Renal sodium retention in cirrhotic rats depends on glucocorticoid-mediated activation of mineralocorticoid receptor due to decreased renal 11β-HSD-2 activity

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Thiesson HC, Jensen BL, Bistrup C, Ottosen PD, McNeilly AD, Andrew R, Seckl J, Skøtt O. Renal sodium retention in cirrhotic rats depends on glucocorticoid-mediated activation of mineralocorticoid receptor due to decreased renal 11β-HSD-2 activity. Am J Physiol Regul Integr Comp Physiol 292: R625–R636, 2007. First published August 17, 2006; doi:10.1152/ajpregu.00418.2005.—Downregulation of the renal glucocorticoid-metabolizing enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) during liver cirrhosis may allow activation of the mineralocorticoid receptor (MR) by glucocorticoids and contribute to sodium retention. We tested this hypothesis in male Wistar rats with decompensated liver cirrhosis and ascites 7 wk after bile duct ligation (BDL). Renal 11β-HSD-2 mRNA, protein, and activity were significantly decreased in decompensated rats. The urinary Na+/K+ ratio was reduced by 40%. Renal epithelial sodium channel (ENaC) mRNA and immunostaining were only slightly affected. Complete metabolic studies, including fecal excretion, showed that the BDL rats had avid renal sodium retention. Treatment of the BDL rats with dexamethasone suppressed endogenous glucocorticoid production, normalized total sodium balance and renal sodium excretion, and reduced ascites formation to the same degree as direct inhibition of MR with K-canrenoate. Total potassium balance was negative in the BDL rats, whereas renal potassium excretion was unchanged. In the distal colon, expression of ENaC was increased in BDL rats. Fecal potassium excretion was increased in cirrhotic rats, and this was corrected by treatment with K-canrenoate but not dexamethasone. We conclude that development of sodium retention and decompensation in cirrhotic rats is associated with downregulation of renal 11β-HSD-2 activity and inappropriate activation of renal sodium reabsorption by endogenous glucocorticoids. In addition, the overall potassium loss in the BDL model is due to increased fecal potassium excretion, which is associated with upregulation of ENaC in distal colon.

ascites; bile duct ligation

DECOMPENSATED LIVER CIRRHOSIS is characterized by avid sodium and water retention. Ascites formation is associated with a high morbidity, and in humans there is a mortality rate of 50% within 3 yr (3). In some patients, activation of the renin-angiotensin-aldosterone system contributes to sodium retention (7, 35, 42), but many patients exhibit low or normal angiotensin-aldosterone-system contributes to sodium retention and potassium wasting was tested by suppression of endogenous glucocorticoids by treatment with the glucocorticoid receptor-specific agonist dexamethasone.

MATERIALS AND METHODS

Animals and Surgical Protocol

Male Wistar rats were obtained from M & B (Ejby, Denmark). The Danish Animal Experiments Inspectorate approved all experimental costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
procedures, and all animals were treated according to the Guide for the Care and Use of Laboratory Animals. The rats were fed a diet with 0.2% sodium and 1.0% potassium (Altromin 1324; Altromin International, Lage, Germany) and had free access to tap water. When rats were in metabolic cages, food was administered as granules (diameter 1.7 mm) to avoid spillage into feces and urine collection vials. Animals were anesthetized by subcutaneous injection with fentanyl citrate (0.25 mg/kg), fluanisone (8 mg/kg), and diazepam (4 mg/kg). The common bile duct was isolated and ligated, and 0.5 cm was excised (26) or manipulated and left intact (sham surgery). Postoperative pain was treated by administration of buprenorphin subcutaneously (0.1 mg/kg). We performed three experimental series in the present study.

At the end of the different treatment protocols, the rats were decapitated and trunk blood was collected. Organs were removed, weighed, and snap frozen for later RNA and protein isolation. Ascites volume was determined by aspiration of free fluid in the abdominal cavity.

Series 1: 5-wk BDL rats. In the first series, six rats had BDL and five rats were sham operated (sham). These rats were followed for 5 wk.

Series 2: 7-wk BDL rats. To study sodium balance in the decompensated phase, the next groups of rats were followed for 7 wk (BDL, n = 18; sham, n = 12). The rats were housed in individual metabolic cages from day 42 to day 49 after BDL. Na⁺, K⁺, and water intake and excretion were followed after a 2-day run-in period. Rats without ascites were excluded from analysis in this series.

Series 3: 7-wk BDL-intervention. In the third series, we tested the effect of dexamethasone and K-canrenoate on Na⁺ and K⁺ balance in decompensated BDL rats (sham-vehicle, n = 12; BDL + vehicle, n = 15; BDL + K-canrenoate, n = 15; BDL + dexamethasone, n = 15). Rats received vehicle, dexamethasone (100 µg·kg⁻¹·day⁻¹; Decadron, Merck Sharp & Dohme, Haarlem, The Netherlands), or K-canrenoate (80 µg·kg⁻¹·day⁻¹; Soldactone, Searle, Denmark) for 5 days in the decompensated stage with sodium retention (days 44–48). A 48-h run-in period in metabolic cages was allowed before administration of drugs. Balance studies began 24 h before drug administration. The experiment was terminated at 7 wk (day 49) after BDL. Rats that did not complete the basal 24-h collections were excluded from the.data analysis.

Plasma and Urine Analyses

Plasma renin and atrial natriuretic peptide (ANP) concentrations were measured by radioimmunoassay as described previously (9, 29). Plasma aldosterone and corticosterone concentrations were measured with commercial kits (Coat-A-Count Aldosterone, DPC, Los Angeles, CA; rat corticosterone assay, Amersham Biotrak, Amersham, Buckinghamshire, UK). Tissue renin concentration was measured by radioimmunoassay as described previously (32). Bilirubin, alanine amino transferase, bile acids, albumin, and creatinine were analyzed using an analyzer (Cobas Mira; Roche Diagnostics, Basel, Switzerland). Platelet count, leucocytes, hematocrit, and hemoglobin were analyzed on a Celltac MEK-6108K (Nihon Koden, Tokyo, Japan). Electrolytes were determined by flame photometry (IL 943 flame photometer; Instrumentation Laboratory, Milan, Italy). Osmolarity was measured by freezing point depression (Osmomat 030D; Gontec, Berlin, Germany). Sodium and potassium balances were calculated as the ratio between oral intake and the sum of losses in urine and feces. To estimate fecal water content, feces were heated until constant weight (24 h) at 100°C. Dried feces were then dissolved in 30 ml of 0.7 M HNO₃, homogenized (Polytron PT300, Kinematica, Kriens-Luzern, Switzerland), and gently shaken for 24 h. Subsequently, electrolytes were determined in the supernatant by flame photometry. Creatinine clearance (CrCl) (mL/min) is a reliable measure of glomerular filtration rate (GFR) in Wistar rats (40), was calculated using urine collected over the last 24 h in a metabolic cage.

Isolation of RNA and Ribonuclease Protection Assay

Total RNA was isolated from rat organs with the Qiagen RNeasy Midi Kit, and mRNA levels were estimated by A/ T1 ribonuclease protection assay (30, 36). Plasmids carrying sequences for renin (234 bp) and 11β-HSD-2 (291 bp) have previously been cloned and validated (22, 30). Partial rat cDNA encoding epithelial sodium channel (ENaC) subunits was cloned by RT-PCR with the following primer sequences (Invitrogen, Paisley, Scotland): α-subunit, 5’-ATTGACCTAGACCTTCAC-3’ as 5’-CAT GCGGTTGTTT-GGA-3’ (accession no. U54700, 263 bp; probe used for RNase protection assay, 193 bp); β-subunit, 5’-AGGCTACACCTCAAGGA-3’ as 5’-AGTTGAGGCTCTGGAAAGC-3’ (accession no. U35175, 222 bp); and γ-subunit, 5’-GCTATTTCTGGCAAGAAC-3’ as 5’-TGAGGTGCTGAGGATGG-3’ (accession no. U37540, 146 bp). Primers were designed to anneal in regions without homology and were synthesized with restriction sites for BamHI (sense) and EcoRI (antisense) in the 5’ direction (+ 15 bp) to allow for directional cloning in vector pSP73 (Promega, Rodovre, Denmark). Plasmids were sequenced by T7 polymerase (Promega) on an ABI Prism genetic analyzer with ready reaction mix from ABI. Plasmids were linearized with HindIII and purified with a Qiagen PCR purification kit. Plasmids yielded radiolabeled antisense cDNA transcripts by incubation with SP6 polymerase (Promega) and [α-32P]GTP (Amer- sham) according to the Promega riboprobe in vitro transcription protocol.

Western Immunoblot Analysis

Protein was isolated from distal colon and kidney as described previously (30). Protein concentration was determined on a spectrophotometer (Bio-Rad protein assay reagent). Protein separation and immunoblotting for 11β-HSD-2 was as described previously (30).

Immunohistochemical Staining for Renin, 11β-HSD-2, and ENaC α-Subunit

In all three series, a limited number of sham and BDL rats were fixed by retrograde perfusion of the aorta with 4% paraformaldehyde. Tissue blocks incubated overnight in paraformaldehyde were and embedded in paraffin using standard techniques. Sections (5 µm) were cut, deparaffinized, brought to a watery medium through successive incubation steps, and dehydrated. Sections were mounted on charged slides and were baked at 60°C for 1 h.

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Measurement of 11β-HSD-2 Activity

11β-HSD-2 is a unidirectional dehydrogenase catalyzing the rapid conversion of active corticosterone to inert 11-dehydrocorticosterone in the presence of NAD⁺. 11β-HSD-2 activity was measured in protein homogenates from renal cortex and distal colon. Protein concentration was quantified colorimetrically using a Bio-Rad protein assay kit. Protein homogenates from kidney (25 µg/ml) and colon (50 µg/ml) were incubated in duplicate at 37°C in 0.1 M Tris buffer, pH 7.6, 0.25 M sucrose, and 5 mM MgCl₂ solution with NAD⁺ (0.5 mM; [3H]corticosterone (10 nM), and unlabeled corticosterone (990 nM) in the presence of 10 mM DTT (19). After 60 (kidney) and 240 min (colon), the reaction was terminated by the addition of ethyl acetate, and the organic phase was reduced to dryness under a stream of oxygen-free nitrogen; steroids in the dried residue were dissolved in the mobile phase (60:15:25 water-acetonitrile-methanol) and quantified by HPLC with online liquid scintillation counting as described.
previously (27). Conditions were optimized for each tissue to ensure first-order kinetics.

Statistical Methods

Differences between two groups were analyzed using an unpaired t-test, and variance was tested with an F-test. Differences between more than two groups were estimated by ANOVA (treatment-series, BDL rats). If data did not have a Gaussian distribution, a Mann-Whitney U-test was used to test differences between two groups, and differences between more than two groups were estimated with the Kruskal-Wallis test. Levels of significance were \( P < 0.05 \).

RESULTS

Characterization of Experimental Model of BDL in Rats

Five weeks after BDL, all rats displayed jaundice with elevated bilirubin and hepatosplenomegaly (Tables 1 and 2). Weight gain was slowed significantly by BDL in all series, and organ weight-to-body weight ratios were significantly higher in both compensated and decompensated BDL rats compared with sham rats (Tables 1 and 2). Histopathological examination of the livers after 5 wk of BDL showed fibrosis and septa formation between portal triads and the central veins with apparently normal liver tissue in between. Seven weeks after BDL, the livers exhibited completely distorted architecture with small regeneration nodules and severe fibrosis. About 80% of the rats accumulated ascites between 5 and 7 wk after BDL (Table 1). This was associated with a mortality of \( \sim 35\% \). In the decompensated stage at 7 wk, plasma hemoglobin, erythrocyte volume fraction, and thrombocytes were lower, whereas the white blood cell count was elevated (Table 2). Serum albumin concentration was lower in BDL rats, whereas serum bilirubin was higher in all BDL groups compared with

Table 1. Basal characteristics of BDL and sham rats after 5 and 7 wk

<table>
<thead>
<tr>
<th></th>
<th>5 wk</th>
<th>7 wk</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>BDL</td>
</tr>
<tr>
<td>Initial number of rats</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rats with/without ascites</td>
<td>0/5</td>
<td>0/6</td>
</tr>
<tr>
<td>Ascites volume, ml</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Final number of rats</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Preoperative weight, g</td>
<td>305 ± 5</td>
<td>289 ± 9</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>367 ± 10</td>
<td>308 ± 18*</td>
</tr>
<tr>
<td>Liver, g/100 g BW</td>
<td>3.2 ± 0.8</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>Spleen, g/100 g BW</td>
<td>0.20 ± 0.01</td>
<td>0.6 ± 0.05**</td>
</tr>
<tr>
<td>Kidney, g/100 g BW</td>
<td>0.57 ± 0.02</td>
<td>0.7 ± 0.05*</td>
</tr>
<tr>
<td>Heart, g/100 g BW</td>
<td>0.29 ± 0.01</td>
<td>0.33 ± 0.02*</td>
</tr>
</tbody>
</table>

All values are expressed as means ± SE. *\( P < 0.05 \); **\( P < 0.01 \). BDL, bile duct ligated; sham, sham-operated control; BW, body weight.

Table 2. Effect of dexamethasone and K-canrenoate on organ weights, plasma electrolytes, blood cells, hormones, and Na⁺/K⁺ ratios in BDL compared with sham rats

<table>
<thead>
<tr>
<th></th>
<th>Sham + Vehicle</th>
<th>BDL + Vehicle</th>
<th>BDL + Dexamethasone</th>
<th>BDL + Canrenoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks after surgery</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Initial number of rats</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Mortality</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Final number of rats</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Preoperative weight, g</td>
<td>262 ± 8</td>
<td>262 ± 6</td>
<td>251 ± 10</td>
<td>265 ± 8</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>386 ± 13</td>
<td>346 ± 10*</td>
<td>285 ± 10†</td>
<td>343 ± 14</td>
</tr>
<tr>
<td>Liver, g/100 g BW</td>
<td>3.8 ± 0.14</td>
<td>7.6 ± 0.6†</td>
<td>8.5 ± 0.7</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td>Spleen, g/100 g BW</td>
<td>0.17 ± 0.02</td>
<td>0.73 ± 0.03†</td>
<td>0.6 ± 0.08</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>Kidney, g/100 g BW</td>
<td>0.61 ± 0.02</td>
<td>0.74 ± 0.02*</td>
<td>0.89 ± 0.05</td>
<td>0.65 ± 0.08</td>
</tr>
<tr>
<td>Heart, g/100 g BW</td>
<td>0.28 ± 0.01</td>
<td>0.39 ± 0.02*</td>
<td>0.42 ± 0.03</td>
<td>0.34 ± 0.03</td>
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<tr>
<td>Adrenal glands, mg/100 g BW</td>
<td>16.6 ± 0.8</td>
<td>20.7 ± 2.5</td>
<td>16.2 ± 2.8</td>
<td>17.1 ± 1.2</td>
</tr>
<tr>
<td>P-sodium, mM</td>
<td>141 ± 0.5</td>
<td>140 ± 0.7</td>
<td>140 ± 0.5</td>
<td>140 ± 0.5</td>
</tr>
<tr>
<td>S-creatinine, mM</td>
<td>28 ± 1.2</td>
<td>27 ± 2.7</td>
<td>26 ± 0.8</td>
<td>27 ± 0.5</td>
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<tr>
<td>B-platelets, 10⁹/lt</td>
<td>743 ± 78</td>
<td>371 ± 66†</td>
<td>370 ± 101</td>
<td>693 ± 88†</td>
</tr>
<tr>
<td>P-albumin, mM</td>
<td>599 ± 10</td>
<td>411 ± 20†</td>
<td>490 ± 45</td>
<td>419 ± 25</td>
</tr>
<tr>
<td>B-leucocytes, 10⁹/lt</td>
<td>5 ± 0.5</td>
<td>28 ± 2.9†</td>
<td>28 ± 1.1</td>
<td>22 ± 1.8</td>
</tr>
<tr>
<td>Hemoglobin, mM</td>
<td>10.3 ± 0.1</td>
<td>8.4 ± 0.4†</td>
<td>9.2 ± 1.0†</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>B-hematocrit, %</td>
<td>47 ± 0.5</td>
<td>40 ± 1.7†</td>
<td>46 ± 5.0†</td>
<td>40 ± 1.7</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kg H₂O</td>
<td>296 ± 1.2</td>
<td>297 ± 1.2</td>
<td>296 ± 0.3</td>
<td>298 ± 0.8</td>
</tr>
<tr>
<td>S-bilirubin, μM</td>
<td>2 ± 0.2</td>
<td>141 ± 16†</td>
<td>166 ± 16</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>P-aldoesterone, mM</td>
<td>0.11 (0.03–0.34)</td>
<td>0.57† (0.19–2.70)</td>
<td>0.46 (0.19–1.14)</td>
<td>0.62 (0.33–2.04)</td>
</tr>
<tr>
<td>PRC, 10⁻³ GU/ml</td>
<td>4.8 (1.9–21.7)</td>
<td>18.7 (3.1–27.0)</td>
<td>1.9 (10.25–7.5)</td>
<td>5.7 (2.3–24)</td>
</tr>
<tr>
<td>Urine Na⁺/K⁺ ratio</td>
<td>0.46 ± 0.01</td>
<td>0.26 ± 0.03†</td>
<td>0.44 ± 0.04†</td>
<td>0.41 ± 0.01*</td>
</tr>
<tr>
<td>Feces Na⁺/K⁺ ratio</td>
<td>0.79 ± 0.07</td>
<td>0.52 ± 0.07†</td>
<td>0.24 ± 0.03</td>
<td>0.67 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Values for aldosterone and plasma renin concentrations (PRC) are geometric means. Differences between the three 7-wk BDL groups were estimated with ANOVA. *\( P < 0.05 \); †\( P < 0.01 \).
Corticosterone in Plasma and Tissue

Effect of BDL on Renin-Angiotensin-Aldosterone and Cholesterol Metabolism

In the BDL rats, PRC was significantly higher than controls. After 5 wk, the control was 100 ± 8.7% and BDL was 97 ± 13.8%. In contrast, after 7 wk of BDL, renocortical 11β-HSD-2 mRNA, protein, and activity levels were significantly lower compared with those of sham rats (Fig. 2, A–C). Immunostaining of sham-control kidney sections for 11β-HSD-2 displayed labeling associated with distal convoluted tubules, connecting tubules, and collecting ducts in cortex, whereas labeling of collecting ducts declined progressively in outer medulla and was absent in inner medulla and papilla, as previously described (Fig. 2D) (19, 30). Intercalated cells in the collecting ducts were not labeled. The overall distribution of 11β-HSD-2 labeling in kidney from BDL rats was similar, albeit with consistently less intensity (Fig. 2D). In marked contrast to kidney, we found that 11β-HSD-2 mRNA levels were significantly increased in distal colon after 7 wk of BDL (Fig. 3A). Indeed, 11β-HSD-2 mRNA expression was threefold elevated in distal colon in the compensated phase after 5 wk of BDL; control was 100 ± 16.9% and BDL was 302 ± 77.5%. The mRNA findings were supported by immunolabeling (Fig. 3C); however, there was no significant difference at the protein level as shown by Western immunoblotting (Fig. 3B). 11β-HSD-2 activity was low in distal colon compared with kidney, and hardly any conversion was detectable in either cirrhotic or control rats.

Effect of BDL on Expression and Distribution of ENaC in Kidney and Distal Colon

In sham-operated control rats, plasma renin concentration (PRC) was not different 5 and 7 wk after surgery (Fig. 1A). Whereas there was an age-dependent decline in renocortical renin mRNA expression between 5 and 7 wk, kidney renin mRNA expression was not significantly different in BDL rats compared with control. After 5 wk, the control was 100 ± 12.6% and BDL was 96.1 ± 18.3%, and after 7 wk, the control was 100 ± 13.9% and BDL was 87.5 ± 21.0%. In the BDL rats, PRC was significantly higher than controls at 5 wk but fell to control level at 7 wk. There was no obvious difference in the distribution pattern of renin-positive cells along afferent arterioles from BDL rats at 7 wk compared with sham (data not shown). The plasma aldosterone concentration was significantly elevated after BDL for 7 wk, whereas corticosterone concentrations were not significantly affected compared with sham-operated controls (Fig. 1B). Urinary Na+/K+ ratio and plasma K+ level were significantly decreased after 7 wk BDL in series 2 (BDL 0.29 ± 0.06 vs. sham 0.48 ± 0.02, P < 0.01; and BDL 5.0 ± 0.2 vs. 5.6 ± 0.2 mM control, P < 0.005, respectively), indicating mineralocorticoid excess.

Effect of BDL on Tissue Expression Level and Cellular Distribution of 11β-HSD-2 in Kidney and Distal Colon of Compensated and Decompensated Rats

Next, we explored whether 11β-HSD-2 was changed by BDL in kidney and distal colon. Renocortical 11β-HSD-2 mRNA expression was not significantly different from sham controls at 5 wk after surgery; the control was 100 ± 8.7% and BDL was 97 ± 13.8%. In contrast, after 7 wk of BDL, renocortical 11β-HSD-2 mRNA, protein, and activity levels were significantly lower compared with those of sham rats (Fig. 2, A–C). Immunostaining of sham-control kidney sections for 11β-HSD-2 displayed labeling associated with distal convoluted tubules, connecting tubules, and collecting ducts in cortex, whereas labeling of collecting ducts declined progressively in outer medulla and was absent in inner medulla and papilla, as previously described (Fig. 2D) (19, 30). Intercalated cells in the collecting ducts were not labeled. The overall distribution of 11β-HSD-2 labeling in kidney from BDL rats was similar, albeit with consistently less intensity (Fig. 2D). In marked contrast to kidney, we found that 11β-HSD-2 mRNA levels were significantly increased in distal colon after 7 wk of BDL (Fig. 3A). Indeed, 11β-HSD-2 mRNA expression was threefold elevated in distal colon in the compensated phase after 5 wk of BDL; control was 100 ± 16.9% and BDL was 302 ± 77.5%. The mRNA findings were supported by immunolabeling (Fig. 3C); however, there was no significant difference at the protein level as shown by Western immunoblotting (Fig. 3B). 11β-HSD-2 activity was low in distal colon compared with kidney, and hardly any conversion was detectable in either cirrhotic or control rats.

Effect of BDL on Expression and Distribution of ENaC in Kidney and Distal Colon

To explore whether the change in 11β-HSD-2 level in kidney and perhaps colon had functional consequences, we determined mRNA level and cellular localization of the key MR-regulated target, the ENaC. In kidney cortex, all three ENaC subunits were detected at significant levels. In BDL, mRNA encoding the β-subunit of ENaC was significantly reduced in abundance, whereas expression of α- and γ-subunits was unchanged (Fig. 2, E and F). In sham rats, only the α-subunit was reproducibly detected in distal colon with the use of 40 μg of total RNA for the hybridization. In contrast to kidney, mRNA abundances for all three ENaC subunits were significantly enhanced in distal colon of decompensated BDL rats (Fig. 3D). Immunolabeling of kidney and distal colon sections with an αENaC subunit-specific antibody showed a distinct distribution of immunoreactive protein that was similar to that observed with the 11β-HSD-2 antibody. Thus, in kidney, αENaC labeling was associated with distal convoluted tubules, cortical collecting ducts, and outer medullary collecting ducts (Fig. 2G). There were no obvious differences in distribution of immunoreactivity between sham and kidneys from BDL rats, but there was a
clear tendency that labeling signals were weaker in the BDL kidneys (Fig. 2G). In distal colon, we did not detect any significant immunolabeling signals for αENaC associated with epithelium or other layers in sham-operated rats, whereas αENaC immunoreactivity and fluorescence were observed in crypt and surface epithelial cells in distal colon from the decompensated BDL rats (Fig. 3E).

Effect of Dexamethasone and K-Canrenoate on Electrolyte and Water Balance in Decompensated BDL Rats

In the next experiments, we tested whether the significant changes in 11β-HSD-2 in kidney resulted in glucocorticoid-mediated effects on sodium balance. Dexamethasone, which has a very low affinity for MR compared with corticosterone,
suppressed endogenous plasma corticosterone (Fig. 4D). The effect of dexamethasone on Na\(^+\), K\(^+\), and water balance in decompensated BDL rats was compared with the effect of K-canrenoate, an MR antagonist. Dexamethasone prevented the development of ascites as efficiently as K-canrenoate (Fig. 4A) and significantly reduced the volume of ascites (Fig. 4B). Plasma ANP concentration reflects changes in extracellular volume; it was significantly higher in the decompensated BDL group compared with sham controls and decreased significantly after treatment with both dexamethasone and K-canrenoate (Fig. 4C). Plasma renin and aldosterone concentrations were not affected by dexamethasone or K-canrenoate administration to BDL rats (Table 2). Next, we performed balance studies by analyzing intake and fecal and renal excretion of electrolytes and water (Table 3). By using special granulated food and measuring both urinary and fecal excretions, we were able to account for 100% of sodium and potassium intake.

Sodium. Sham control rats were in sodium balance (intake of 680 ± 16 μmol Na\(^+\)·100 g rat\(^{-1}\)·24 h\(^{-1}\)), and they excreted 85% of the daily sodium intake in the urine and 15% in feces (Fig. 5A–C). In contrast, the BDL rats at 7 wk were in positive sodium balance (Fig. 5A). This was the result of a significant decrease in the urinary sodium excretion to 55% of the intake (Fig. 5B), which was not counteracted by any significantly increased fecal sodium loss: 23% of intake (Fig. 5C). Treatment with dexamethasone or K-canrenoate did not affect so-
Effect of Dexamethasone and K-Canrenoate on Urinary and Feces $\text{Na}^+/\text{K}^+$ Concentration Ratios

The urinary $\text{Na}^+/\text{K}^+$ ratio was significantly lower in decompensated BDL rats compared with sham-operated controls (Fig. 6A). Treatment of the BDL rats with dexamethasone or K-canrenoate led to a significant increase in urinary $\text{Na}^+/\text{K}^+$ ratio to a level not different from that of sham-operated controls. Feces $\text{Na}^+/\text{K}^+$ ratio was also significantly reduced in the BDL rats compared with sham-operated controls. Treatment with dexamethasone led to a further reduction of the feces $\text{Na}^+/\text{K}^+$ ratio, whereas treatment with K-canrenoate led to a significant increase in $\text{Na}^+/\text{K}^+$ ratio compared with the decompensated BDL + vehicle rats (Fig. 6B).

Effect of Dexamethasone and K-Canrenoate on Plasma and Urine Osmolality and Creatinine Clearance in Decompensated BDL Rats

Plasma osmolalities were similar in all groups (sham + vehicle, $296 \pm 1.2$ mosmol/kgH$_2$O; BDL + vehicle, $297 \pm 1.2$ mosmol/kgH$_2$O). The sham control rats were in potassium balance (Fig. 5D). The decompensated group had a negative potassium balance, indicating excess MR activation. Potassium loss was caused by higher fecal loss, whereas urinary potassium excretion was not different from control level. The fecal potassium loss was decreased by treatment with K-canrenoate but not dexamethasone (Fig. 5F). Of note, renal handling of potassium was similar in all four groups and unaffected by treatments (Fig. 5E). Thus excess potassium wasting occurred exclusively through fecal losses in BDL rats. The time courses of absolute values of potassium intake and outputs are shown in Table 3.
mosmol/kgH2O; BDL + dexamethasone, 296 ± 0.3 mosmol/kgH2O; BDL + K-canrenoate, 298 ± 0.8 mosmol/kgH2O). Urine osmolality decreased significantly in the K-canrenoate-treated group as expected from the increase in water excretion (BDL + canrenoate, 0.8 ± 0.1 mosmol/kgH2O, compared with sham + vehicle, 1.6 ± 0.1 mosmol/kgH2O, P < 0.05; BDL + vehicle, 1.5 ± 0.2 mosmol/kgH2O, and BDL + dexamethasone, 1.5 ± 0.2 mosmol/kgH2O). Osmolar clearance (Cl\text{osmol}) was lower in the BDL + vehicle group with the sham + vehicle group (12.0 ± 1.3 vs. 16.7 ± 1.2 μL/min, P < 0.05). Cl\text{osmol} increased to the control level after dexamethasone and K-canrenoate (17.7 ± 0.7 and 15.1 ± 0.5 μL/min, respectively). Cl\text{crea} was higher in the sham + vehicle group (0.30 ± 0.03 ml·min⁻¹·100 g body wt⁻¹) than in the decompensated BDL + vehicle group (0.20 ± 0.03 ml·min⁻¹·100 g body wt⁻¹). In the dexamethasone-treated BDL group, Cl\text{crea} was increased to 0.32 ± 0.03 ml·min⁻¹·100 g body wt⁻¹, whereas in the K-canrenoate-treated BDL group, Cl\text{crea} was unchanged (0.25 ± 0.03 ml·min⁻¹·100 g body wt⁻¹).

**Effect of Dexamethasone and K-Canrenoate on Weight Gain, Organ Weights, and Blood Chemistry**

Treatment with dexamethasone or K-canrenoate did not correct the significantly slower rate of growth of BDL rats. The dexamethasone-treated BDL rats actually had a significantly lower weight at completion of the study compared with the K-canrenoate-treated BDL rats despite a similar food intake and natriuretic effect (Table 2). This was probably caused by the overall catabolic effect of the potent glucocorticoid. The organ weight-to-body weight ratios in the decompensated BDL rats remained or were further elevated after treatment with K-canrenoate or dexamethasone compared with sham controls. Hemoglobin and eryth-
rocyte volume fractions were lower in the vehicle-treated BDL and K-canrenoate-treated BDL groups but increased in the dexamethasone-treated BDL group (Table 2). Plasma sodium and creatinine levels were equal in all groups (Table 2). Plasma bilirubin concentration was similar in BDL rats and in the BDL rats treated with K-canrenoate and dexamethasone.

**DISCUSSION**

In the present study, we used the rat model of BDL to study mechanisms of sodium and water retention in the transition from compensated to decompensated liver cirrhosis. The data showed that augmented renal sodium reabsorption was responsible for a positive sodium balance despite increased fecal...
sodium losses. The enzyme 11β-HSD-2 was downregulated in kidney, and suppression of endogenous corticosterone by treatment with dexamethasone corrected the decreased urinary Na⁺/K⁺ ratio, augmented urinary sodium excretion, and reduced ascites formation. This indicates that excess activation of the MR by corticosterone contributes to the exaggerated renal sodium reabsorption during decompensated stages of BDL.

Consistent with reports from rats with carbon tetrachloride-induced cirrhosis (12), we observed that ENaC mRNA expression in the kidney was unchanged or slightly reduced at the same time as plasma aldosterone was elevated. Increased cell surface expression of the ENaC subunits after MR activation (28) may explain the increased renal ENaC activity despite unchanged ENaC mRNA expression (2), or dysregulated expression and activity of aldosterone-induced/repressed proteins may lead to prolonged surface expression of ENaC. In a recent study, serum and glucocorticoid-inducible kinase-1, which have a central role in recruitment of ENaC to the cell surface, were unchanged in 4-wk BDL rats, whereas Nedd-4, a ubiquitin ligase protein that promotes internalization and degradation of ENaC, was reduced >50% (44). During the development of decompensation in the period from 5 to 7 wk, there was a decrease in renin synthesis and secretion, whereas plasma aldosterone concentration was significantly increased in the decompensated rats at 7 wk. This could contribute to increased sodium reabsorption.

In contrast to kidney, all ENaC subunits were markedly elevated in distal colon of BDL rats. This upregulation correlated with increased fecal potassium and sodium losses, suggesting a shift to low-capacity, high-affinity NaCl transport. Fecal NaCl excretion was decreased to control level by dexamethasone, but fecal potassium wasting was not. Thus sodium and potassium handling were differentially changed in kidney and distal colon with progression of bile duct-occlusive liver disease.

Urinary Na⁺/K⁺ ratio was decreased in the decompensated BDL rats, which indicated a MR-mediated effect on the collecting ducts. This was supported by the fact that administration of an MR antagonist, K-canrenoate, increased the urinary Na⁺/K⁺ ratio, normalized sodium balance, reduced the incidence and severity of ascites, and led to suppression of the plasma ANP concentration, which is an indicator of intravascular volume. Several mechanisms may contribute to the activation of the MR. Plasma aldosterone concentration was significantly increased after 7 wk of BDL compared with the sham-operated controls. On the other hand, there also was a significant fall in the expression of renal 11β-HSD-2 in from 5 to 7 wk, when decompensation develops. This would allow corticosterone to gain access to renal MR. In accordance with this notion, we observed that suppression of plasma corticosterone (but not aldosterone) concentration with dexamethasone corrected the urinary Na⁺/K⁺ ratio, normalized sodium balance, reduced the incidence and severity of ascites, and suppressed plasma ANP concentration to a similar degree as direct blockade of MR with K-canrenoate. A similar effect of dexamethasone on urinary Na⁺/K⁺ ratio was observed in mice with targeted deletion of the 11β-HSD-2 gene (25).

Dexamethasone binds to the MR in vitro with an affinity close to that of glucocorticoids (20), suggesting that dexamethasone could act as a MR antagonist. However, dexamethasone does not functionally bind to the renal MR in vivo (14, 16), probably because of a high off-rate of dexamethasone bound to the MR in contrast to the stable binding of dexamethasone to the glucocorticoid receptor (21, 34). Furthermore, the sodium transport response to dexamethasone in the mCCDcl1 cell line is not inhibited by the MR antagonist spironolactone (17). Thus a direct antagonist action of dexamethasone is less likely to explain the present results. Dexamethasone increased creatinine clearance, suggesting that an increase in GFR and tubular flow rate could increase distal potassium secretion and thereby normalize the Na⁺/K⁺ ratio. However, the absolute urinary potassium excretion was not affected by dexamethasone treatment, making this possibility less likely. Together, the data are consistent with the hypothesis that inappropriate activation of MR by corticosterone contributed to renal salt and water retention in decompensated liver cirrhosis. This confirms the functional significance of reduced renal 11β-HSD-2 level, observed in the present study during decompensation and in previous studies during the early preaschatic phase of liver impairment (1, 10, 23, 24). Inhibition of 11β-HSD-2 may be mediated by the high concentration of bile acids (1, 31, 33, 37) or other processes. However, it is interesting that the process was fairly specific for the nephron and affected 11β-HSD-2 mRNA and protein levels as well as enzyme activity. In this line, licorice-based inhibitors have been suggested to down-regulate 11β-HSD gene transcription, although the molecular mechanism is moot. This raises the intriguing possibility that treatments to induce renal 11β-HSD-2 activity as well as those that block cortisol production may be useful in hepatic failure-induced sodium and water retention.

Distal colon is another target for mineralocorticoids that has not previously been investigated in the context of the BDL model. The total balance studies showed a marked increase in
fetal NaCl losses after BDL, and in contrast to the kidney, 11β-HSD-2 mRNA expression was significantly upregulated in colon, whereas protein levels were unchanged. In addition, we found an increased expression of ENaC as also observed in the colon of portal vein-ligated rats (13). It has been hypothesized that downregulation of renal 11β-HSD-2 activity in liver cirrhosis could be mediated by the high plasma concentration of bile acids (1, 31, 33). The predominant mechanism of sodium reabsorption in the normal distal rat colon is a low-affinity, high-capacity electroneutral sodium transport through combined Na+/H+ and Cl-/HCO₃⁻ exchangers that are stimulated by glucocorticoids (4). In conditions with increased MR activation, the transport mechanism of distal colon changes from the low-affinity, high-capacity electroneutral sodium transport to a high-affinity, low-capacity transport through ENaC (5). The increased expression of ENaC in BDL rats is consistent with such a transition. The electrogenic transport through ENaC varies as a function of mineralocorticoid status. Increased activation of ENaC results in a lumen-negative potential, which favors leak of potassium back into the lumen. This also is consistent with the observed 50% decrease in intestinal sodium absorption in the cirrhotic rats and with the stimulation of intestinal sodium absorption by dexamethasone, which stimulates glucocorticoid receptors. In keeping with this interpretation, dexamethasone did not correct the intestinal potassium loss. The increased ENaC expression in colon of compensated rats was associated with a higher potassium loss into feces as one would predict from the transport characteristics. This finding could explain the lower plasma potassium concentration, which was not explained by potassium loss in the urine. In keeping with this, intestinal potassium loss, but not sodium loss, was corrected by treatment with K-canrenone. Thus, under the conditions investigated in the present study, changes in intestinal potassium transport account fully for the effects on total potassium homeostasis in BDL rats.

We conclude that development of liver cirrhosis in the BDL rat is associated with sequential changes in the renin-angiotensin-aldosterone system, but our results suggest that renal downregulation of 11β-HSD-2 and inappropriate activation of sodium absorption by glucocorticoids plays a major functional role for development of overt decompensation. In addition, our results point to an unexpected regulatory role of colon for potassium and sodium homeostasis in liver cirrhosis.

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