Renal water handling in rats with decompensated liver cirrhosis

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Jonassen, Thomas E. N., Sten Christensen, Tae-Hwan Kwon, Susanna Langhoff, Nanna Salling, and Søren Nielsen. Renal water handling in rats with decompensated liver cirrhosis. Am J Physiol Renal Physiol 279: F1101–F1109, 2000.—The present study was performed to investigate the renal handling of water in rats with decompensated liver cirrhosis. Liver cirrhosis was induced by intraperitoneal administration of carbon tetrachloride twice weekly for 16 wk. Control rats were treated with vehicle. The cirrhotic rats developed severe disturbances in water homeostasis: urine production was decreased and hyperosmotic, the rats had significantly decreased plasma sodium concentration and ascites, and the ability to excrete an intravenous water load was significantly impairel. Plasma concentrations of vasopressin and aldosterone were increased. Mean arterial pressure, glomerular filtration rate (GFR), and fractional lithium excretion were decreased. Acute vasopressin type 2-receptor blockade with the selective nonpeptide antagonist OPC-31260 (800 μg/kg h) was performed during conditions whereby volume depletion was prevented by computer-driven, servo-controlled intravenous volume replacement with 150 mM glucose. The aquaretic response to OPC-31260 was similar in cirrhotic and control rats. However, the OPC 31260-induced rises in fractional water excretion (ΔV/GFR; +24%) and fractional distal water excretion (ΔV/CLi; +46%) were significantly increased in the cirrhotic rats, where V is flow rate and Δ is change. This suggests that vasopressin-mediated renal water reabsorption capacity was increased in the cirrhotic rats. Semiquantitative immunoblotting revealed that the expression of the vasopressin-regulated water channel aquaporin-2 was unchanged in membrane fractions of both whole kidney and inner medulla from cirrhotic rats. Together, these results suggest a relative escape from vasopressin on collecting duct water reabsorption in rats with decompensated liver cirrhosis.

aquaporin-2; OPC-31260; collecting ducts; carbon tetrachloride; vasopressin type 2 receptor

A NUMBER OF PATHOPHYSIOLOGICAL conditions are associated with increased plasma levels of vasopressin and extracellular fluid expansion. Experimental studies in rats with congestive heart failure, induced by left coronary artery ligation (31, 40), showed that severe decompensated congestive heart failure with hyponatremia was associated with an increased abundance of the vasopressin-regulated water channel aquaporin-2 (AQP2). However, rats with nephrotic syndrome induced by puromycin aminonucleoside, had a significantly decreased AQP2 abundance despite high levels of plasma vasopressin (1). Studies from rats with liver cirrhosis have shown conflicting results with regard to the renal expression of AQP2. Initial studies showed increased expression of AQP2 mRNA and protein in CCl4-induced cirrhotic rats with ascites (2, 13). However, we have recently found decreased abundance of AQP2 protein in rats with liver cirrhosis induced by common bile duct ligation (16, 18). The rats had sodium retention, slightly increased plasma levels of vasopressin, normal plasma sodium concentrations, normal 24-h urine production, and no sign of ascites. Renal clearance experiments showed that the decreased AQP2 abundance was associated with a significantly decreased aquaretic response to acute, selective vasopressin type 2 (V2)-receptor blockade, which suggests that vasopressin-mediated renal water reabsorption is decreased in the common bile duct ligation model of liver cirrhosis. Recently, Fernandez-Llama et al. (11) confirmed that AQP2 abundance is significantly decreased in rats with liver induced by common bile duct ligation. Compared with our model, these rats had ascites and hyponatremia, which indicate that water retention with the formation of hyponatremia can occur in the absence of increases in AQP2 abundance.

One question to be answered is whether the reported differences in AQP2 expression in experimental cirrhosis are due to the model used or the fact that the rats were investigated at different stages of the disease, i.e., that AQP2 expression may be downregulated in the preascitic state and during early decompensation and then increased in terminal conditions with severe decompensation.

The present study was made in rats with decompensated liver cirrhosis induced by CCl4. Vehicle-treated...
rats were used as controls. The cirrhotic rats had significantly decreased plasma sodium concentration (hyponatremia), ascites, and increased plasma levels of vasopressin and showed an impaired ability to excrete an intravenous (iv) water challenge. \( V_2 \) receptor-mediated water reabsorption in the collecting duct was examined as the aquaretic response to acute, selective \( V_2 \)-receptor blockade in chronically instrumented rats. \( V_2 \)-receptor blockade was achieved in the absence of changes in fluid balance by use of a computer-driven, servo-controlled iv volume replacement system. The renal abundance of the AQP2 protein was determined by semiquantitative immunoblotting in additional groups of rats.

**METHODS**

**Materials**

Barrier-bred and specific pathogen-free female Wistar rats (210–230 g) were obtained from the Department of Experimental Medicine, Panum Institute, University of Copenhagen, Denmark. The animals were housed in a temperature- and moisture-controlled room with a 12:12-h light-dark cycle (lights on from 6:00 AM to 6:00 PM). All animals were given free access to tap water and a pelleted rat diet containing \( \sim 140 \) mmol/kg sodium, 275 mmol/kg potassium, and 23% protein (Altromin catalogue no. 1310, Altromin, Lage, Germany).

**Animal Preparation**

Liver cirrhosis was induced by intraperitoneal (ip) injection of a solution of \( \text{CCl}_4 \) in groundnut oil (1:1), 1 ml/kg body wt twice a week throughout the experimental period. Control rats received ip injections of groundnut oil 0.5 ml/kg body wt. To accelerate the generation of cirrhosis, all rats received phenobarbital in the drinking water (350 mg/ml) throughout.

After 14-wk treatment with \( \text{CCl}_4 \) or vehicle, rats that were subjected to renal function studies were anesthetized with halothane-nitrous oxide, and permanent medical-grade Tygon catheters were implanted into the abdominal aorta and into the inferior caval vein via a femoral artery and vein. A permanent suprapubic bladder catheter was implanted into the urinary bladder and sealed with a silicone-coated stainless steel pin after flushing of the bladder with ampicillin (0.6 mmol/ml, Anhypen, Nycomed Pharma, Oslo, Norway). Catheters were produced, fixed, and sealed as previously described (14–19, 33). All surgical procedures were performed during aseptic conditions. To relieve postoperative pain, rats were treated with buprenorfin, 0.2 mg/kg body wt ip twice daily for 2 days (Anorfin, GEA, Copenhagen, Denmark). After instrumentation, the animals were housed individually.

**Urine Production and Sodium Balance**

In the beginning of study week 16, the rats were placed in metabolic cages (Techniplast, model 1700, Scandbur, Lellinge, Denmark). The rats received demineralized water and granulated standard diet (Altromin catalogue no. 1310, Altromin) that contained 133 mmol Na/kg. Sodium intake was calculated from the amount of diet ingested per 24 h, and sodium loss was estimated from the amount of sodium excreted in the urine within the same 24 h. Twenty-four-hour urine production was measured gravimetrically, and then the metabolic cage was rinsed with 40–50 ml of demineralized water to optimize the recovery of sodium. The sodium content was measured in the combined volume of urine and demineralized water, and 24-h sodium balance was then calculated as sodium intake minus urinary sodium losses. After 2 days of adaptation, 24-h sodium balance was measured for 3 consecutive days, and the average of the three values was used.

**Renal Clearance Experiments**

Renal function was examined by clearance techniques at the end of study week 16. Before the renal clearance experiments, all rats were adapted to the restraining cage used for these experiments by training them for two periods of 2 h each. To examine the rats at the same level of hydration, all experiments were started at 9:00 AM. Renal function was examined by clearance techniques whereby \( ^{14} \text{C} \)-tetraethylammonium bromide clearance was used as a marker for the effective renal plasma flow (32), \(^{3} \text{H} \)inulin clearance as a marker for glomerular filtration rate (GFR), and lithium clearance as a marker for the delivery of fluid from the proximal tubule (38). Renal clearances and fractional excretions were calculated by the standard formula

\[
C = U \cdot V/P; \quad FE = C/GFR
\]

where \( C \) is renal clearance, \( U \) is concentration in urine, \( V \) is urine flow rate, \( FE \) is fractional excretion, and \( P \) is plasma concentration (for further details, see Refs. 14–19). During the clearance experiment, mean arterial pressure (MAP) was measured continuously as described earlier (14–19).

After a 90-min equilibration period, urine was collected during two 30-min control periods. Arterial blood samples of 300 \( \mu \)l each were collected into ammonium-heparinized capillary tubes at the end of the equilibration period and at the end of the control periods. At the beginning of the equilibration period (i.e., at 9:00 AM), a 0.2-ml blood sample was collected for measurement of plasma sodium and potassium concentrations and plasma osmolality, and, for measurement of the plasma concentration of vasopressin, a 1.0-ml blood sample was collected in a prechilled test tube with 20 \( \mu \)l 0.5 M EDTA, pH 7.4, and 10 \( \mu \)l 20 \( \times \) \( 10^4 \) IB/ml aprotinin. After centrifugation at 4°C, plasma was transferred to a prechilled test tube and stored at −20°C for later determination. All blood samples was replaced immediately with heparinized blood from a normal donor rat. After the second control period, the rats where subjected to either protocol 1 or protocol 2.

**Protocol 1: Renal function during iv water loading.** The ability to excrete an iv water challenge was examined in six vehicle- and six \( \text{CCl}_4 \)-treated animals. After the control period, the rats received an iv infusion of 10 ml 50 mM glucose (infusion rate 1 ml/min). Urine collections were made in a 15-min period during the next 3 h. Arterial blood samples of 300 \( \mu \)l each were collected 1, 2, and 3 h after the start of the water loading.

**Protocol 2: Renal function before and during acute \( V_2 \) receptor blockade.** The aquaretic response to acute \( V_2 \)-receptor blockade was examined in eight vehicle- and eight \( \text{CCl}_4 \)-treated animals. Infusion (iv) of the selective \( V_2 \)-receptor antagonist OPC-31260 (prime: 400 \( \mu \)g/kg body wt; 800 \( \mu \)g·kg\(^{-1}\)·h\(^{-1}\); Otsuka America Pharmaceuticals) (42) was started at the end of the control periods. Total body water content was kept constant during \( V_2 \)-receptor blockade by iv replacement of urine losses with 150 mM glucose. Volume replacement was performed as described earlier by use of a computer-driven, servo-control system (3, 16, 18). Urine collections were made in one 60-min period followed by three 30-min periods. A steady-state diuresis was achieved 45–60
min after onset of the OPC-31260 infusion. Arterial blood samples of 300 μl each were collected 1 h after OPC-31260 administration was started and at the end of the experiment. After the clearance experiment (protocols 1 and 2), all catheters were sealed, the bladder was flushed with ampicillin (0.6 mg/ml), and the animals were returned to their home cages. Two days later, an additional 800-μl blood sample was drawn into a prechilled test tube for measurements of the plasma aldosterone concentration. The blood sample was centrifuged immediately at 4°C, and plasma was transferred to a prechilled test tube and stored at −20°C until analysis.

Analytic Procedures

Urine volume was determined gravimetrically. Concentrations of sodium, potassium, and lithium in plasma and urine were analyzed with Student’s unpaired t-test. 3HInulin and 14Ctetraethylammonium bromide in plasma and urine were determined by dual-label liquid scintillation. The plasma concentration of aldosterone was measured by RIA using a commercial kit (Coat-A-Count Aldosterone, DPC, Los Angeles, CA). Vasopressin was extracted from plasma on C18 Sep-Pak cartridges and measured by RIA as described earlier (20).

Membrane Fractionation for Immunoblotting

Two additional series of rats were prepared for semiquantitative immunoblotting. The rats were anesthetized with halothane-nitrous oxide and, in one group of animals, the right kidney was removed and immediately frozen in liquid nitrogen and stored at −80°C before analysis. In the second group of animals, the inner medulla from the right kidney was rapidly dissected and stored at −80°C before analysis. The kidney/inner medulla was homogenized (0.3 M sucrose, 25 mM imidazole-1, mM EDTA, pH 7.2/8.5), and the homogenates were centrifuged at 4,000 g for 15 min. Then, the supernatant was centrifuged at 200,000 g for 1 h to produce a pellet containing both plasma membrane and intracellular vesicle fractions (23, 26). Gel samples were prepared by using Laemmli sample buffer containing 2% SDS.

Electrophoresis and Immunoblotting

Samples of membrane fractions (~2 μg/lane) were run on 12% polyacrylamide minigels (Bio-Rad Mini Protein II). For each gel, an identical gel was run in parallel and subjected to Coomassie staining to assure identical loading (37). The other gel was subjected to immunoblotting. Blots were blocked with 5% milk in PBS-T (80 mM Na2HPO4, 20 mM Na2HPO4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated with affinity-purified anti-AQP2 [40 ng IgG/μl IgG (see Refs. 6, 26–28)]. The labeling was visualized with horseradish peroxidase-conjugated secondary antibody (P448; DAKO; diluted 1:3,000), using an enhanced chemiluminescence system (Amersham). Controls were prepared with replacement of the primary antibody with an antibody preabsorbed with immunizing peptide IgG, or with nonimmune IgG.

Quantitation of AQP2 Expression

Enhanced chemiluminescence films with bands within the linear range were scanned (24) by using an AGFA scanner (ARCUS II). For AQP2, both the 29- and the 55- to 50-kDa band, corresponding to the nonglycosylated and the glycosylated species (36), were scanned as described earlier (12, 23, 26, 28, 37). The labeling density in cirrhotic rats was quantitated (23, 26) from blots run on a gel, along with control material taken from vehicle-treated control animals. The labeling density was corrected by densitometry of identical Coomassie-stained gels run in parallel. AQP2 labeling in samples from cirrhotic rats was expressed relative to the mean expression in the corresponding control material run on the same gel.

Statistics

Data are presented as means ± SE. To evaluate the effects of V2 receptor blockade, the average value during the two 30-min control periods was compared with the average value during the last two 30-min periods during OPC-31260-induced diuresis. Within-group comparisons were analyzed with Student’s paired t-test. Between-group comparisons were analyzed with Student’s unpaired t-test. Differences were considered significant at the 0.05 level.

RESULTS

Body Weight Gain, Urine Production, and Sodium Balance

The average daily weight gain during the first 14 wk was significantly higher in control than in the cirrhotic rats (control: 0.63 ± 0.04 vs. cirrhosis: 0.48 ± 0.06 g/day, P < 0.05) (Fig. 1). Thereafter, the control rats showed no further weight gain from week 14 until the termination of the study at the end of week 16 (0.07 ± 0.21 g/day), whereas cirrhotic rats showed a marked increase in daily weight gain (1.27 ± 0.30 g/day). The metabolic cage study in experimental week 16 showed that the increased weight gain in the cirrhotic rats was associated with sodium retention relative to the control animals (0.81 ± 0.10 vs. 0.38 ± 0.01 mmol Na/day, P < 0.01), increased urinary concentration, as reflected by a decreased urine output (4.73 ± 2.28 vs. 9.28 ± 1.33 ml·day−1·100 g body wt−1; P < 0.01) and production of highly concentrated urine (1,564 ± 101 vs. 975 ± 57 mosmol/kgH2O; P < 0.01). Exposure of the abdomen at the termination of the study showed that all the cirrhotic rats had ascites, as evidenced by visible pools of fluid in the lateral abdominal gutters.

Plasma Biochemistry

Plasma levels of vasopressin (6.4 ± 1.4 vs. 1.7 ± 0.3 pg/ml, P < 0.01) and aldosterone (6.3 ± 0.6 vs. 2.2 ± 0.3 nM, P < 0.01) were significantly increased, and plasma sodium (140.5 ± 0.8 vs. 145.6 ± 1.2 mM, P < 0.01) significantly decreased in the cirrhotic rats (Fig. 2).

Baseline Values for Systemic and Renal Hemodynamics and Tubular Function

Table 1 shows systemic and renal hemodynamics and tubular function during control conditions in the renal clearance experiments. The cirrhotic rats had decreased MAP, and renal function was significantly attenuated: effective renal plasma flow (ERPF), GFR, and the fractional lithium excretion were all significantly decreased, and so were V and tubular sodium and potassium handling.
Renal Function During IV Water Loading

Water loading (10 ml water bolus, infusion rate: 1 ml/min iv) did not affect MAP, ERPF, GFR, and the renal handling of lithium and sodium in both groups (data not shown). However, the cirrhotic rats showed marked changes in renal water handling: the time profile for the cumulated excretion of the water load was significantly delayed (Table 2), and the maximal increases in diuresis and free water clearance were significantly attenuated (maximal \( \Delta V \): 100.5 \pm 12.9 vs. 73.9 \pm 5.0 ml/day\(^{-1}\) \cdot 100 g body wt\(^{-1} \), \( P < 0.05 \); maximal \( \Delta C_{H_2O} \): 89.7 \pm 8.0 vs. 71.9 \pm 5.3 ml/day\(^{-1}\) \cdot 100 g body wt\(^{-1} \), \( P < 0.05 \) ) (Fig. 3), where \( \Delta \) is change. The decreased and delayed water excretion in the cirrhotic rats was associated with a significantly delayed increase in the fractional distal water excretion (V/CLi).

Effect of V2-Receptor Blockade on Renal Water Handling

Systemic and renal hemodynamics, GFR, and the renal handling of sodium, potassium, and lithium

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Fig. 1. Daily urine production (A), urine osmolality (B), and sodium balance (C) in cirrhotic and control rats. Cirrhosis was induced by 16-wk intraperitoneal (ip) treatment with CCl4. Control rats were treated with vehicle (groundnut oil). Daily sodium balance was calculated as sodium intake minus urinary sodium excretion. Values are means \( \pm \) SE; \( n = 27 \) and 32, respectively. b.w., Body wt. *\( P < 0.01 \) vs. control.

Fig. 2. Plasma concentrations of sodium (A), aldosterone (B), and vasopressin (C) in cirrhotic and control rats. Cirrhosis was induced by 16-wk ip treatment with CCl4. Control rats were treated with vehicle (groundnut oil). Values are means \( \pm \) SE; \( n = 16 \) in both groups. *\( P < 0.01 \) vs. control.
were, as previously shown (15, 18), unchanged during acute iv treatment with the V2-receptor antagonist OPC-31260 (data not shown). V2-receptor blockade induced marked changes in the renal water handling with significant increases in V, CH2O, V/GFR, and V/C Li in both groups (Fig. 4). The increases in V and CH2O were not different in the cirrhotic and control rats. However, when expressed as a fraction of GFR or distal delivery, the aquaretic effect of OPC-31260 was significantly enhanced in the cirrhotic rats: ΔV/GFR: +124% (7.99 ± 0.56 vs. 6.46 ± 0.64%; P < 0.05), ΔV/C Li: +41% (35.37 ± 2.66 vs. 25.04 ± 2.97%; P < 0.01). Thus the results indicate a marked increase in vasopressin-mediated distal water reabsorption in the cirrhotic rats.

Renal Expression of AQP2 Protein

Figures 5 and 6 show immunoblots of membrane fractions (2 μg/lane) from whole kidney preparations (Fig. 5) and preparations of inner medulla (Fig. 6). As previously shown, the affinity-purified anti-AQP2 protein antibody recognizes the 29- and the 35- to 50-kDa band, corresponding to nonglycosylated and glycosylated AQP2 protein, respectively. As shown in Figs. 5A (whole kidney) and 6A (inner medulla), similar labelings of both the 29- and the 35- to 50-kDa AQP2 bands were observed in the control and the cirrhotic rats. This was confirmed by densitometry of all samples (Figs. 5B and 6B) that showed no difference in the expression of AQP2 protein between cirrhotic and control rats. Thus, despite significantly increased plasma levels of vasopressin and marked water retention, AQP2 expression remained normal in the cirrhotic rats.

Table 1. Baseline values of systemic and renal hemodynamics, GFR, and renal tubular function in rats with liver cirrhosis induced by CCl4 treatment and in vehicle-treated control rats

<table>
<thead>
<tr>
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<th>Control (n = 14)</th>
<th>Cirrhosis (n = 14)</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>109 ± 2</td>
<td>100 ± 2*</td>
</tr>
<tr>
<td>ERPF, μl·min−1·100 g bw−1</td>
<td>3.467 ± 203</td>
<td>2.923 ± 220*</td>
</tr>
<tr>
<td>GFR, μl·min−1·100 g bw−1</td>
<td>9.07 ± 31</td>
<td>689 ± 73*</td>
</tr>
<tr>
<td>V, μl·min−1·100 g bw−1</td>
<td>15.08 ± 1.14</td>
<td>9.84 ± 2.09*</td>
</tr>
<tr>
<td>UNaV, μmol·min−1·100 g bw−1</td>
<td>0.48 ± 0.07</td>
<td>0.19 ± 0.06*</td>
</tr>
<tr>
<td>FE Na%</td>
<td>0.42 ± 0.03</td>
<td>0.16 ± 0.08*</td>
</tr>
<tr>
<td>FE K%</td>
<td>19.42 ± 1.55</td>
<td>10.52 ± 1.91*</td>
</tr>
<tr>
<td>FE Li%</td>
<td>27.07 ± 2.04</td>
<td>17.83 ± 2.31*</td>
</tr>
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Values are means ± SE. MAP, mean arterial pressure; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; V, urine flow rate; UNaV, sodium excretion rate; FE Na, fractional sodium excretion; FE K, fractional potassium excretion; FE Li, fractional lithium excretion. Experiments were performed after 16-wk treatment with CCl4. *P < 0.05 vs. control.

Table 2. Water excretion in percentage of infused amount in vehicle-treated control rats and rats with liver cirrhosis induced by CCl4 treatment

<table>
<thead>
<tr>
<th></th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
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<tbody>
<tr>
<td>Control (n = 6)</td>
<td>23.8 ± 5.1</td>
<td>42.7 ± 5.0</td>
<td>79.6 ± 4.8</td>
<td>100.1 ± 6.1</td>
<td>103.2 ± 6.3</td>
</tr>
<tr>
<td>Cirrhosis (n = 6)</td>
<td>6.1 ± 2.7*</td>
<td>14.5 ± 4.6*</td>
<td>59.1 ± 5.5*</td>
<td>86.9 ± 5.9*</td>
<td>98.9 ± 10.6</td>
</tr>
</tbody>
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Values are means ± SE. The rats were chronically instrumented and received an intravenous water challenge (10 ml, 50 mM glucose, infusion rate: 1 ml/min). Experiments were performed after 16-wk treatment with CCl4. *P < 0.05 vs. control.
DISCUSSION

This study was designed to elucidate renal mechanisms in the disturbed water metabolism in rats with decompensated liver cirrhosis and hyponatremia induced by chronic administration of CCl4. The animal model showed marked alterations in water homeostasis: urine production was decreased, urine osmolality increased, and the rats had decreased plasma sodium concentration and ascites. Plasma vasopressin concentrations were increased, and the rats showed an impaired ability to excrete an iv water load. The frac-

Fig. 4. Effect of acute V2-receptor blockade with OPC-31260 (800 μg·kg⁻¹·h⁻¹ iv) on urine flow rate (ΔV; top left), free water clearance (ΔCwco; top right), fractional water excretion (ΔV/GFR; bottom left), and fractional distal water excretion (ΔV/Cl; bottom right) in conscious, chronically instrumented, cirrhotic, and control rats, where Δ is change. Cirrhosis was induced by 16-wk ip treatment with CCl4. Control rats were treated with vehicle (groundnut oil). Volume depletion during V2-receptor blockade was prevented by computer-driven, servo-controlled iv volume replacement with 150 mM glucose. Values are means ± SE; n = 8/group. *P < 0.01 vs. control.

Fig. 5. Immunoblots of membrane fractions (2 μg/lane) from whole kidney preparations from cirrhotic and control rats. Cirrhosis was induced by 16-wk ip treatment with CCl4. Control rats were treated with vehicle (groundnut oil). A: immunoblot was reacted with affinity-purified anti-aquaporin-2 (AQP2) and revealed 29- and 35- to 50-kDa AQP2 bands. B: densitometry performed on all rats. Values are means ± SE.

Fig. 6. Immunoblots of membrane fractions (2 μg/lane) from inner medulla preparations from cirrhotic and control rats. Cirrhosis was induced by 16-wk treatment with CCl4. Control rats were treated with vehicle (groundnut oil). A: immunoblot was reacted with affinity-purified anti-AQP2 and reveals 29- and 35- to 50-kDa AQP2 bands. B: densitometry performed on all rats. Values are means ± SE.
tional aquaretic response to acute selective vasopressin-$V_2$-receptor blockade was increased, but the renal expression of AQP2 was unchanged. Together, these results show that AQP2 expression, despite compensation and increased plasma vasopressin levels, is unchanged in rats with CCl$_4$-induced liver cirrhosis.

Regulation of AQP2 Expression

Vasopressin regulates water permeability in the renal collecting duct by short-term and long-term regulation. Collecting duct water permeability increases within a few minutes in response to an acute increase in plasma vasopressin concentration, and this is mediated by shuttling of AQP2 from intracellular vesicles into the apical plasma membrane via exocytosis (27, 28, 34, 41). For long-term regulation of body water, the total amount of AQP2 protein in the principal cells is increased (28), along with increased AQP2 mRNA levels (22) due, at least in part, to increased AQP2 gene transcription (30). Conversely, in the absence of vasopressin, e.g., in vasopressin-deficient homozygous Brattleboro rats, AQP2 expression is suppressed (6). From several studies it has become clear that both vasopressin-dependent and vasopressin-independent mechanisms operate to modulate AQP2 expression levels (for recent review, see Ref. 29). Long-term treatment of vasopressin-deficient Brattleboro rats with vasopressin resulted in 1) a marked increase in AQP2 expression levels, 2) increased osmotic water permeability of inner medullary collecting ducts, and 3) complete restoration of the urinary concentration defect (6). However, vasopressin-independent regulation of AQP2 also seems to be present in rats with lithium-induced nephrogenic diabetes insipidus; thirsting (or water deprivation) produces a much greater increase in AQP2 expression than 7 days of 1-desamino-8-D-arginine vasopressin (dDAVP) treatment (24). Water loading of rats with clamped high levels of plasma dDAVP causes an escape from the renal effects of dDAVP associated with a significant reduction in renal AQP2 levels (8, 9). Thirsting of rats in the presence of chronic $V_2$-receptor blockade (OPC-31260) increases AQP2 expression levels (24). Together, these studies have given support to the view that vasopressin-independent mechanisms may play a significant role in modulating AQP2 expression levels, and several studies suggest that this pathway may be involved in various water balance disorders.

AQP2 Expression in Conditions with Disturbances in Renal Water Handling

Dysregulation of AQP2 expression has been shown to be associated with several diseases or conditions with disturbances in renal water handling. Experimental studies in rats have shown that conditions with increased solute-free urine production, such as acquired nephrogenic diabetes insipidus (23), hypokalemia (25), hypercalcemia (7, 35), ureteral obstruction (12), and chronic aldosterone-receptor blockade (18), all display a significant downregulation of renal AQP2 expression. Furthermore, it has been shown that mutant, nonfunctional AQP2 was the cause of very severe non-X-linked inherited nephrogenic diabetes insipidus in humans (5). Together, this points to AQP2 dysfunction/regulation as a major common component in conditions with defects in renal concentrating ability.

The role of AQP2 in conditions with extracellular volume expansion is more unclear. Rats with severe congestive heart failure associated with hyponatremia and increased plasma vasopressin levels (31, 40) have a significantly increased AQP2 expression. However, rats with nephrotic syndrome induced by pyromycin aminonucleotide (1) or adriamycin (10) have a significant downregulation of renal AQP2 expression. The mechanisms behind this downregulation is unknown.

AQP2 Expression in Liver Cirrhosis

Initial studies made in rats with severe CCl$_4$-induced liver cirrhosis showed a significant increase in AQP2 expression (2, 13). However, we recently found a significant downregulation of AQP2 expression in rats with compensated liver cirrhosis induced by common bile duct ligation (16, 18). These rats had sodium retention, slightly increased plasma levels of vasopressin, and normal GFR, but no sign of ascites. Accordingly, Fernandez-Llama and coworkers (11) recently reported a significant downregulation of AQP2 in rats with liver cirrhosis induced by common bile duct ligation where decompensation was induced by giving the rats free access to sweetened water. The present study showed a normal expression of AQP2 in rats with decompensated liver cirrhosis induced by CCl$_4$ administration. The discrepancy between the present and the previously reported findings in the CCl$_4$ model of decompensated liver cirrhosis is unclear. One possible explanation could be that the initial studies (2, 13) showing increased AQP2 expression were made in rats with very severe water disturbances in the terminal state of the disease. Unfortunately, no information about renal function in terms of renal perfusion, GFR, or segmental tubular function were reported in these studies.

Our present observation, that rats with decompensated cirrhosis developed water retention with the formation of ascites and decreased plasma sodium concentration (hyponatremia) in the absence of increases in the AQP2 expression, raises the question of how water retention occurs in this model of liver cirrhosis. We recently demonstrated that rats with compensated liver cirrhosis (induced by common bile duct ligation) and significantly decreased AQP2 expression have increased furosemide-sensitive sodium reabsorption and tubular hypertrophy in the thick ascending limb of Henle’s (TAL) (14–16). These functional and structural changes were associated with sodium retention and an increased interstitial sodium concentration in the renal medulla (16). In addition, we have shown that rats with CCl$_4$-induced cirrhosis with ascites and hyponatremia have the same functional changes in the TAL as the common bile duct-ligated rats (19). Furthermore,
the present study demonstrates that the fractional water excretion in response to acute V₂-receptor blockade is significantly increased in the CCl₄ rats, suggesting that the vasopressin-sensitive water reabsorption capacity is increased in these water-retaining rats. Therefore, we propose that the increased sodium reabsorption in the TAL induces an increased cortical-peritubular interstitial osmotic gradient and thus increases the driving force for collecting duct water reabsorption. The lack of increased AQP2 expression despite increased plasma vasopressin levels could then be a compensatory mechanism aimed at limiting excessive collecting duct water reabsorption and thereby preventing avid water intoxication. The mechanism behind such a compensatory “escape” from vasopressin stimulation in the collecting ducts is, however, unknown.

Summary

In summary, the present study demonstrates that rats with CCl₄-induced liver cirrhosis and increased plasma vasopressin levels compensate with the formation of decreased plasma sodium concentration and ascites in the absence of changes in the renal expression of AQP2. However, functional studies with acute selective vasopressinV₂-receptor blockade in chronic catheterized rats indicate that the vasopressin-sensitive water reabsorption capacity is increased in the cirrhotic rats. Together, these results indicate that vasopressin-mediated water reabsorption in the collecting ducts is increased in rats with CCl₄-induced cirrhosis despite an unchanged expression of AQP2.

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REFERENCES


