

Brief Report

DEFECTIVE URINARY CONCENTRATING ABILITY DUE TO A COMPLETE DEFICIENCY OF AQUAPORIN-1

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AQUAPORIN-1, the archetypal water-channel protein,¹ was initially identified in red cells and renal proximal tubular epithelium.² The gene for aquaporin-1 (*AQP1*) on chromosome 7 co-localizes with the Colton blood-group antigen,^{3,4} and the Colton blood-group antigen polymorphism was identified as a substitution of a single amino acid in an extracellular domain of aquaporin-1.⁵ The International Blood Group Reference Laboratory has confirmed the existence of only six kindreds who lack the Colton blood group. Members of three of these kindreds were found to be homozygous for different mutations in the *AQP1* gene, and their red-cell membranes had a complete absence or a marked reduction of aquaporin-1.^{6,7} Surprisingly, aquaporin-1 deficiency had no obvious clinical consequence in these people.

Since aquaporin-1 is abundant in renal proximal tubular epithelium, the thin descending limb of the loop of Henle, and the descending vasa recta of the kidney,^{8,9} we hypothesized that people with a deficiency of aquaporin-1 have defects in water homeostasis in the kidneys that can be identified only under conditions of stress. We studied two unrelated subjects with a deficiency of aquaporin-1 and found that they had impaired urinary concentrating ability, suggesting that aquaporin-1 has a physiologic role in renal function.

CASE REPORTS

Subject 1

Subject 1 was a 37-year-old woman who had no active medical problems but was homozygous for a deletion of exon 1 of the *AQP1* gene.⁶ In 1980 she had a miscarriage during the first trimester of her first pregnancy. During her second pregnancy, routine testing demonstrated the presence of antibodies against the Colton blood group. She gave birth to a healthy baby at 34 weeks of gestation,

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but the infant subsequently required three blood transfusions. In 1987, a third pregnancy was complicated by marked hemolysis in the fetus, which necessitated five intrauterine transfusions. After delivery at 31 weeks of gestation, the baby required two transfusions.

Subject 1 had occasional edema of the lower legs, for which she infrequently took furosemide or indapamide; neither diuretic had been taken within the previous month at the time of our study. She drank three to four liters of fluid per day. The results of a physical examination were normal, except for trace nonpitting edema of the lower legs.

Subject 2

Subject 2 was a 57-year-old woman who was homozygous for a frame-shift mutation in exon 1 of the *AQP1* gene.⁶ Her medical history was unremarkable. She had had four uncomplicated pregnancies. Antibodies against the Colton blood group were detected on routine blood screening. She drank two liters of fluid per day and urinated two to three times daily, without nocturia. The results of a physical examination were normal.

METHODS

Subjects were evaluated in the Johns Hopkins General Clinical Research Center. Both subjects provided written informed consent. Blood chemical and hematologic studies and urinalyses were performed; the urine of Subject 1 was screened for diuretics. During a base-line period of 24 hours, vital signs, weight, serum and urine osmolality, and plasma vasopressin levels were measured at regular intervals, and fluid intake was recorded. The daily urine volume and creatinine clearance were measured in a 24-hour collection, and the glomerular filtration rate was measured with [^{99m}Tc]diethylenetriamine pentaacetic acid (DTPA). Renal and bladder ultrasonography was performed.

Water deprivation was initiated at 9 a.m. on the day after the base-line evaluation. After 21 to 23 hours of water deprivation, subjects received 1 μ g of desmopressin subcutaneously, and urine osmolality was determined hourly for 2 hours. Subject 2 then received intravenous desmopressin (30 mU per kilogram of body weight per hour) and 3 percent sodium chloride (10 ml per minute). Serum and urine sodium levels and osmolality were measured every 30 minutes. Subject 1 received hypertonic saline after 15 hours of water deprivation in a second study but did not receive desmopressin because of a possible allergic rash during the first study. Safety end points, including changes in blood chemical values, development of symptoms, and duration of the study, were predefined; studies were discontinued when established safety end points were met.

After three days of ad libitum intake of fluids, lithium clearance, maximal rate of urine flow, and free-water clearance were measured as described.¹⁰ On the morning after an evening dose of lithium chloride (300 mg in the case of Subject 1 and 600 mg in the case of Subject 2), the subjects drank 20 ml of tap water per kilogram over a period of 30 minutes. The hourly urine output during the four-hour study was replaced with an equal amount of water. Lithium clearance was calculated according to the following equation: lithium clearance = $(U_{Li} \div P_{Li})\dot{V}$, where U_{Li} is the lithium level of the pooled urine sample, P_{Li} the plasma lithium level, and \dot{V} the urinary flow rate; the serum lithium level was calculated according to the following formula: $(P_1 - P_2) \div (2.3 \times \log[P_1 \div P_2])$, where P_1 and P_2 are the initial and final serum lithium levels, respectively. The steady-state values of maximal urinary flow rate and free-water clearance are reported.

Osmolal clearance during infusion of hypertonic saline was calculated according to the following equation: osmolal clearance = $(U_{osm} \div P_{osm})\dot{V}$, where U_{osm} and P_{osm} are the urine and plasma osmolality, respectively. Free-water clearance was calculated according to the following equation: free-water clearance = $\dot{V}(1 - [U_{osm} \div P_{osm}])$; negative values represent free-water reabsorption.

Laboratory studies were performed in Johns Hopkins clinical laboratories. Arginine vasopressin, renin, and aldosterone levels were measured by radioimmunoassay (Quest Diagnostic Laboratories, Baltimore). Immunoblots of red-cell membranes and urine sediment

were performed as described previously¹¹ and probed with antibodies against aquaporin-1¹² or aquaporin-2.¹³

RESULTS

Blood chemical and hematologic values, results of urinalyses, creatinine clearance rates, glomerular filtration rates, and kidney and bladder sizes were normal in both subjects. Ad libitum intake of fluid was 1.9 liters per day in Subject 1 and 1.6 liters per day in Subject 2.

The urine volume was 1.5 liters per 24 hours in Subject 1 and 1.2 liters per 24 hours in Subject 2.

After water deprivation, Subject 1 lost 2.7 kg (3.3 percent of body weight) and Subject 2 lost 2.0 kg (2.7 percent of body weight). Serum osmolality increased from 280 mOsm per kilogram to 287 mOsm per kilogram after water deprivation in Subject 1 and from 288 mOsm per kilogram to 294 mOsm per kilogram in Subject 2 (Fig. 1). Correspondingly, vaso-

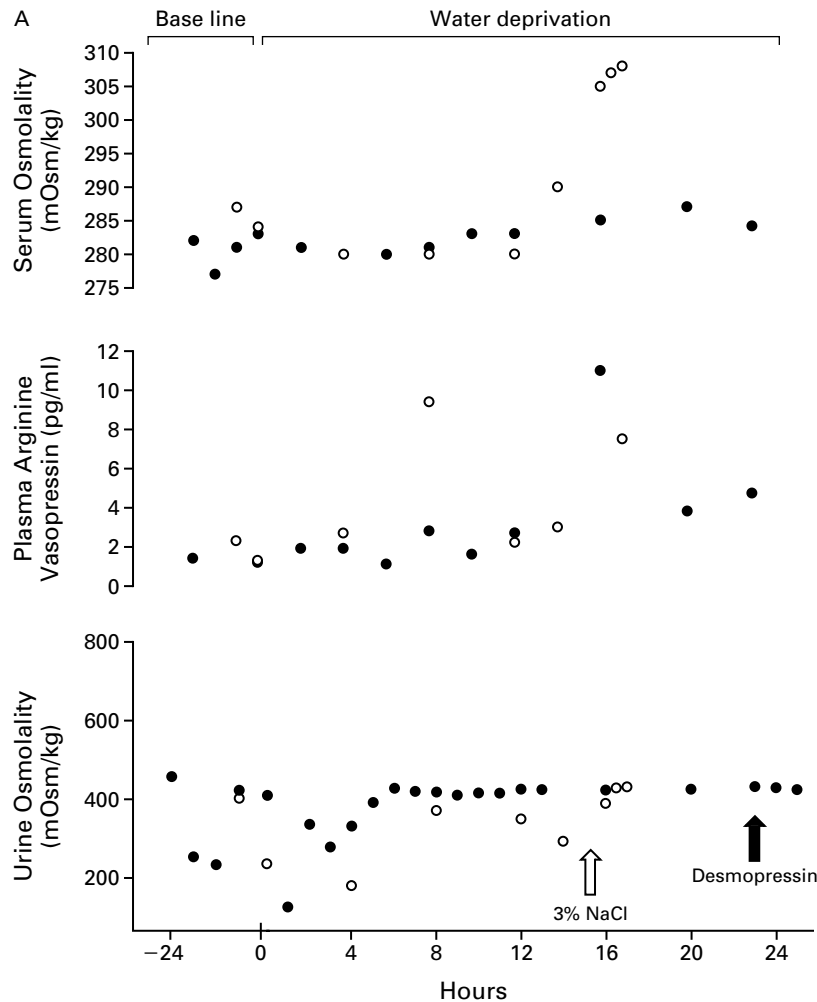


Figure 1. Water Deprivation in Two Subjects with a Complete Deficiency of Aquaporin-1. Subject 1 (Panel A) and Subject 2 (Panel B, facing page) were evaluated over a base-line period of 24 hours during which they had ad libitum access to water and food; samples were taken at 6-hour or 12-hour intervals for measurements of serum osmolality, plasma arginine vasopressin levels, and urine osmolality. After the base-line monitoring period, subjects were deprived of water, and samples were collected. After 23 (Subject 1) and 21 (Subject 2) hours of water deprivation, both subjects were given 1 μ g of desmopressin subcutaneously, and urine osmolality was measured each hour for 2 hours. Subject 2 was then given an intravenous infusion of hypertonic (3 percent) sodium chloride and desmopressin, and serum and urine osmolality were measured at 30-minute intervals for the next hour. Subject 1 received an infusion of hypertonic saline after 15 hours of water deprivation as part of a separate study (open circles and open arrow in Panel A). All plasma vasopressin levels were measured before the administration of desmopressin. The scale on the horizontal axis differs before and after the zero point.

pressin levels increased from 1.5 pg per milliliter to 4.7 pg per milliliter in Subject 1 and from 1.6 pg per milliliter to 5.3 pg per milliliter in Subject 2; these increases were within the range reported for normal persons.¹⁴ Despite these changes, urine osmolality failed to increase normally after water deprivation, reaching only 431 mOsm per kilogram in Subject 1 and 460 mOsm per kilogram in Subject 2. Urine osmolality was not increased in either subject by the administration of desmopressin.

After water loading, urine became maximally dilute (less than 80 mOsm per kilogram) in both subjects (Table 1). Maximal urinary flow rate and the ratio of maximal urinary flow rate to creatinine clearance were within the range reported for normal persons.^{15,16} Both the clearance and fractional excretion of lithium^{10,17,18} were normal in Subject 2 but could not be calculated in Subject 1 because of undetectable serum levels of lithium.

During hypertonic saline loading, free-water reabsorption increased in both subjects as solute excretion (osmolal clearance) increased (Fig. 2). However, as

compared with normal controls,¹⁹ free-water reabsorption was reduced at all values of osmolal clearance in the two subjects.

Immunoblots confirmed the absence of aquaporin-1 from red-cell membranes and urine sediment⁶ and the presence of aquaporin-2 in the urine sediment of both subjects (data not shown).

DISCUSSION

The pathophysiology associated with the aquaporin family of water-channel proteins includes mutations in some patients with nephrogenic diabetes insipidus (*AQP2*)²⁰ and cataracts (*AQP0*)²¹ and abnormal transport of aquaporin-5 in patients with Sjögren's syndrome.^{22,23} We found that aquaporin-1 is essential for maximal urinary concentrating ability.

Although the two subjects with a complete deficiency of aquaporin-1 did not have polyuria, both had an impaired ability to concentrate their urine maximally when deprived of water. Despite normal increases in serum osmolality and plasma vasopressin levels in both subjects, urine osmolality after water deprivation was

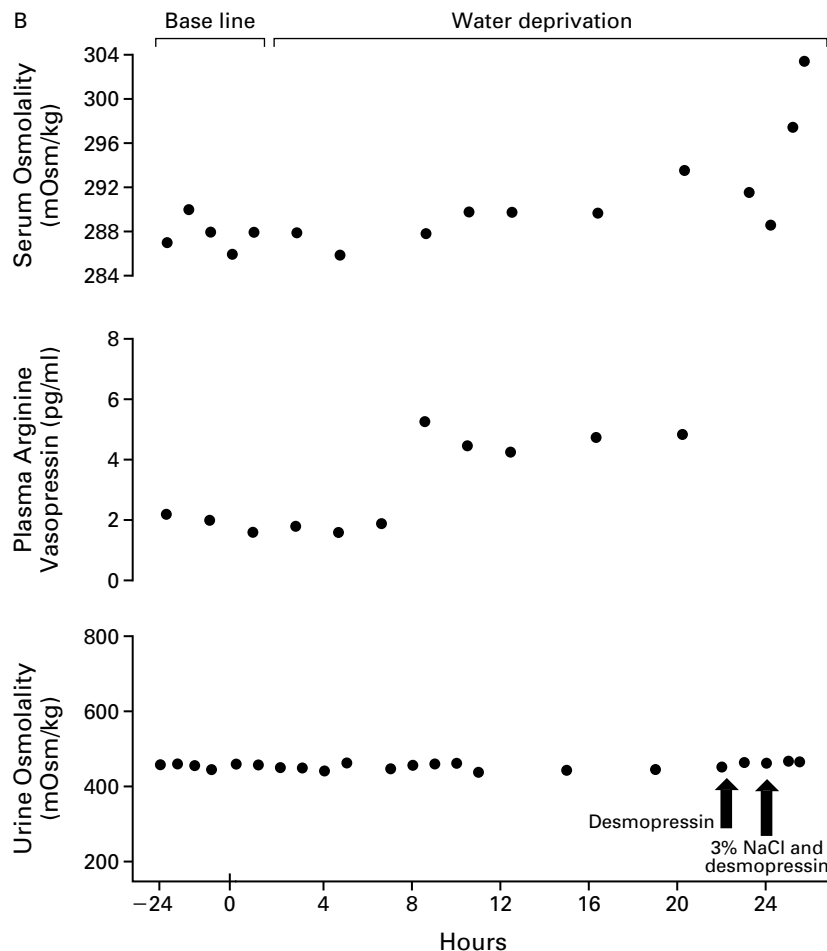


TABLE 1. MAXIMAL FREE-WATER DIURESIS AND LITHIUM CLEARANCE.*

VARIABLE	SUBJECT 1	SUBJECT 2	NORMAL RANGE
\dot{V}_{\max} (ml/min/1.73 m ²)	10.5	10.0	9.7–23.7†
Creatinine clearance (ml/min)	146.3	128.9	
Ratio of \dot{V}_{\max} to creatinine clearance (%)	7.2	7.8	7.5–18.2†
Lithium clearance (ml/min)‡		29	16.0–44.1§
Ratio of lithium clearance to creatinine clearance (%)‡		22	20–30¶
Urine osmolality at steady state (mOsm/kg)	64	39	

* \dot{V}_{\max} denotes the maximal rate of urine flow.

†Values are from Kleeman et al.¹⁵ and Levinsky and Lieberthal.¹⁶

‡Values could not be calculated for Subject 1 because blood levels of lithium were undetectable.

§Values are from Thomsen.¹⁷

¶Values are from Rombola et al.¹⁰ and Thomsen.¹⁸

in the range reported for patients with partial nephrogenic diabetes insipidus. At vasopressin levels of 4 to 5 pg per milliliter, urine osmolality reaches 775 to 1200 mOsm per kilogram in normal persons,²⁴ as compared with a maximal urine osmolality of approximately 460 mOsm per kilogram in our subjects. Unlike patients with central or nephrogenic diabetes insipidus,²⁵ our subjects had aquaporin-2 in their urine sediment. The low daily fluid intake and urine output exclude the possibility that urine concentration was impaired because of the medullary washout that occurs in patients with primary polydipsia.²⁴

Aquaporin-1 is normally abundant in renal proximal tubular epithelium,⁸ but our data suggest that impaired water reabsorption due to the absence of aquaporin-1 does not account for the impaired urinary concentrating ability in our subjects. During water diuresis, the maximal urinary flow rate and the fractional excretion of water, as well as lithium clearance, are indexes of the reabsorption of fluid in the proximal tubule.^{10,15,16,18} These measurements were normal in our subjects, who also had normal glomerular filtration rates. The preservation of glomerular filtration also suggests that reabsorption of fluid in the proximal tubule was not affected; if reabsorption were decreased, increased delivery of chloride to the macula densa would secondarily decrease the glomerular filtration rate through tubuloglomerular feedback. Our findings in subjects with a complete deficiency of aquaporin-1 contrast with observations in mice with a complete deficiency of aquaporin-1, since such mice have polyuria, severe dehydration in response to fluid deprivation, defects in the reabsorption of fluid in the proximal tubule, and a reduced glomerular filtration rate.^{26,27} This suggests

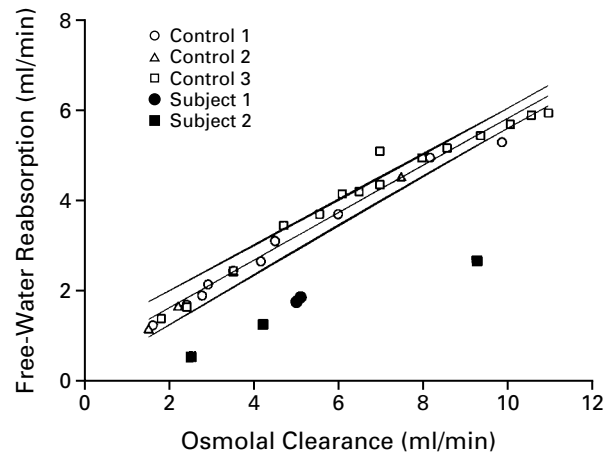


Figure 2. Free-Water Reabsorption Plotted against Osmolal Clearance in the Two Subjects and Three Historical Controls.

Subjects 1 and 2 were given intravenous infusions of hypertonic saline, and free-water clearance and osmolal clearance were calculated from the urine and blood samples collected as described in the Methods section. For comparison, data from three control subjects¹⁸ are shown, as are the regression line (middle line) and 95 percent confidence intervals (upper and lower lines) for the control group (analyzed with Stata software, version 6.0, Stata, College Station, Tex.).

that people with a complete deficiency of aquaporin-1 have unidentified mechanisms of fluid reabsorption in the proximal tubules that compensate for the deficiency of aquaporin-1.

Urinary concentration depends on normal function of the countercurrent multiplier, which requires both active transport of sodium chloride in the thick ascending limb of the loop of Henle and osmotic equilibration of water across the epithelium of the descending limb into the interstitium. The latter process is almost certainly mediated by aquaporin-1. Two measurements of the reabsorption of sodium chloride in the distal nephron that were made during water diuresis, the rate of formation of free water (the ratio of free-water clearance to maximal urinary flow rate, 77 percent in Subject 1 and 86 percent in Subject 2) and the difference between lithium clearance and sodium clearance (28 ml per minute in Subject 2), were normal.^{17,28,29} This suggests that reabsorption of sodium chloride in the distal nephron is intact in our subjects and, therefore, does not contribute to the defect in urinary concentrating ability.

The calculated free-water reabsorption represents the rate of osmotically driven transfer of solute-free water out of the medullary collecting duct. When measured during water deprivation at increased osmolar loads, free-water reabsorption depends on the reabsorption of sodium chloride in the distal nephron, as well as the maintenance of a hypertonic inter-

stitial gradient and an intact response of the collecting duct to vasopressin. In our subjects, free-water reabsorption during hypertonic saline loading increased with increasing solute clearance; this increase was largely parallel to that reported for normal persons but at a lower level (Fig. 2). Although the range of solute clearance was somewhat narrow, the results suggest that the absence of aquaporin-1 in our subjects impairs the small fraction of free-water reabsorption required to produce maximally concentrated urine at the papilla.

In mice with a complete deficiency of aquaporin-1, the absence of aquaporin-1 in the thin descending limb of the loop of Henle impairs water transfer into the interstitium and limits the maximal urinary concentrating ability. In addition, aquaporin-1-mediated osmotic transfer of water from the descending vasa recta into the interstitium reduces blood flow into the papillary tip and prevents washout of solute in that region.³⁰ The absence of aquaporin-1 may consequently increase both medullary blood flow and papillary washout, resulting in decreased maximal urinary concentrating ability.

Our observations show an important physiologic role for aquaporin-1 in renal concentrating ability and provide an example of an impairment in maximal urinary concentration arising from a molecular defect in transport in the thin descending limb of the loop of Henle, the descending vasa recta, or both.

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