Molecular analysis of impaired urinary diluting capacity in glucocorticoid deficiency

Weidong Wang, Chunling Li, Sandra N. Summer, Sandor Falk, Melissa A. Cadnapaphornchai, Yung-Chang Chen, and Robert W. Schrier. Molecular analysis of impaired urinary diluting capacity in glucocorticoid deficiency. Am J Physiol Renal Physiol 290: F1135–F1142, 2006. First published December 13, 2005; doi:10.1152/ajprenal.00356.2005.—Urinary diluting ability and protein abundance of inner medullary AQP2 and pAQP2 in association with improved urinary diluting capacity in glucocorticoid-deficient (GD) rats were examined at baseline and in response to oral water loading. Protein expression of inner medullary AQP2 was significantly upregulated in GD compared with CTL rats (all P < 0.05). GD rats also demonstrated a marked reduction in urinary Na⁺ excretion compared with pair-fed CTL rats. Protein abundance of inner medullary AQP2 (148 ± 18%), phosphorylated AQP2 (pAQP2, 156 ± 13%), and AQP3 (145 ± 8%) was significantly upregulated in GD compared with CTL rats (all P < 0.05). GD rats also demonstrated a marked reduction in urinary Na⁺ excretion compared with pair-fed CTL rats. Na⁺-K⁺-2Cl⁻ cotransporter, Na⁺/H⁺ exchanger type 3, and cortical β- and γ-subunits of the epithelial Na⁺ channel were significantly upregulated in GD rats. At 1 h after an acute water load (40 ml/kg by oral gavage), GD rats demonstrated a decrease in percent water excretion (5 ± 1 vs. 33 ± 9%, P < 0.01) and urinary output (33 ± 12 vs. 250 ± 65 μl·kg⁻¹·min⁻¹, P < 0.05) and an increase in Uosm (1,894 ± 292 vs. 316 ± 92 mosmol/kgH₂O, P < 0.001) compared with CTL rats. Plasma AVP was increased (1.6 ± 0.2 vs. 0.9 ± 0.2 pg/ml, P < 0.05), as was protein expression of inner medullary AQP2 (149 ± 5%) and pAQP2 (177 ± 9%, P < 0.01), in GD compared with CTL rats; apical expression of AQP2 was maintained in GD rats. The vasopressin V₂ receptor antagonist OPC-31260 increased percent water excretion and urinary output and reduced Uosm compared with vehicle-treated GD rats. OPC-31260 also reversed the increased abundance and apical trafficking of inner medullary AQP2 and pAQP2 protein in GD rats. In conclusion, enhanced protein abundance of Na⁺ transporters and Na⁺ channels with Na⁺ retention occurred with GD. OPC-31260 reversed upregulation and apical trafficking of AQP2 and pAQP2 in association with improved urinary diluting capacity and increased water excretion after oral water loading.

arginine vasopressin; aquaporin; impaired urinary dilution

THE PATHOGENESIS OF ALTERED salt and water metabolism in adrenal insufficiency is complex, with proposed mechanisms in mineralocorticoid deficiency that are quite different from those in glucocorticoid deficiency (GD). Thus these hormonal disorders must be selectively and independently investigated. Both conditions are associated with hyponatremia and impaired urinary dilution. Mineralocorticoid deficiency, however, is associated with urinary Na⁺ wasting, diminished extracellular fluid volume, nonosmotic release of arginine vasopressin (AVP) (7), and upregulation of the AVP-responsive aquaporin (AQP)-2 (AQP2) water channel in the renal collecting duct (18). It has been shown that maintenance of Na⁺ balance during mineralocorticoid deficiency abolishes water retention and hyponatremia (18), whereas administration of vasopressin V₂ receptor antagonists markedly improves, but does not totally correct, the associated impairment in water excretion (7). Thus water retention in mineralocorticoid deficiency can be attributed to excess AVP and secondary intrarenal factors.

In contrast, in GD, Na⁺ wasting does not occur, but decreased cardiac output and mean arterial pressure are observed (2, 12). Several studies have suggested that the nonosmotic release of AVP plays a critical role in the water retention associated with GD. Specifically, the messenger RNA for hypothalamic AVP has been shown to be increased in GD (20). There also appears to be a central effect of glucocorticoid hormone to suppress AVP promoter activity (10). Finally, peptide (7) and nonpeptide V₂ receptor antagonists (23) have been shown to dramatically enhance the renal capacity to excrete solute-free water in GD. Thus the nonosmotic release of AVP during GD may involve direct hypothalamic and peripheral baroreceptor pathways.

The effect of GD on renal AQP2 expression is less clear (11, 23). However, in a recent study, Saito et al. (23) demonstrated an AVP-dependent increase in renal medullary AQP2 expression in GD rats. To fully evaluate urinary diluting capacity, it is necessary to also investigate solute transport in the water-impermeable medullary ascending limb of Henle’s loop and the rate of solute excretion.

In the present investigation, selective GD was examined in pair-fed rats during chronic Na⁺ and water balance studies as well as during acute water loading. The protein expression of the major renal AQP and ion transporters and channels was examined in GD rats and compared with that in control rats. To delineate the role of AVP- and non-AVP factors, studies were also performed in GD rats with and without an orally active, nonpeptide vasopressin V₂ receptor antagonist.

MATERIALS AND METHODS

Experimental animals. The study protocol was approved by the University of Colorado Institutional Animal Care and Use Committee. Male Wistar rats (160–180 g body wt) were allowed to acclimate to...
Denver, CO, altitude (1,500 m) for 4 days before the experimental protocols. All animals were acclimatized to metabolic cages for a continuous 2-day period before initiation of study. The animals were housed individually in metabolic cages and exposed to a 12:12-h light-dark cycle and constant ambient temperature.

Protocol 1. Twenty-five rats were divided randomly into two study groups: GD (n = 13) and control (CTL, n = 12). Under anesthesia with ketamine (40 mg/kg body wt ip) and xylazine (5 mg/kg body wt ip), all animals were adrenalectomized through bilateral retroperitoneal incisions. Simultaneously, all rats were implanted with osmotic minipumps (Alzet model 2ML4, Durect, Cupertino, CA) containing aldosterone (Research Plus, Bayonne, NJ) at a dose calculated to deliver 17 μg·kg⁻¹·day⁻¹ into the peritoneal cavity for 12 days (26). CTL rats were also treated with dexamethasone (Research Plus) dissolved in peanut oil at 12 μg·kg⁻¹·day⁻¹ sc for 12 days starting immediately after adrenalectomy. This dose of dexamethasone has been reported to maintain normal weight gain, glomerular filtration rate (GFR), and fasting plasma glucose and insulin levels in adrenalectomized rats (24). After adrenalectomies, all rats were pair fed plain powdered rat chow containing 0.23% sodium (Harlan Teklad Bioproducts, Indianapolis, IN) and received tap water ad libitum. Daily water intake, urine volume, and food intake were monitored.

Protocol 2. The animals underwent the treatment described for protocol 1 and then subjected to water loading. On day 12, the GD and CTL rats were subjected to an acute oral water load for determination of the effect of GD on renal water excretion. The water load was administered 3 h after removal of food and water. The nonpeptide vasopressin V₂ receptor antagonist 5-dimethylamino-1-[4-(2-methylbenzoylamino)-benzoyl]-2,3,4,5-tetrahydro-1H-urazole (OPC-31260, Otsuka Pharmaceutical; 30 mg/kg) was administered 3 h after removal of food and water. The nonpeptide vasopressin V₂ receptor antagonist 5-dimethylamino-1-[4-(2-methylbenzoylamino)-benzoyl]-2,3,4,5-tetrahydro-1H-urazole (OPC-31260, Otsuka Pharmaceutical; 30 mg/kg) was administered to eight GD rats by oral gavage (GD hydrochloride (OPC-31260, Otsuka Pharmaceutical; 30 mg/kg) was methylbenzoylamino)-benzoyl]-2,3,4,5-tetrahydro-1H-Urinary flow rate and minimal urinary osmolality for the 1 h after oral urine excreted in 1 h percent water load excreted was determined as follows: (volume of collected, volume was recorded, and osmolality was assessed. The Each urine sample spontaneously voided over the next 1 h was into the peritoneal cavity for 12 days (26).

At the end of protocols 1 and 2, rats (n = 17 in protocol 1 and 8 in protocol 2) were killed under anesthesia, and blood was collected from the abdominal aorta at the time of death for measurement of serum osmolality and Na⁺ and creatinine concentrations. For each animal, the left kidney was rapidly removed and dissected into cortex and outer stripe of outer medulla (cortex + OSOM), inner stripe of outer medulla (ISOM), and inner medulla (IM) and processed for membrane fractionation and semiquantitative immunoblotting. In protocol 2, trunk blood was collected by decapitation for plasma AVP concentration (5 rats in each group). Decapitation was performed to avoid any influence of anesthesia on plasma AVP concentration (2, 6).

Antibodies. Antibodies to AQP2 (16), AQP2 phosphorylated at the protein kinase A phosphorylation consensus site (Ser²⁵⁶; pAQP2, kindly provided by Dr. Søren Nielsen, University of Aarhus, Aarhus, Denmark) (3), AQP3 (5), Na⁺/H⁺ exchanger isoform 3 (NHE3) (8), Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2, kindly provided by Dr. Mark A Knepper, National Institutes of Health, Bethesda, MD) (9), and α₁-, β₁-, and γ-subunits of the epithelial Na⁺ channel (ENaC, kindly provided by Dr. Bernard C. Rossier, Université de Lausanne, Lausanne, Switzerland) have been previously characterized (13, 15). Anti-Na⁺-K⁺-ATPase α₁-antibody was obtained from Upstate Biotechnology (Lake Placid, NY). Anti-AQP1 antibody was obtained from Chemicon International (Temecula, CA).

Protein isolation. Kidneys were placed in ice-cold isolation solution containing 250 mM sucrose, 25 mM imidazole, and 1 mM EDTA, pH 7.2, with 0.1 vol% protease inhibitors (0.7 μg/ml peptatin, 0.5 μg/ml leupeptin, and 1 μg/ml aprotonin) and 200 μM phenylmethylsulfonyl fluoride. As noted above, kidneys were dissected on ice into cortex + OSOM, ISOM, and IM. Tissue samples were immediately homogenized in a glass homogenizer at 4°C. After homogenization, protein concentration was determined for each sample by the Bradford method (Bio-Rad, Richmond, CA). Tissue protein was utilized for immunoblotting for AQP water channels and Na⁺ transporters and channels.

Western blot analysis. SDS-PAGE was performed on 10% acrylamide gels for NKCC2 and ENaC and on 12% acrylamide gels for AQP, NHE3, and Na⁺-K⁺-ATPase α₁-subunit proteins. After transfer by electroelution to polyvinylidene difluoride membranes (Millipore, Bedford, MA), the blots were blocked with 5% nonfat dry milk in PBS and then probed with the respective antibodies at 4°C overnight.
The membranes were washed with buffer containing PBS with 0.1% Tween 20 (J. T. Baker, Phillipsburg, NJ) and then exposed to secondary antibody for 1 h at room temperature. Subsequent detection of the specific proteins was carried out by enhanced chemiluminescence (Amersham, Arlington Heights, IL) according to the manufacturer's instructions. Densitometric results were reported as integrated values (area/density of band) and expressed as a percentage compared with the mean value in controls (100%). Blots are representative of results obtained from all samples. Densitometry reflects means ± SE of all samples.

**Immunohistochemical studies.** The right kidneys from rats in protocols 1 and 2 (n = 8 each) were fixed by retrograde perfusion via the aorta with 3% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). Kidney blocks containing all kidney zones were dehydrated, embedded in paraffin, and cut into 2-μm-thick slices. Staining was carried out using indirect immunoperoxidase. Briefly, the sections were dewaxed and rehydrated. Endogenous peroxidase was blocked by 0.5% H2O2 in absolute methanol for 10 min at room temperature. The sections were incubated overnight at 4°C with primary antibodies diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. The sections were then washed with PBS and incubated in horseradish peroxidase-conjugated goat anti-rabbit secondary antibodies (Dako) diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. The sections were examined with a Leica DMRE light microscope.

**Biochemical measurements.** Plasma AVP concentration was assessed by RIA as described previously (22, 25). Serum and urinary osmolality were measured by freezing-point depression (Advanced Instruments, Norwood, MA). Serum and urinary creatinine were measured (Beckman Instruments, Fullerton, CA). Creatinine clearance at 24 h was used as an estimate of GFR. Serum Na⁺ concentration was measured by flame photometry.

**Statistical methods.** Statistical analysis of results was performed using the unpaired t-test. Values are means ± SE. P < 0.05 was considered significant.

**RESULTS**

Chronic (12-day) GD was associated with impaired water excretion. GD rats demonstrated a significantly impaired capacity to excrete solute-free water compared with CTL animals. Specifically, GD rats exhibited significantly lower urin-
nary output, higher urinary osmolality, and higher urine-to-plasma osmolality ratio than CTL rats (Fig. 1, A–C). In the first 5 days after adrenalectomy, although daily water intake was comparable between the two groups, urinary output was lower in the GD rats. This water retention no doubt explained why body weight was significantly higher in GD than in CTL rats (Fig. 1D). Plasma osmolality was decreased in GD compared with CTL rats but did not reach statistical significance: 298 ± 2 vs. 304 ± 3 mosmol/kgH₂O (P = 0.08). GFR, as assessed by creatinine clearance, was lower in GD rats (1.4 ± 0.1 vs. 2.0 ± 0.3 ml/day in CTL, P = 0.09), but the difference did not reach significance.

Impaired water excretion in chronic (12-day) GD was associated with upregulation of AQP2, pAQP2, and AQP3 in the IM. The abundance of AQP2 (Fig. 2A) and pAQP2 (Fig. 2B) was significantly increased in the IM of GD rats, as was AQP3 (Fig. 2C). AQP2 in the cortex + OSOM [102 ± 9 vs. 100 ± 4%, P = not significant (NS)] and AQP3 (95 ± 6 vs. 100 ± 14%, P = NS) were not different between CTL and GD rats. AQP1 expression in the cortex + OSOM significantly de-

Fig. 3. Semiquantitative immunoblots of Na⁺-K⁺-2Cl⁻ cotransporter (NKCC2) from kidney proteins prepared from cortex and outer stripe of outer medulla (CO/OSOM, A) and inner stripe of outer medulla (ISOM, B) and immunoperoxidase microscopy of NKCC2 in medullary thick ascending limb cells (C and D). GD rats (n = 7) showed a significant increase in protein abundance of CO/OSOM (A) and ISOM (B) NKCC2 compared with CTL rats (n = 6). Immunohistochemistry revealed abundant NKCC2 labeling (arrows) in apical plasma membrane domains of thick ascending limb cells in OSOM in GD and CTL rats. Labeling densities of NKCC2 were markedly increased in GD (D) compared with CTL (C) rats. Magnification ×400.

Fig. 4. Semiquantitative immunoblots of Na⁺/H⁺ exchanger type 3 (NHE3) and β- and γ-subunits of epithelial Na⁺ channel (β- and γ-ENaC) from kidney proteins prepared from cortex + OSOM. GD rats (n = 7) showed a significant increase in protein abundance of NHE3 (A), β-subunit of ENaC (B), and γ-subunit of ENaC (C) in cortex + OSOM compared with CTL rats (n = 6).

Fig. 5. Treatment of GD rats with the vasopressin V₂ receptor antagonist OPC-31260 (GD + OPC, n = 8) resulted in a marked increase in percent excretion of water load (A) and a significant decrease in minimal urinary osmolality (B) 1 h after a 40 ml/kg water load by oral gavage compared with treatment with vehicle (GD, n = 8).
Table 2. Expression of AQP1 and Na⁺ transporters in GD, GD + OPC, and CTL rats: protocol 2

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Values are means ± SE expressed as percentage of control (CTL); n = 5 in each group. OPC, OPC-31260; IM, inner medulla; pAQP2, phosphorylated AQP2. <sup>a</sup>P < 0.01; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.05 vs. CTL. <sup>d</sup>P < 0.01; <sup>e</sup>P < 0.05 vs. GD + OPC.

Increased in GD compared with CTL rats: 82 ± 2 vs. 100 ± 3% (P < 0.01; Table 1).

Effect of chronic (12-day) GD on renal ion transporters. Medullary NKCC2 is a major factor in generation of hypotonic tubular fluid; thus urinary dilution, i.e., NaCl transport, occurs in the water-impermeable thick ascending limb (TAL). GD was associated with a substantial increase in NKCC2 protein expression in the cortex + OSOM and ISOM (Table 1, Fig. 3, A and B). Immunohistochemistry confirmed increased expression of NKCC2 in TAL cells in the cortex (not shown) and ISOM (Fig. 3, C and D) in GD rats. NHE3 (Fig. 4A) and β- and γ-subunits of ENaC (Fig. 4, B and C) were also increased in the cortex + OSOM of GD compared with CTL rats. In contrast, the abundance of Na⁺-K⁺-ATPase did not change in any zones (Table 1). Consistent with increased expression of several Na⁺ transporters, urinary Na⁺ excretion (U₁Na⁺V) was significantly reduced in GD compared with CTL rats (2.8 ± 0.5 vs. 4.2 ± 0.4 mmol·min⁻¹·kg⁻¹, P < 0.04), despite pair feeding. Moreover, urinary osmolal excretion (U₁osm × V, where V is urinary flow) was significantly reduced in GD compared with CTL rats: 0.43 ± 0.04 vs. 0.55 ± 0.03 mosmol/min (P < 0.02). There was no difference in plasma aldosterone concentration between GD and CTL rats: 21 ± 4 and 27 ± 6 ng/dl, respectively (P = 0.4).

Impaired urinary dilution and water excretion in response to an oral water load in GD: reversal by vasopressin V₂ receptor antagonist. With oral water loading, significantly less water was excreted in 1 h by GD than by CTL rats: 5 ± 1 vs. 33 ± 9% (P < 0.01). This finding was associated with higher minimal urinary osmolality (1,894 ± 292 vs. 316 ± 92 mosmol/kgH₂O, P < 0.001) in GD rats within 1 h of water loading as well as a significantly greater decrease in serum osmolality (281 ± 5 vs. 299 ± 2 mosmol/kgH₂O, P < 0.02) than in CTL rats. However, despite this hyposmolality in GD rats, plasma AVP concentration remained significantly higher in GD than in CTL rats: 1.6 ± 0.2 vs. 0.9 ± 0.2 pg/ml (P < 0.04). Administration of the vasopressin V₂ receptor antagonist OPC-31260 resulted in a profound reversal of the urinary diluting defect in GD rats, with a marked increase in percent water excretion (26 ± 9% in GD + OPC vs. 5 ± 1% in GD, P < 0.03) within 1 h after the oral water load (Fig. 5A). The defect in urinary dilution in GD rats, as assessed by minimal urinary osmolality, was also significantly improved with OPC-31260 treatment: 340 ± 127 and 1,894 ± 292 mosmol/kgH₂O.
in GD + OPC and GD, respectively ($P < 0.001$; Fig. 5B). OPC-31260, however, did not alter $U_{\text{Na}}V: 3.0 \pm 1.1$ and $3.6 \pm 1.3 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ in GD + OPC and GD, respectively ($P > 0.05$). There was a statistically significant difference in serum Na$^+$ levels between GD and GD + OPC rats: $140 \pm 2$ and $135 \pm 1 \text{ mmol/l}$ in GD + OPC and GD, respectively ($P < 0.01$).

**Increased abundance and apical trafficking of AQP2 in GD rats was abolished by V$_2$ antagonist.** Expression of renal AQP$s$ and Na$^+$ transporters 1 h after an acute oral water load in GD + OPC rats was investigated by immunoblot and compared with that in vehicle-treated GD and CTL rats (Table 2). Generally, the patterns of protein changes were similar to those observed in GD rats before water load (Table 2). Specifically, IM AQP2 and pAQP2 expression and cortical NHE3 and outer medullary NKCC2 expression were upregulated in GD rats. AQP3 expression was increased, but did not reach statistical significance. Importantly, 2 h after administration of the V$_2$ receptor antagonist OPC-31260, the previously observed increases in IM AQP2 and pAQP2 protein abundance (Fig. 6, A and B) were abolished in the GD rats. OPC-31260 treatment was also associated with the absence of AQP2 labeling in apical plasma membrane domains in cortical and medullary collecting duct principal cells in GD rats treated with OPC-31260, as shown by light microscopy (Figs. 7 and 8).

**DISCUSSION**

GD in the absence of mineralocorticoid deficiency has been shown to impair the renal capacity to excrete solute-free water (2, 7, 12). Several factors are important in maximal urinary dilution and solute-free water excretion, including 1) the fluid volume delivered to the distal diluting segment of the nephron, 2) NaCl reabsorption in the water-impermeable TAL, as mediated by NKCC2, which can result in fluid osmolality of 50–100 mosmol/kg$\text{H}_2\text{O}$ reaching the cortex from the medulla (1), and 3) water permeability in the cortical and medullary collecting ducts, which is the final modulator of solute-free

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**Fig. 7.** Immunoperoxidase microscopy of AQP2 in inner medullary collecting ducts from CTL, GD, and GD + OPC rats 1 h after an acute oral water load. AQP2 labeling in inner medullary collecting ducts was substantial in cytoplasmic vesicles and less prominent in plasma membrane of CTL rats (A). In GD rats, AQP2 labeling was markedly increased and associated with clear apical plasma membrane domains (arrows, B). In GD + OPC rats, clear apical labeling in plasma membrane domains disappeared and AQP2 labeling densities were comparable with CTL rats (C). Magnification $\times 1,000$.

**Fig. 8.** Immunoperoxidase microscopy of AQP2 in cortical collecting ducts (CCD) from GD and GD + OPC rats 1 h after an acute oral water load. In GD rats, AQP2 labeling was clearly seen in apical plasma membrane domains (arrows) and intracellular domains in CCD (A and C). OPC treatment caused disappearance of AQP2 labeling from apical plasma membrane domains of CCD and strong labeling of AQP2 in basolateral domains in CCD principal cells (B and D) in GD + OPC rats. *, Intercalated cells. Magnification $\times 400$ (A and B) and $\times 1,000$ (C and D).
water reabsorption and, thus, the final determinant of urinary dilution (1, 4). The discovery of water channels in the collecting duct has allowed for molecular insights into the mechanisms whereby AVP regulates the protein expression and trafficking of the AQP2 water channel to the apical membrane of the collecting duct (17). There is also evidence for AVP-independent regulation of AQP2 expression and trafficking (17). The AQP3 water channel on the basolateral surface of the collecting duct also appears to be regulated by AVP (5), and AQP3-knockout mice are known to exhibit nephrogenic diabetes insipidus (14).

In the present study, we investigated the molecular mechanisms that contribute to defective urinary dilution and solute-free water excretion in chronic (12-day) GD in rats. During the first 5 days of GD, daily fluid intake was comparable to that of control animals, yet urinary output was significantly decreased and urinary osmolality and urine-to-plasma osmolality ratios were significantly increased, which led to water retention and a greater body weight in the GD rats. NHE3 ion transporter in the proximal tubule was increased. As one of the determinants of fluid delivery to the distal nephron, this effect would be expected to impede maximal solute-free water excretion. The observed decrease in AQP1 in GD rats would, however, be expected to attenuate this effect.

NKCC2 was not downregulated in GD rats and, in fact, was upregulated. The upregulation of NKCC2 would result in increased ion reabsorption in TAL and would be expected to increase ion reabsorption in TAL and would be expected to increase urinary dilution was excluded as a factor in the water retention. The β- and γ-subunits of ENaC were also significantly increased in the GD rats in association with lower UNaV, despite pair feeding. Thus, as expected, the upregulation of NHE3, NKCC2, and ENaC was associated with a significant decrease in UNaV and solute excretion. Increased solute excretion, which is known to impair urinary dilution (4), therefore, can also be excluded as a factor in the water retention in the GD rats. In contrast, the significant upregulation of the IM AQP2 and AQP3 water channel proteins in GD rats provided an important potential explanation for impaired urinary dilution.

In addition to these 12-day chronic studies, acute water-loading studies were performed. As in the chronic studies, the acute studies demonstrated a profound impairment in water excretion and urinary dilution in GD, which led to a significantly greater fall in plasma osmolality in the GD rats with acute water loading. Despite more severe hyposmolality, the plasma AVP concentrations were significantly higher in the GD rats, thus indicating the nonosmotic release of AVP. Similar to the chronic studies, AQP2 expression was significantly higher in GD than in CTL rats. Phosphorylation of Ser256 of AQP2 via the cAMP-protein kinase A pathway has been shown to mediate trafficking of the water channel to the apical membrane (3). In this regard, pAQP2 was higher during the acute water-loading studies in GD than in CTL rats.

There is in vitro evidence for a direct effect of glucocorticoid hormone on water transport in the collecting duct (21). Moreover, AQP2 in the IM has been shown to be upregulated during water deprivation in Brattleboro rats, which have central diabetes insipidus and undetectable plasma AVP concentration (19). Thus, on this background, it was important to study the response of GD rats to water loading in the presence of a V2 receptor antagonist administered 1 h before the water load. Within 2 h after V2 antagonist administration, the increased AQP2 trafficking to the apical membrane in GD rats was significantly decreased as assessed by immunohistochemistry. This observation also was associated with a reversal of the increase in AQP2 and pAQP2 protein expression in the IM of GD rats. These AQP2 changes in GD rats treated with the V2 receptor antagonist were associated with a profound improvement in minimal urinary dilution 1 h after water loading compared with untreated GD rats (1.894 vs. 340 mosmol/kgH2O, P < 0.001) and the percent excretion of the water load (5 vs. 33%, P < 0.01). In GD rats, the increase in AQP3 during acute water loading was also decreased with V2 receptor antagonist administration, thus supporting a role for nonosmotic AVP regulation of AQP3 (5). Although NKCC2 can be stimulated by AVP (9), the lack of reversal by the V2 receptor antagonist suggested an alternative mechanism in the GD rats.

In summary, the impairment of maximal solute-free water excretion in GD rats was attributable to several factors. Upregulation of the primary proximal tubule ion transporter NHE3 in GD would be expected to limit distal fluid delivery and, thus, impair maximal solute-free water excretion. The initiators of tubular fluid dilution, NKCC2, was upregulated in the GD rats and, therefore, did not contribute to the defect in urine dilution. However, the major defect in urinary dilution in the GD rats appeared to relate to upregulation and phosphorylation of collecting duct AQP2 in the chronic state and in response to acute water loading. In the latter setting, confirmation of the significant role of AVP in defective urinary dilution was obtained when administration of an orally active, nonpeptide V2 receptor antagonist dramatically improved the impaired urinary dilution in the GD rat in association with significant downregulation of AQP2 and pAQP2 and decreased AQP2 trafficking to the apical membrane of the collecting duct.

GRANTS

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REFERENCES


