and vasopressin-induced antidiuresis

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Liu J, Sharma N, Zheng W, Ji H, Tam H, Wu X, Manigrasso MB, Sandberg K, Verbalis JG. Sex differences in vasopressin V2 receptor expression and vasopressin-induced antidiuresis. Am J Physiol Renal Physiol 300: F433–F440, 2011. First published December 1, 2010; doi:10.1152/ajprenal.00199.2010.—The renal vasopressin V2 receptor (V2R) plays a critical role in physiological and pathophysiological processes associated with arginine vasopressin (AVP)-induced antidiuresis. Because clinical data suggests that females may be more prone to hyponatremia from AVP-mediated antidiuresis, we investigated whether there are sex differences in the expression and function of the renal V2R. In normal Sprague-Dawley rat kidneys, V2R mRNA and protein expression was 2.6- and 1.7-fold higher, respectively, in females compared with males. To investigate the potential physiological implications of this sex difference, we studied changes in urine osmolality induced by the AVP V2R agonist desmopressin. In response to different doses of desmopressin, there was a graded increase in urine osmolality and decrease in urine volume during a 24-h infusion. Females showed greater mean increases in urine osmolality and greater mean decreases in urine volume at 0.5 and 5.0 ng/h infusion rates. We also studied renal escape from antidiuresis produced by water loading in rats infused with desmopressin (5.0 ng/h). After 5 days of water loading, urine osmolality of both female and male rats escaped to the same degree (63% and 73%, respectively) than in males (32% and 48%, respectively). By the end of the 5-day escape period, renal V2R mRNA and protein expression were reduced to the same relative levels in males and females, thereby abolishing the sex differences in V2R expression seen in the basal state. Our results demonstrate that female rats express significantly more V2R mRNA and protein in kidneys than males, and that this results physiologically in a greater sensitivity to V2R agonist administration. The potential pathophysiological implications of these results are that females may be more susceptible to the development of dilutional hyponatremia because of a greater sensitivity to endogenously secreted AVP.

renal escape; desmopressin; hyponatremia

THE PRIME DETERMINANT OF WATER homeostasis in animals and man is the regulation of urinary free water excretion by circulating plasma levels of the hormone arginine vasopressin (AVP). AVP is a nine-amino acid peptide that is synthesized in magnocellular neural cells located in the hypothalamus. The synthesized peptide is enzymatically cleaved from its prohormone and is transported to the posterior pituitary where it is stored within neurosecretory granules until specific stimuli cause secretion of AVP into the bloodstream (23). Antidiuresis then occurs via interaction of the circulating hormone with AVP V2 receptors (V2Rs) in the kidney, which results in increased water permeability of the collecting duct through the insertion aquaporin-2 (AQP2) water channels into the apical membranes of renal collecting duct principal cells (19). The importance of AVP in water homeostasis is underscored by the pathophysiology that occurs when AVP, or AVP-mediated receptor activation, is either deficient or excessive (27).

The most common disorder of AVP dysregulation encountered in clinical medicine is hypoosmoluric hyponatremia. Studies of hyponatremia in hospitalized patients have suggested incidences as high as 15–30% in both acutely (5, 9, 17) and chronically (18) hospitalized patients using a serum [Na+] < 135 mmol/l to define hyponatremia. While most studies of hyponatremia have been conducted in men, studies suggest that women may have more adverse complications from hyponatremia than men (2), raising the possibility that important sex differences exist in the mechanisms underlying this disorder and its adverse consequences. For example, females are more prone to water imbalance disorders such as exercise-induced hyponatremia (1). Such considerations underscore the importance of elucidating the mechanisms underlying the pathogenesis of hypoosmolality in both males and females.

Although available clinical data suggests that females may be more prone to hyponatremia from AVP-mediated antidiuresis, studies of AVP secretion in females and males have not shown very large differences in basal or stimulated plasma AVP levels between the sexes (10, 30). Because the renal V2R plays a critical role in the urinary concentrating process (19), we hypothesized that renal V2R expression might be higher in female compared with male animals, and that the higher levels of V2R expression in females than in males may have physiological consequences. To evaluate this hypothesis, we measured V2R mRNA (measured by real-time PCR) and protein (measured by Western blot analysis) expression in normal female and male Sprague-Dawley rat kidneys. To investigate the potential physiological implications of this sex difference, we compared urine volume and urine osmolality in males and females under basal conditions and during infusion of graded doses of the AVP V2R agonist desmopressin.

Renal epithelial cells undergo pronounced volume regulation in response to changes in extracellular osmolality using the same mechanisms of electrolyte and organic osmolyte fluxes as found in the brain (13). The kidney, however, employs additional mechanisms to adapt to induced antidiuresis and water retention. Chief among these is the phenomenon of renal escape from antidiuresis. In animal models of sustained AVP administration and in patients with...
the syndrome of inappropriate antidiuretic hormone secretion (SIADH), water loading typically results in free water retention and progressive hyponatremia for several days, which is then followed by escape from the AVP-induced antidiuresis (4, 16). With the onset of vasopressin escape, water excretion increases despite sustained administration of AVP, allowing water balance to be reestablished and the serum [Na+] to be stabilized at a steady, albeit decreased level. Kidney water channels have been shown to play a crucial role in AVP-induced antidiuresis (19). AQP2 is the major water channel expressed in the apical membrane of collecting duct principal cells (12) and is highly regulated by AVP. States of inappropriate antidiuresis are associated with increased kidney AQP2 expression, including animal models of SIADH (11) and congestive heart failure (31). In addition, AQP2 protein and mRNA levels are markedly downregulated during renal escape from antidiuresis in a temporal pattern that correlates closely with the physiological parameters of escape (7).

Experimental data has suggested that the renal AVP V2R plays a key role in the physiological and pathophysiological processes underlying renal escape from AVP-induced antidiuresis in male rats (21). In contrast, much less information is known about the mechanisms underlying renal escape in females. We therefore performed additional studies of renal escape from AVP-induced antidiuresis in female and male rats to evaluate whether changes in basal V2R expression affected the escape process by changing the time course or magnitude of downregulation of V2R mRNA and protein expression, as well as subsequent effects on urine osmolality and volume.

MATERIALS AND METHODS

Animals and desmopressin dose response experiment. Sprague-Dawley male and female rats (Taconic Farms, Germantown, NY) weighing 225–250 g were acclimated individually in metabolic cages (Lab Products, Seaford, DE) and fed ad libitum a liquid diet (AIN-76A; Bioserv, Frenchtown, NJ) prepared in water with 0.2% saccharin (Sigma-Aldrich, St. Louis, MO) and tap water for 2 days. After this period, the rats (n = 6 males and 6 females for each dose tested) were anesthetized using Aerrane (isosufurane; Baxter Healthcare, Deerfield, IL) for 3–5 days prior to experimentation. Under light isoflurane anesthesia, osmotic minipumps were implanted subcutaneously to deliver 5 ng/h of desmopressin. After 2 days of desmopressin administration, during which time all rats received ad libitum pelleted chow and water, the experimental groups were water loaded by substituting daily feedings of a liquid formula (AIN-76) in a volume of 100 ml. This amount of liquid diet provides sufficient calories for weight maintenance in adult rats. Thus, to maintain their caloric intake, the rats were forced to consume substantial quantities of water as well. The rats were maintained in metabolic cages, allowing quantitative urine collections. Urine volume and osmolality were measured daily. On day 5 after water loading, rats were euthanized by decapitation and the left kidneys were rapidly dissected and homogenized for immunoblotting.

Immunoblotting. Left whole kidneys were homogenized in chilled membrane-isolation solution containing 250 mM sucrose, 10 mM triethanolamine, 1 μM/ml leupeptin (Sigma-Aldrich), and 0.1 mg/ml PMSF (United States Biochemical, Cleveland, OH) adjusted to pH 7.6. Protein concentration was measured by using Protein Assay Dye Reagent (Bio-Rad Laboratories, Hercules, CA). Samples were then diluted to 2 μg/μl with 5× sample buffer (7.5% SDS, 30%glycerol, 50 mM Tris pH 6.8, 30 μg/ml DTT). SDS-PAGE was carried out on 10% Tris-glycine polyacrylamide gels using a Bio-Rad Criterion System. The proteins were transferred from the gels to polyvinylidene fluoride membranes electrophoretically using the Bio-Rad Criterion System. The polyvinylidene fluoride membranes were probed overnight at 4°C with rabbit polyclonal anti-vasopressin V1R (H-80) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) or polyclonal anti-AQP2 antibodies [gift from Dr. Carolyn Ecelbarger (6)]. The secondary antibody was goat anti-rabbit IgG conjugated to horseradish peroxidase (KPL, Gaithersburg, MD) used at a concentration of 0.1 μg/ml. To visualize sites of antibody-antigen reaction, blots were exposed to a luminol-based enhanced chemiluminescent reagent (LumiGLO; KPL) or Supersignal West Dura horseradish peroxidase detection kit (Pierce, Rockford, IL) before exposure to X-ray film (FUJI Film, Kanagawa, Japan). Image densitometric analysis of the protein bands was performed using the OptiQuant image analysis software (PerkinElmer Life Sciences). For all studies, equal numbers of both male and female kidney extracts were run on the same gels to allow quantitative comparisons of the densitometry results.

Statistical analysis. Data are presented as means ± SE. Urine data were statistically analyzed by two-way, repeated-measures ANOVA followed by Bonferroni multiple comparisons. Results from immunoblot densitometry were analyzed by one-way ANOVA followed by Student Newman-Keuls multiple comparison. Differences were considered statistically significant at P < 0.05.

RESULTS

Basal V2R gene expression in female and male rat kidney cortex. To detect V2R gene expression levels, mRNA and protein were isolated from the kidneys of adult female (n = 6)
and male \((n = 6)\) Sprague-Dawley rats. Both real-time PCR and immunoblotting demonstrated that female rats expressed higher levels of \(V_2R\) mRNA \([\text{arbitrary units (AU)}]: \text{female, 2.8} \pm 0.57; \text{male, 1.1} \pm 0.15; \text{\(P < 0.05\)}\) and protein \([\text{AU}]: \text{female, 1.7} \pm 0.12; \text{male, 1.0} \pm 0.22; \text{\(P < 0.05\)}\) than males. \(V_2R\) mRNA (Fig. 1A) and protein (Fig. 1B) were 2.6-fold and 1.7-fold higher in the females compared with the males, respectively.

Desmopressin dose response. Female \((n = 6)\) and male \((n = 6)\) rats were water loaded with liquid diet. All rats excreted \(> 60\ \text{ml/24}\) with a urine osmolality \(< 500\ \text{mosmol/kgH}_2\text{O}\), and there were no significant differences between the females and males at baseline \((0\ \text{ng/h desmopressin dose})\). In response to desmopressin, there was a graded increase in urine osmolality (Fig. 2A) and decrease in urine volume (Fig. 2B) during the 24-h desmopressin infusion. Females showed greater mean increases in urine osmolality and greater mean decreases in urine volume only at the two highest infusion rates \((0.5 \text{ and } 5.0\ \text{ng/h})\). Analysis by two-way ANOVA showed a significant effect of dose on urine osmolality \((F = 31.63, \text{\(P < 0.001\)}}\) and urine volume \((F = 7.29, \text{\(P = 0.003\)}}\). Analysis by two-way ANOVA also showed a significant effect of sex on urine volume after the onset of water loading \((F = 7.03, \text{\(P = 0.0085\)}}\) (Fig. 3B). As reported previously using this model \((21)\), urine volume began to increase and urine osmolality began to decrease by \text{day 2} of water loading in both females and males, indicating the onset of renal escape from antidiuresis. Urine osmolality remained higher in the female rats on the second day after water loading, but by \text{day 3} onward there were no significant differences in urine osmolality (Fig. 3A) or urine volume (Fig. 3B) between the female and male rats.
Changes in V2R gene expression in female and male rat kidney after renal escape. At day 5 after the onset of water loading, mRNA and protein were isolated from kidneys of desmopressin-treated male and female rats. V2R mRNA was significantly reduced in both sexes compared with basal V2R mRNA levels [AU: female (basal), 1.0 ± 0.07; male (basal), 1.0 ± 0.085] but the magnitude of the fall was substantially greater in females (63%) compared with males (32%) (Fig. 4A). A similar pattern of change was observed for V2R protein expression at day 5 of escape compared with basal levels measured in a subset of the females and males [AU: female (basal), 1.0 ± 0.07; male (basal), 1.0 ± 0.15] but the magnitude of the fall was substantially greater in females (73%) compared with males (48%) (Fig. 4B).

Comparison of V2R and AQP2 expression in female and male rats after escape from antidiuresis. Real-time PCR result showed that following escape from antidiuresis, female (n = 24) and male (n = 21) V2R mRNA reached the same levels of expression [AU: female (after escape), 0.77 ± 0.063; male (after escape), 0.70 ± 0.074; not significant (NS)] (Fig. 5A), despite marked differences in basal V2R expression (Fig. 1A). Expression of V2R protein also reached the same level in female (n = 9) and male (n = 9) rats after escape from antidiuresis [AU: female (after escape), 0.44 ± 0.11; male (after escape), 0.54 ± 0.16; NS] (Fig. 5A), despite marked differences in basal V2R protein (Fig. 1B). Similarly, kidney AQP2 protein levels (measured as the sum of the glycosylated and nonglycosylated bands by Western blot analysis) also showed the same relative levels of expression between female (n = 14) and male (n = 14) rats after escape from antidiuresis [AU: female (after escape), 0.50 ± 0.092; male (after escape), 0.50 ± 0.096; NS] (Fig. 5B). However, under basal conditions, AQP2 expression was not significantly different between male and female rats (data not shown). These results are consistent with the physiological results indicating identical parameters of renal escape (i.e., urine osmolality and volume) in the female and the male rats after 5 days of water loading (Fig. 3).

Fig. 2. Response of urine volume and urine osmolality to desmopressin in female and male rats implanted subcutaneously with osmotic minipumps that delivered desmopressin dosages of 0, 0.1, 0.5, and 5.0 ng/h. Daily urine collections and water intake were recorded. All rats were euthanized 24 h after minipump implantation, and plasma was collected for osmolality and sodium levels. In response to desmopressin, there was a graded increase in urine osmolality (A) and decrease in urine volume (B) during the 24-h desmopressin infusion. Values are expressed as means ± SE; *P < 0.05 compared with males.

Fig. 3. Physiological parameters of renal function during escape from antidiuresis in female and male rats. A: 24-h urine osmolality. B: urine volumes in female and male rats during renal escape from desmopressin-induced antidiuresis. All rats were implanted with desmopressin osmotic minipumps at day 2, and all had access to pelleted rat chow and water ad libitum. Beginning on day 0, all rats were water loaded by substituting daily feedings of a liquid formula for the pelleted chow. Values are expressed as means ± SE; *P < 0.05 compared with males.
DISCUSSION

These studies demonstrate the presence of marked sex differences in renal V2R expression in female compared with male rats. The 2.6-fold greater V2R mRNA and 1.7-fold greater V2R protein levels in female rats are consistent with the hypothesis that renal V2R gene expression is higher in female compared with male animals under basal conditions.

The higher levels of renal V2R expression in females compared with males was manifested physiologically by a higher desmopressin-stimulated urine osmolality in female rats compared with males (Fig. 2). Although a significant sex difference was not found in desmopressin-stimulated decreases in urine output, this is not surprising given the relative insensitivity of urine volume to changes in urine osmolality at high levels of AVP stimulation. Interestingly, the higher urine osmolality in female rats compared with males was observed predominantly at higher doses of desmopressin administration more so than at lower doses, or under basal conditions where neither the baseline urine osmolality nor renal AQP2 expression was significantly different between female and male rats. This suggests that the increased V2R receptor expression in females may be of greater functional significance during situations of...
high ligand concentrations. This may indicate that under basal conditions of submaximal AVP stimulation the limiting factor in urine concentration is not V2R receptor number, but rather circulating AVP levels. Another possibility to explain these findings is that under basal or low AVP concentrations, much of the increased V2R in females may be in the form of “spare receptors” that are not functionally coupled to G protein signaling proteins (8, 15), whereas at higher AVP concentrations increased G protein coupling of the receptors occurs. In either case, the result would be that female rats manifest greater maximal responses to high AVP concentrations.

Because of these initial findings, we undertook additional studies of escape from AVP-induced antidiuresis, since previous studies from our laboratory have indicated that renal escape is accompanied by a marked downregulation of V2R expression (26), with decreased AVP-stimulated signal transduction in collecting tubule principal cells and resultant downregulation of AQP2 expression (7). We hypothesized that because females had higher basal levels of V2R expression, they would undergo escape from antidiuresis more slowly and to a lesser absolute degree relative to the males. Although to some extent this was found to be true, i.e., urine osmolality remained significantly higher in the female rats on days 1 and 2 of water loading (Fig. 3A), these differences were relatively small and did not reach statistical significance on all subsequent days of escape. More importantly, both the female and male rats reached the same level of escape by completion of escape process, as reflected by the day 5 urine osmolalities and volumes. Interestingly, the relative changes in kidney V2R expression mirrored the physiological results in that the female rats experienced a greater downregulation of V2R mRNA and protein compared with the males. The magnitude of this effect can be best appreciated by the observation that after 5 days of escape, the profound sex differences in basal V2R expression were completely abolished (Fig. 5A).

The most reliable correlate of renal escape from antidiuresis has been decreased renal expression of AQP2 protein, which is the final mediator of AVP-stimulated antidiuresis (7). Any decrease in AVP-mediated signal transduction is capable of causing decreased water reabsorption by the kidney, as demonstrated by the effect of AVP V2R receptor antagonists to produce an aquaresis in animals (22, 25, 29) and humans (14, 29). Thus, decreased AVP-stimulated signal transduction as a result of decreased V2R expression should also be accompanied by decreased kidney AQP2 expression, which was found to an equivalent degree in both the female and male rats in this study (Fig. 5B).

Although sexually dimorphic differences in V2R and AQP2 expression in response to continuous desmopressin administration were not observed in this study, the escape process represents an adaptation to chronic AVP administration that takes days to occur. The more acute desmopressin stimulation studies (Fig. 2) indicate that the increased V2R expression in
females does alter the renal responses to shorter-term changes in AVP levels and/or V2R activation.

In normal XX female mammals, the second X chromosome is silenced in each cell by a mechanism called “X inactivation”; however, some genes escape X inactivation and are expressed on both the active and inactive X chromosome in the same cell. Genes that escape X inactivation are potential contributors to sexually dimorphic traits and to the phenotypic variability among females heterozygous for X-linked conditions, as well as to clinical abnormalities in patients with abnormal X chromosomes. In 2005, Carrel and Willard (3) published the X inactivation status of 95% of the assayable genes on the X chromosome. The V2R gene scored 9 out of 9 in the X-inactivation tests in heterozygous human fibroblasts, suggesting that this gene has a high probability of escaping X inactivation. Thus, one potential explanation for our findings is that the V2R gene might escape X-inactivation in females, thereby allowing expression from both X chromosomes in females compared with expression from a single X chromosome in males. However, this hypothesized sex chromosomal effect will require further studies for definitive proof, since intact females and males manifest different levels of gonadal hormones, which could also contribute to the sex differences in V2R expression.

The results of some studies have several important implications both molecularly and physiologically. First, our results demonstrate physiologically significant ramifications of sex differences in renal V2R expression. Understanding the underlying mechanisms responsible for increased V2R expression in females may in turn provide insights into observed sex differences in other diseases such as hypertension and cardiovascular disease (20). Second, these results add additional support to V2R regulation as the predominant mechanism that regulates escape from antidiuresis (28), since the changes in V2R expression in females and males in this study accurately predicted the physiological manifestations of escape. Finally, our results suggest that the molecular mechanisms underlying V2R, and possibly other G protein-coupled receptor, regulation act in a manner capable of downregulating mRNA and protein expression to an absolute degree, rather than simply to a relative degree; if the latter were true, we would have expected to find proportional decreases in V2R mRNA and protein during escape from antidiuresis in both males and females rather than a greater degree of downregulation in females. All of these findings will require further studies to fully understand the significance of sex differences in AVP V2R expression, and the mechanisms leading to the loss of this sexual dimorphism during escape from antidiuresis.

Clinically, increased V2R expression in females may cause greater sensitivity to nonosmotically stimulated AVP thereby leading to hyponatremia from SIADH more frequently, which has not been carefully studied to date. Similarly, increased V2R expression and function in females might cause a relatively greater resistance to the aquaretic effect of acutely administered AVP V2R antagonists, which are now in clinical use (14, 24). Finally, our results raise the possibility that females may be more sensitive to the renal effects of exogenously administered AVP or desmopressin, in which case lower doses may achieve acceptable antidiuretic effects in females with less risk of hyponatremia. These and other potential clinical implications await further study.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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