Decreased vasopressin-mediated renal water reabsorption in rats with compensated liver cirrhosis

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Jonassen, Thomas E. N., Søren Nielsen, Sten Christensen, and Jørgen Søberg Petersen. Decreased vasopressin-mediated renal water reabsorption in rats with compensated liver cirrhosis. Am. J. Physiol. 275 (Renal Physiol. 44): F216–F225, 1998.—Experiments were performed to investigate vasopressin type 2 receptor (VP2)-mediated renal water reabsorption and the renal expression of the vasopressin-regulated water channel aquaporin-2 (AQP-2) in cirrhotic rats with sodium retention but without ascites. In addition, the expression of the furosemide-sensitive type 1 Na-K-2Cl cotransporter (BSC-1) and the natriuretic response to an intravenous test dose furosemide (7.5 mg/kg) during acute VP2-receptor blockade was measured. Acute VP2-receptor blockade with the selective nonpeptide antagonist OPC-31260 (800 µg·kg−1·h−1) was performed during conditions in which volume depletion was prevented by computer-driven, servo-controlled intravenous volume replacement with 150 mM glucose. OPC-31260 produced a significantly smaller increase in urine flow rate (−26%) and free water clearance (−18%) in cirrhotic rats than in control rats. The natriuretic response to an intravenous test dose furosemide (7.5 mg/kg) was significantly increased in cirrhotic rats (−52%), but pretreatment with OPC-31260 did not affect the natriuretic response to furosemide in neither cirrhotic nor in control rats. Semiquantitative immunoblotting showed a significant downregulation of AQP-2 in the renal cortex (−72%) and in the outer medulla (−44%). The relative expression of BSC-1 in the outer medulla was unchanged in cirrhotic rats. The corticopapillary gradient of Na was significantly increased in cirrhotic rats. Since daily urine flow rate was similar in cirrhotic and sham-operated rats, we suggest that non-vasopressin-mediated water reabsorption is increased in cirrhotic rats probably as a result of an increased corticomedullary gradient due to exaggerated NaCl reabsorption in the thick ascending limb of Henle’s loop.

Vasopressin plays a central role in the kidneys concentration ability by stimulation of water reabsorption in the collecting duct. In the collecting duct principal cell, vasopressin binds to vasopressin type 2 receptors (VP2 receptors) in the basolateral plasma membrane and increases intracellular cAMP concentration, which in turn increases the water permeability (26). Several studies have demonstrated that the vasopressin-sensitive aquaporin-2 (AQP-2) water channel plays a central role in the regulation of collecting duct water permeability. AQP-2 is localized in the luminal plasma membrane and in cytoplasmic vesicles (26). Acute increases in the plasma vasopressin concentration are associated with insertion of AQP-2 from cytoplasmic vesicles into the luminal plasma membrane (24, 25, 32, 44), whereas prolonged increases in plasma vasopressin levels are associated with marked increases in AQP-2 expression (26).

In addition, in vitro studies on isolated segments of medullary TAL from rats and mice have shown that vasopressin increases adenylate cyclase activity and stimulates transepithelial sodium transport in this nephron segment (34, 40). Like the effect of vasopressin on the collecting duct, the effect of vasopressin on the medullary TAL is mediated by VP2 receptors (3). These findings suggest that vasopressin not only increases the renal concentration ability by stimulation of collecting duct water permeability, but also by increasing the corticomedullary osmotic gradient through VP2-Receptor-mediated stimulation of NaCl reabsorption in the TAL.

In the present study, VP2-receptor-mediated water reabsorption in the collecting ducts was examined in

LIVER CIRRHOSIS is a chronic disease with marked progressive changes in systemic and renal hemodynamics. Initially, patients with cirrhotic liver disease have peripheral vasodilation and increased cardiac output but without clinical signs of fluid retention (the compensated state). During the late decompensated state, liver cirrhosis is associated with sodium retention, edema, and ascites. The renal mechanisms that initiate sodium retention during the early compensated stage of liver cirrhosis are still unknown. Experimental studies suggest that the sodium retention that initiates edema and ascites formation in cirrhosis begins 1–2 wk before ascites become detectable (13, 19). The early sodium retention seems to be mediated by an increased tubular NaCl reabsorption, since the glomerular filtration rate (GFR) is unaltered at this stage of the disease (18, 41, 42). In rats with secondary biliary cirrhosis induced by common bile duct ligation (CBL), we recently reported that rats with sodium retention but without ascites, i.e., compensated liver cirrhosis, had an exaggerated natriuretic response to furosemide and an increased volume of the thick ascending limb of Henle’s loop (TAL) epithelium in the inner stripe of the outer medulla (14). These functional and structural changes suggest that increased NaCl reabsorption in the TAL may be involved in the early sodium retention observed during liver cirrhosis. Furthermore, plasma vasopressin levels were unchanged in CBL rats, but since both the functional and the structural changes in the TAL were absent in CBL rats with hereditary vasopressin deficiency, these findings suggested that vasopressin plays a permissive role for the adaptive changes in the TAL in cirrhotic rats.
chronically instrumented rats with compensated liver cirrhosis. Liver cirrhosis was induced by CBL, and sham-operated rats (sham-CBL) were used as controls. V2-receptor blockade was induced by intravenous treatment with the selective V2-receptor antagonist, OPC-31260. V2-receptor blockade was achieved in absence of changes in fluid balance, by use of a computer-driven, servo-controlled intravenous volume replacement system, which replaced urinary losses momentarily by intravenous infusion of 150 mM glucose. In an additional group of animals with CBL or sham-CBL, the expression of the vasopressin-sensitive water channel AQP-2 was determined in cortex, outer, and inner medulla by semiquantitative immunoblotting.

The role of V2-receptor-mediated NaCl reabsorption in the TAL was examined by studying the natriuretic response to a test dose of furosemide during acute V2-receptor blockade with OPC-31260. Furthermore, to examine the expression of the furosemide-sensitive Na-K-2Cl cotransporter (BSC-1) in cirrhotic rats, semiquantitative immunoblotting was performed on renal outer medulla from CBL and sham-CBL rats. Finally, to examine whether the increased furosemide-sensitive NaCl reabsorption in the medullary TAL in cirrhotic rats was associated with an increased corticopapillary gradient, interstitial solute concentrations were measured in the cortex, in the inner stripe of the outer medulla, and in the papilla in CBL and sham-CBL rats.

METHODS

Materials. Barrier-bred and specific pathogen-free female Wistar rats (220–240 g) were obtained from the Department of Experimental Medicine, the Panum Institute, University of Copenhagen, Denmark. The animals were housed in a temperature-controlled (22–24°C) and moisture-controlled (40–70%) room with a 12-h light-dark cycle (light on from 6:00 A.M. to 6:00 P.M.). All animals were given free access to tap water and pelleted rat diet containing 20% fat, 45% carbohydrate, 35% protein (catalog no. 1314; Altromin International, Lage, Germany). Three days before the clearance experiments, the rats diet was changed to a similar diet to which lithium citrate (12 mmol Li/kg dry diet) was added. When Li is given by this mode of administration and in this dose, it does not influence renal function (20).

Animal preparation. During halothane-nitrous oxide anesthesia, CBL or sham-CBL was performed as previously described by Kountouras et al. (16). Three weeks later, rats used for renal clearance experiments 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, which was sealed with a silicone-coated stainless steel pin after flushing the bladder with ampicillin, 0.6 mg/ml (Anhyphen; Nycomed Pharma, Oslo, Norway). Catheters were produced, fixed, and sealed as described previously (31). After instrumentation, the animals were housed individually. All surgical procedures were performed during aseptic conditions. To relieve postoperative pain, rats were treated postoperatively with buprenorphin, 0.2 mg/kg body wt ip (Anorfin; GE A, Copenhagen, Denmark), and to accelerate postoperative recovery, animals were given access to 1.5% sodium chloride in addition to tap water until they reached preoperative weight (3–4 days later).

Renal clearance study. Renal function was examined by clearance techniques 5 wk after CBL or sham-CBL. On the day of the experiment, the animal was transferred to a restraining cage, and intravenous infusion (150 mM glucose, 13 mM sodium chloride, 3 mM lithium chloride; 2.0 ml/h) with [1H]inulin (Amersham, Buckinghamshire, UK; batch no. 145 457, specific activity 0.10 GBq/mmol, infusion rate 1.5 µCi/h) was started. After a 90-min equilibration period, urinary was collected during two 30-min control periods. Next, intravenous infusion of the selective V2-receptor antagonist OPC-31260 (prime, 400 µg/kg body wt; 800 µg·kg⁻¹·h⁻¹; Otsuka America Pharmaceuticals) (43) or vehicle (150 mM glucose) was started. This dose of OPC-31260 was chosen based on dose-response experiments which demonstrated that 800 µg·kg⁻¹·h⁻¹ produced a diuretic response that was ~90% of the maximal response to OPC-31260, and since higher doses caused sedation, this dose was used. Extracellular fluid volume was kept constant during V2-receptor blockade by intravenous replacement of urine losses with 150 mM glucose. Volume replacement was performed by a computer-driven servo-control system in which urine output was monitored continuously by collecting urine in test tubes placed on an electronic balance (type MC 1; Sartorius, Gottingen, Germany) that was connected to an IBM-compatible computer, which in turn controlled the infusion rate of an infusion pump (Perfusor Secura; B. Braun, Melsungen, Germany) that was connected to a 12-h light-dark cycle (light on from 6:00 A.M. to 6:00 P.M.). All animals were given free access to tap water and pelleted rat diet containing 20% fat, 45% carbohydrate, 35% protein (catalog no. 1314; Altromin International, Lage, Germany). Three days before the clearance experiments, the rats diet was changed to a similar diet to which lithium citrate (12 mmol Li/kg dry diet) was added. When Li is given by this mode of administration and in this dose, it does not influence renal function (20).

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During the clearance experiment, mean arterial pressure (MAP) and heart rate (HR) were measured continuously using Baxter Uniflow pressure transducers (Bentley Laboratories, Ulm, Germany) connected to pressure and heart rate couplers (Hugo Sachs, Hugstetten, Germany). Signals were displayed on a model WR 3101 Linearcorder Mark VII (Watanabe Instruments, Tokyo, Japan) and sampled on-line using a data acquisition program written in LabView (National Instruments, Austin, TX) and developed in collaboration with Bie Data, Copenhagen, Denmark. After the clearance experiment, all catheters were sealed, the bladder was flushed with ampicillin (0.6 mg/ml), and the animals were returned to their home cages. To replace furosemide-induced sodium losses, rats were given free access to 1.5% sodium chloride in addition to tap water for 24 h after the renal function study.
Experimental groups. For renal clearance experiments, 9 sham-operated rats (sham-CBL) were used for control experiments and 13 rats with CBL were used to study responses in cirrhotic rats. Renal function studies were performed in the following four groups: sham-CBL/vehicle (n = 8), sham-CBL/OPC-31260 (n = 9), CBL/vehicle (n = 8), and CBL/OPC-31260 (n = 9). Rats that were used for both control and OPC-31260 experiments were allowed a 3-day recovery period between experiments.

Analytical procedures. Urine volume was determined gravimetrically. Concentrations of sodium, potassium, and lithium in plasma and urine were determined by atomic absorption spectrophotometry using a Perkin-Elmer (Allerød, Denmark) model 2380 atomic absorption spectrophotometer. Urine and plasma osmolality were determined by use of a cryometric osmometer (model 3 CII; Advanced Instruments, Needham Heights, MA). [3H]inulin and [14C]tetraethylammonium bromide was visualized with horseradish peroxidase-conjugated secondary antibody (P448; Dako; diluted 1:3,000) using an ECL film (Amersham, Buckinghamshire, UK). Plasma concentrations of bilirubin and alanine aminotransferase (ALT) were measured by reflowmetry using a Reflotron (Boehringer, Mannheim, Germany). Vasopressin was extracted from plasma on C18 Sep-Pak cartridges and measured by a radiimmunoassay as described earlier (15).

Table 1. Plasma levels of vasopressin, bilirubin, ALT, sodium, and potassium

<table>
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<tr>
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<th>Sham-CBL (n = 9)</th>
<th>CBL (n = 13)</th>
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<tbody>
<tr>
<td>P_{EVP}, pg/ml</td>
<td>1.61 ± 0.61</td>
<td>2.05 ± 0.25</td>
</tr>
<tr>
<td>P_{bilirubin}, µM</td>
<td>&lt;0.05</td>
<td>129 ± 8</td>
</tr>
<tr>
<td>PALAT, U/l</td>
<td>147.6 ± 0.6</td>
<td>148.5 ± 0.9</td>
</tr>
<tr>
<td>PNa, mM</td>
<td>147.0 ± 0.6</td>
<td>147.5 ± 0.9</td>
</tr>
<tr>
<td>PK, mM</td>
<td>4.81 ± 0.09</td>
<td>4.98 ± 0.05</td>
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</table>

Values are means ± SE at the day of the renal clearance experiments 5 wk after common bile duct ligation (CBL) or sham-CBL; n = no. of animals. P_{EVP}, P_{bilirubin}, PALAT, PNa, PK, plasma levels of vasopressin, bilirubin, alanine aminotransferase, sodium, and potassium, respectively. *P < 0.01 vs. sham-CBL. †Value below detection limit (8.55 µM).
The effects of V2-receptor blockade, the average value during
after CBL or sham-CBL

<table>
<thead>
<tr>
<th>Table 2. Daily urine flow and sodium balance 5 wk after CBL or sham-CBL</th>
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<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>24-h Urine flow, ml·24 h−1·100 g body wt−1</td>
</tr>
<tr>
<td>24-h Urine osmolality, mosmol/kg H2O</td>
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<tr>
<td>24-h Sodium balance, µmol/24 h</td>
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Values are means ± SE. All values are means of a 3 days urine collection period prior to immunocytochemical examination. Sodium balance was calculated as 24-h sodium intake minus 24-h urinary sodium excretion. *P < 0.05 vs. sham-CBL.

Reabsorption of filtered lithium may be reabsorbed in the TAL, and therefore only changes of FE Li in excess of 2–5% can be attributed to changes in proximal tubular sodium reabsorption (9, 35). However, when comparisons are performed between groups in which all animals are treated with furosemide, any difference among groups can be ascribed to changes in proximal tubular sodium reabsorption, since there is no evidence for lithium reabsorption beyond the early distal convoluted tubules in sodium replete rats (9, 30). Like most other clearance markers, the validity of the use of lithium clearance as a marker for proximal tubular sodium transport has not been examined in cirrhotic animals, but in animal models with normal plasma levels of vasopressin there is no evidence for increased lithium reabsorption in the TAL, distal convoluted tubules, or in the collecting ducts (39).

Statistics. Data are presented are 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 15-min periods during OPC-31260-induced diuresis. The response during the period with furosemide-induced peak diuresis was used to evaluate the effect of furosemide. Within-group comparisons were analyzed with Student’s paired t-test. Between-group comparisons were performed by one way analysis of variance followed by Fisher’s least significant difference test. Differences were considered significant at the 0.05 level.

RESULTS

Organ weights, sodium balance, diuresis, and plasma biochemistry. Body weights at the end of the study were similar in CBL and sham-CBL rats, and the average weight gain during the 5-wk experimental period was 1.0 ± 0.1 g/day in both groups. There were no signs of ascites in rats with CBL when the abdomen was exposed. Plasma concentrations of vasopressin, bilirubin, ALAT, Na, and K in rats used for the renal clearance experiments are shown in Table 1. Plasma vasopressin concentrations were similar in CBL and sham-CBL animals. Plasma concentrations of bilirubin and ALAT were significantly increased in CBL rats. Plasma concentrations of Na and K were unchanged in cirrhotic rats. Table 2 shows daily urine flow, urine osmolality, and Na balance in CBL and sham-CBL animals. Cirrhotic rats had Na retention relative to sham-operated animals, whereas 24-h urine flow and urine osmolality were similar in cirrhotic and sham-operated control animals.

Systemic and renal hemodynamics before and during V2-receptor blockade. Table 3 shows systemic and renal hemodynamics before and during treatment with OPC-31260 or vehicle. During the control period, HR were similar in all groups (not shown). CBL rats treated with vehicle had a slightly lower ERPF and GFR than CBL rats treated with OPC-31260. The reason for these differences in systemic and renal hemodynamics is unclear, but it is probably a reflection of the vehicle group being closer to the stage of decompensation (decreased ERPF and decreased GFR) than the OPC-treated group of CBL animals. However, none of the CBL rats had any signs of ascites, decreased ERPF, or decreased GFR, and therefore all CBL animals were considered to be in the clinically compensated stage of cirrhosis. Overall, ERVR was significantly decreased in CBL rats compared with sham-operated control animals. (13.5 ± 1.0 vs. 19.8 ± 1.6 mmHg·ml−1·min−1·100 g body wt−1; P < 0.01), suggesting peripheral vasodilatation. All systemic and renal hemodynamic parameters were unchanged during treatment with OPC-31260 and vehicle.

Renal tubular electrolyte handling before and during V2-receptor blockade. Data on the renal handling of Na, K, and Li before and during treatment with OPC-31260 or vehicle are shown in Table 4. During baseline conditions, urinary sodium excretion rate (UNaV), FENa, FEK, and FE Li were similar in CBL and sham-CBL rats.

Table 3. Systemic and renal hemodynamics

<table>
<thead>
<tr>
<th>n</th>
<th>MAP, mmHg</th>
<th>ERPF, ml·min−1·100 g body wt−1</th>
<th>GFR, µl·min−1·100 g body wt−1</th>
<th>EFF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-CBL</td>
<td>9</td>
<td>112 ± 3</td>
<td>3.66 ± 0.23</td>
<td>998 ± 49</td>
</tr>
<tr>
<td>CBL</td>
<td>9</td>
<td>108 ± 2</td>
<td>5.30 ± 0.58*</td>
<td>954 ± 31</td>
</tr>
<tr>
<td>sham-CBL</td>
<td>8</td>
<td>118 ± 4</td>
<td>3.74 ± 0.14</td>
<td>991 ± 41</td>
</tr>
<tr>
<td>CBL</td>
<td>8</td>
<td>105 ± 2*</td>
<td>4.20 ± 0.28</td>
<td>825 ± 42*</td>
</tr>
</tbody>
</table>

Values are means ± SE before (control) and during intravenous administration of the V2-receptor antagonist OPC-31260 (OPC, 800 µg·kg−1·h−1) or vehicle (150 mM glucose) 5 wk after CBL or sham-CBL. MAP, mean arterial pressure; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; EFF, effective filtration fraction; *P < 0.05 vs. sham-CBL.
renal handling of water during baseline conditions and during OPC-31260 treatment is shown in Fig. 1. During baseline conditions, urine flow rate, \( C_{\text{H}_{2}\text{O}} \), and the fractional excretion of water (V/GFR), as well as the fractional distal excretion of water (V/CLi), were similar in cirrhotic and sham-operated animals. V_2-receptor blockade significantly increased these parameters, but compared with sham-operated control animals, the increases were significantly attenuated in the cirrhotic rats, as follows: V, \( 26\% \) (64 \pm 5 vs. 86 \pm 4 \mu\text{L}\cdot\text{min}^{-1}\cdot\text{100 g body wt}^{-1}; P < 0.001); C_{\text{H}_{2}\text{O}}, \( 18\% \) (69 \pm 6 \mu\text{L}\cdot\text{min}^{-1}\cdot\text{100 g body wt}^{-1}; P < 0.01); V/GFR, \( 19\% \) (6.66 \pm 0.46 vs. 8.19 \pm 0.33; P < 0.01); and V/CLi, \( 26\% \) (22.4 \pm 2.1 vs. 27.4 \pm 1.6; P < 0.05).

Effect of furosemide. MAP, HR, ERPF, GFR, EFF, and ERVR were unchanged in all groups during furosemide-induced peak diuresis. Furosemide significantly increased urine flow rate and fractional sodium excretion in all groups (Fig. 2). However, the diuretic and natriuretic responses to furosemide were significantly increased in the cirrhotic animals compared with the sham-operated control animals. In relative terms, the

### Table 4. Renal handling of sodium, potassium, and lithium

|                  | Sham-CBL | OPC | Sham-CBL | Vehicle | Sham-CBL | Vehicle | Control | Vehicle | Control | Vehicle |
|------------------|----------|-----|----------|---------|----------|---------|---------|---------|---------|---------|---------|
| U_{\text{NaV}}, \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{100 g body wt}^{-1} | | | | | | | | | | | | |
| FE_{\text{Na}}, % | 0.19 \pm 0.05 | 0.30 \pm 0.09 | 0.13 \pm 0.04 | 0.19 \pm 0.06 | 16.6 \pm 1.4 | 14.9 \pm 1.8 | 28.5 \pm 1.7 | 30.1 \pm 1.6 |
| FE_{\text{K}}, % | 0.29 \pm 0.07 | 0.16 \pm 0.04 | 0.21 \pm 0.05 | 0.12 \pm 0.03 | 21.3 \pm 1.6 | 9.8 \pm 1.4*† | 29.7 \pm 2.0 | 30.8 \pm 2.2 |
| FE_{\text{Li}}, % | | | | | | | | | | | | |

Values are means \( \pm \) SE before (control) and during intravenous administration of the V_2-receptor antagonist OPC-31260 (OPC, 800 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}) or vehicle (150 mM glucose) 5 wk after CBL or sham-CBL. U_{\text{NaV}}, urinary sodium excretion rate; FE_{\text{Na}}, fractional sodium excretion; FE_{\text{K}}, fractional potassium excretion; FE_{\text{Li}}, fractional lithium excretion. *P < 0.05 vs. sham-CBL. †P < 0.05 vs. control.
The diuretic response to furosemide was increased by 41% (169.7 ± 6.4 vs. 120.4 ± 8.8 µl·min⁻¹·100 g body wt⁻¹; P < 0.001) and the natriuretic response was increased by 52% (19.4 ± 0.7 vs. 12.8 ± 1.4 µmol·min⁻¹·100 g body wt⁻¹; P < 0.001) in cirrhotic rats. The change in fractional sodium excretion was 75% higher in CBL than in sham-CBL rats (15.4 ± 1.2 vs. 8.8 ± 0.9%; P < 0.001).

Pretreatment with OPC-31260 did not affect the natriuretic response to furosemide in cirrhotic or in sham-operated rats. Although V₂-receptor blockade did not affect the diuretic response to furosemide in CBL rats, the diuretic response to furosemide was significantly attenuated in sham-operated control rats pretreated with OPC-31260 (90.7 ± 7.5 vs. 120.4 ± 8.8 µl·min⁻¹·100 g body wt⁻¹; P < 0.01).

The furosemide-induced increases in Cₗᵢ and FEₗᵢ were similar in all four groups. However, the furosemide-induced increase in Cₙₐ/Cₗᵢ was significantly increased in CBL compared with sham-CBL rats (26.2 ± 1.8% vs. 15.5 ± 1.7%; P < 0.001). The furosemide-induced increase in FEₖ was similar in all four groups.

Renal expression of aquaporins. Figures 3 and 4 show immunoblots of membrane fractions (20 µg/lane) from the renal cortex (Fig. 3) and the outer medulla (Fig. 4). As previously shown, the affinity-purified anti-AQP-2 antibody recognizes the 29-kDa and the 35- to 50-kDa band (23, 36), corresponding to nonglycosylated and glycosylated AQP-2, respectively (33).

As shown in Fig. 3A and 4A, a significant decrease of both the 29-kDa and the 35- to 50-kDa AQP-2 bands was observed in the cortex as well as in the outer medulla from cirrhotic rats, whereas inner medullary tissue from cirrhotic and control rats produced bands of similar density (not shown). Relative to kidneys from sham-operated control rats, densitometry revealed a marked decrease in AQP-2 expression in cirrhotic rats in the renal cortex (100 ± 28% vs. 28 ± 7%; P < 0.01) and in the outer medulla (100 ± 14% vs. 56 ± 10%; P < 0.01), whereas the expression was similar to control rats in the inner medulla (100 ± 14% vs. 99 ± 17%; not significant). Recently reported data from our laboratory
have shown selective hypertrophy of the inner stripe of the outer medulla in CBL rats with increased volume of the TAL as well as the collecting duct epithelium. However, there was sign of cortical or inner medulla hypertrophy in Wistar CBL rats (14). Together these results suggest that the AQP-2 downregulation is most pronounced in the renal cortex.

Renal expression of BSC-1. Figure 5 shows immunoblots of membrane fractions (20 µg/lane) from renal outer medulla prepared from female Wistar rats subjected to CBL (solid bars) or sham operation (open bars) 5 wk earlier. A: immunoblot was reacted with affinity-purified anti-AQP-2 and revealed 29-kDa and 35- to 50-kDa AQP-2 bands. B: densitometry was performed on all sham-operated (n = 8) and all cirrhotic rats (n = 12). Values are means ± SE. *P < 0.01 vs. sham-CBL.

DISCUSSION

The present results demonstrate that rats with compensated liver cirrhosis and normal plasma concentrations of vasopressin have decreased expression of the vasopressin-regulated water channel AQP-2 along with a decreased diuretic response to selective V2-receptor blockade with the V2-receptor antagonist, OPC-31260. These results suggest that vasopressin-mediated renal water reabsorption is decreased in rats with compensated liver cirrhosis. Furthermore, as we have shown previously (14), cirrhotic rats had an increased diuretic and natriuretic response to a test dose of furosemide along with an increased corticopapillary Na gradient as reflected by an increased interstitial Na concentration in the inner stripe of the outer medulla and in the papilla. The expression of BSC-1 per microgram membrane protein was unchanged in cirrhotic rats, but since the volume of the TAL epithelium is significantly increased in the outer medulla in rats with compensated cirrhosis (14), these results suggest the total amount of BSC-1 is increased in rats with compensated liver cirrhosis. These results are compatible with our observation that the furosemide-sensitive tubular Na reabsorption in the TAL is increased in compensated cirrhosis. Since daily urine flow rate and proximal tubular Na handling were similar in cirrhotic and sham-operated rats, the blunted diuretic response to OPC-31260 in cirrhotic rats suggests that non-vasopressin-mediated renal water reabsorption is increased in rats with compensated liver cirrhosis. The increased corticopapillary interstitial gradient due to an exaggerated NaCl reabsorption in the TAL may explain the increased driving force for non-vasopressin-mediated water reabsorption in compensated cirrhosis.

Vasopressin regulates water permeability in the renal collecting duct by different mechanisms in short-term and in long-term regulation. Collecting duct water permeability increases within a few minutes after an acute increase in plasma vasopressin concentration, and it is mediated by shuttling of AQP-2 from intracellular vesicles into the apical plasma membrane via exocytosis (24, 25, 32, 44). Mechanisms involved in the effects of vasopressin on long-term regulation of water

**Fig. 4.** Immunoblots of membrane fractions (20 µg/lane) from renal outer medulla prepared from female Wistar rats subjected to CBL (solid bars) or sham operation (open bars) 5 wk earlier. A: immunoblot was reacted with affinity-purified anti-AQP-2 and revealed 29-kDa and 35- to 50-kDa AQP-2 bands. B: densitometry was performed on all sham-operated (n = 8) and all cirrhotic rats (n = 12). Values are means ± SE. *P < 0.01 vs. sham-CBL.

**Fig. 5.** Immunoblots of membrane fractions (20 µg/lane) from renal outer medulla prepared from female Wistar rats subjected to CBL or sham operation 5 wk earlier. Immunoblot was reacted with affinity-purified anti-BSC-1 and revealed a 161-kDa BSC-1 band.
Interstitial water content and concentrations of sodium, potassium, and urea

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<th>Cortex</th>
<th>ISOM</th>
<th>Papilla</th>
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<tr>
<td></td>
<td>Sham-CBL</td>
<td>CBL</td>
<td>Sham-CBL</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Water content, %</td>
<td>75.1 ± 0.2</td>
<td>74.0 ± 0.4</td>
<td>83.4 ± 0.5</td>
</tr>
<tr>
<td>Na, mM</td>
<td>74.9 ± 3.2</td>
<td>77.7 ± 1.7</td>
<td>111.9 ± 6.8</td>
</tr>
<tr>
<td>K, mM</td>
<td>104.2 ± 1.7</td>
<td>101.8 ± 4.0</td>
<td>74.5 ± 4.6</td>
</tr>
<tr>
<td>Urea, mM</td>
<td>29.1 ± 4.1</td>
<td>47.4 ± 12.2</td>
<td>191.6 ± 19.6</td>
</tr>
</tbody>
</table>

Values are means ± SE in the renal cortex, inner stripe of the outer medulla (ISOM), and in the papilla 5 wk after CBL or sham-CBL.

* P < 0.05 vs. sham-CBL.

permeability are activated during prolonged (>24 h) increases in the plasma vasopressin concentration. During prolonged elevations of the plasma vasopressin level, the density of AQP-2 in the principal cells is increased (26) along with increased AQP-2 mRNA levels (21) due to increased AQP-2 gene transcription (27).

Defects in the long-term regulation of AQP-2 expression have been described in a number of experimental conditions with impaired renal ability to handle water. Conditions with impaired concentration ability, like central diabetes insipidus in the homozygous Brattleboro rat (5), acquired diabetes insipidus due to lithium intoxication (22), prolonged hypokalemia (23), bilateral ureteral obstruction (10), and nephrotic syndrome due to puromycin aminonucleoside toxicity (1), have a decreased renal expression of AQP-2. Recently, it was demonstrated that AQP-2 expression is increased in conditions associated with avid water retention and hyponatremia, as in rats with uncompensated congestive heart failure (28, 43), rats with uncompensated liver cirrhosis (11), and in a model of syndrome of inappropriate antidiuretic hormone secretion (11). During conditions with water retention and hyponatremia, the development of hyponatremia is limited by "vasopressin escape," which is the term used to describe the situation where the water-retaining action of vasopressin is impaired (17). With the onset of vasopressin escape, water excretion increases despite high levels of vasopressin, thereby allowing a new steady-state water balance during conditions with low plasma sodium concentration. During water loading of rats treated with high doses of the selective V2-receptor antagonist desmopressin, Ecelbarger et al. (7) recently found that the renal AQP-2 expression as well as AQP-2 mRNA levels significantly decreased from day 2 of water loading, whereas AQP-2 trafficking was intact. These results suggest that vasopressin-independent mechanisms downregulate AQP-2 levels and decrease collecting duct water permeability during vasopressin escape. Similar to these findings, Apostol et al. (1) found that AQP-2 expression was significantly decreased in rats with puromycin aminonucleoside-induced nephrotic syndrome, despite an increased plasma vasopressin concentration.

In the present study, cirrhotic rats with sodium retention, but a normal plasma vasopressin concentration, had an impaired long-term regulation of renal collecting duct water permeability with a significant downregulation of AQP-2 expression in the renal cortex and in the outer medulla. Thus it may be speculated that this downregulation occurs as a physiological compensatory response like in vasopressin escape and in puromycin aminonucleoside-induced nephrotic syndrome. The mechanisms behind the altered long-term regulation of AQP-2 in the models of vasopressin escape, puromycin aminonucleoside-induced nephrotic syndrome, as well as in the present model of compensated liver cirrhosis, are still unknown. The AQP-2 gene contains a putative cAMP regulatory element (38), suggesting that intracellular cAMP concentrations may be involved in the long-term regulation of AQP-2 expression.

Results from the present renal clearance studies showed that V2-receptor blockade with the highly selective V2-receptor antagonist OPC-31260 significantly increased the urine flow rate, C,H2O, fractional water excretion (V/GFR), and fractional distal water excretion (V/Ci,1) without any changes in systemic or renal hemodynamics or renal tubular sodium or lithium handling. These results suggest that OPC-31260 is a highly selective aquarectic agent. The attenuated aquarectic response in CBL rats is in accordance with the downregulation of AQP-2 in this model. Together these results strongly suggest that vasopressin-mediated renal water reabsorption is decreased in rats with compensated liver cirrhosis induced by CBL.

Stimulation of vasopressin V2-receptors in medullary TAL of rats and mice causes activation of the adenylate cyclase, which results in stimulation of tubular sodium transport and increased transepithelial voltage (34, 40). We recently demonstrated that the functional and structural changes in TAL found in rats with compensated liver cirrhosis and normal plasma concentration of vasopressin were completely absent in homozygous Brattleboro rats with CBL (14). Therefore, we suggested that vasopressin has a permissive role for the observed changes in this nephron segment; i.e., the presence of vasopressin is required for the expression of increased tubular NaCl reabsorption and structural changes in the TAL in cirrhotic rats. To examine the effects of acute changes in the vasopressin tonic on furosemide-sensitive NaCl reabsorption in rats with compensated liver cirrhosis, a test dose of furosemide (7.5 mg/kg body wt iv) was given during steady-state V2-receptor blockade. This experiment showed that furosemide produced similar hemodynamic, diuretic, natriuretic, and lithiumuretic responses in CBL rats pre-
treated with vehicle and OPC-31260. This suggests that acute V₂-receptor blockade does not modify the exaggerated natriuretic response to furosemide in rats with compensated liver cirrhosis.

Sodium chloride transport across the apical plasma membrane in the TAL is mediated by an Na-K-2Cl cotransporter (alternatively termed BSC-1) that is directly inhibited by furosemide as shown by Greger (12). The outer medulla is the renal zone with the highest density of TAL segments, and to examine whether the exaggerated natriuretic response in CBL rats was associated with changes in the renal expression of BSC-1, we performed semiquantitative immunoblotting on renal outer medulla in CBL and sham-operated control rats. The expression of BSC-1 in this renal zone was found to be unchanged in the cirrhotic rats. Recent morphometric examinations of in vivo perfused kidneys demonstrated a 47% increase in the volume of the inner stripe of the outer medulla, with a 55% increase in the volume of TAL epithelium in cirrhotic rats relative to controls (14). Thus, along with our previous findings, the immunoblotting data suggest that the total amount of BSC-1 is increased in rats with compensated cirrhosis. These findings suggest that the increased nonvasopressin-mediated renal water reabsorption in CBL rats is due to an increased corticopapillary interstitial gradient due to an exaggerated sodium chloride reabsorption in the TAL.

In summary, the aquarectic response to a near-maximal dose of the selective V₂-receptor antagonist OPC-31260 was significantly attenuated in rats with compensated liver cirrhosis compared with sham-operated control rats. This was paralleled by a significant decrease in AQP-2 expression in the renal cortex and outer medulla of cirrhotic rats. These results suggest that vasopressin-mediated renal water transport is decreased in rats with compensated liver cirrhosis. Furthermore, acute V₂-receptor blockade did not modify the natriuretic response to furosemide, neither in cirrhotic rats nor in sham-operated control rats. These findings suggest that acute changes in V₂-receptor stimulation do not influence furosemide-sensitive NaCl reabsorption in the TAL.

We gratefully acknowledge Dr. J. Warberg for performing the plasma vasopressin analyses. We also acknowledge the technical assistance of Anette Francker, Lisette Knoth-Nielsen, Iben Nielsen, Mette Vestisen, Trine Møller, and Gitte Christensen.

This work received financial support from The Danish Medical Research Council, The Novo Nordic Foundation, The P. Carl Petersen Foundation, The Eva and Robert Voss Hansen Foundation, The Knud Øster-Jørgensen Foundation, and The Helen and Ejnar Bjernow Foundation.

This study was presented in preliminary form at the annual meeting of the Federation of American Societies for Experimental Biology, Experimental Biology 97, New Orleans, LA, April 6–9, 1997. Address for reprint requests: T. E. N. Jonassen, Dept. of Pharmacology, The Panum Institute, Univ. of Copenhagen, 3 Blegdamsvej, Bldg. 186, DK-2200 Copenhagen N, Denmark.

Received 1 December 1997; accepted in final form 23 April 1998.

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