Intrarenal octreotide treatment prevents sodium retention in liver cirrhotic rats: evidence for direct effects within the thick ascending limb of Henle’s loop

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Intrarenal octreotide treatment prevents sodium retention in liver cirrhotic rats: evidence for direct effects within the thick ascending limb of Henle’s loop. *Am J Physiol Renal Physiol* 291: F537–F545, 2006. First published January 17, 2006; doi:10.1152/ajprenal.00226.2005.—We have previously shown that systemic treatment with the somatostatin analog octreotide has marked beneficial effects on renal function in rats with liver cirrhosis induced by common bile duct ligation (CBL; Jonassen TEN, Christensen S, Sørensen AM, Marcussen N, Flyvbjerg A, Andreasen F, and Petersen JS. *Hepatology* 29: 1387–1395, 1999). In the present study, we tested the hypothesis that octreotide has a direct effect on renal tubular function. Rats (CBL or Sham-CBL) were intrarenally treated with low-dose octreotide in a long-acting release formulation, which had no systemic actions (100 μg/kg body wt as a single dose). Rats receiving low-dose octreotide (sc) were used as controls. The rats were chronically instrumented, and renal function was examined 4 wk after CBL or Sham-CBL. Intrarenal octreotide administration (IROA) prevented sodium retention in CBL rats without changes in renal plasma flow, glomerular filtration rate, or circulating levels of aldosterone and vasopressin. Renal clearance studies revealed that IROA normalized the increased natriuretic efficacy of furosemide found in CBL rats. Furthermore, IROA protected against the development of hypertrophy of the inner stripe of the outer medulla and thereby increased the volume of thick ascending limb of Henle’s loop (TAL) epithelium found in CBL rats. Finally, Western blot analyses of outer medullary homogenates showed increased abundance of the furosemide-sensitive Na-K-2Cl (NKCC2) cotransporter. IROA did not affect the abundance of NCKK2 within the outer medulla. Together with the histological findings, these results indicate that IROA reduces the total number of NKCC2 within the outer medulla. In conclusion, the results indicate a direct intrarenal effect of octreotide on TAL function and morphology in cirrhotic rats.

octreotide-LAR; inner stripe of outer medulla; sodium retention; furosemide

**Somatostatin** is a 14-amino acid peptide that is present in the hypothalamus, the gastrointestinal tract, as well as in the kidneys (7). It serves as a paracrine inhibitor of growth hormone secretion from the hypothalamus and of the release of glucagon, insulin, and gastrin in the gastrointestinal tract (25, 31, 37). In addition to this, it has been shown that the long-acting analog octreotide has the ability to reverse systemic vasodilatation and ameliorate renal sodium retention in rats with carbon tetrachloride-induced liver cirrhosis (39).

Somatostatin and the octreotide have been shown to have antiproliferative effects within the kidneys in experimental models of diabetes mellitus (DM) and liver cirrhosis. In both mice and rats with streptozotocin-induced DM, octreotide treatment significantly reduce both glomerular and proximal tubular hypertrophy by a mechanisms that has been suggested to be involve inhibition of insulin like growth factor 1 (IGF1) hypersecretion (33). In rats with liver cirrhosis induced by common bile duct ligation (CBL), we have recently shown that long-time treatment with octreotide completely abolished the development of hypertrophy of the thick ascending limb of Henle’s loop (TAL) within the inner stripe of the outer medulla (ISOM) that is a marked characteristic for this model of cirrhosis (16, 19, 21). Moreover, the prevention of hypertrophy was associated with a complete normalization of sodium balance and normalization of increased natriuretic efficacy of furosemide, another characteristic of this model of cirrhosis. In contrast to the finding in the streptozotocin-induced DM animals, octreotide treatment did not have any effect on the circulating levels of growth hormone or IGF1 (19).

In addition to these renal effects of octreotide treatment in DM and liver cirrhosis, a number of studies indicate direct tubular actions of somatostatin and thereby also octreotide. Recently, the renal segmental expression pattern of the type 1 and 2 somatostatin receptors (sst1 and sst2) in human kidneys has been reported by Balster and co-workers (3) showing that Gs-coupled sst2, the somatostatin receptor subtype with the highest affinity for octreotide (13), is present within the TAL. In vitro studies have shown that somatostatin inhibits the vasopressin (AVP)-stimulated cAMP generation in microdissected rat TAL and collecting ducts (CD) (14, 40) associated with a marked inhibitory effect of somatostatin on the antidiuretic effect of AVP in vivo (40). Moreover, intrarenal infusion of somatostatin or octreotide in anesthetized dogs produces a fast increase in urine flow rate and free water clearance (28, 29, 34). Finally, Turman and Apple (38) have shown that human proximal tubules in culture express somatostatin in a regulated fashion. Together, these findings suggest that somatostatin might work in a paracrine way within the kidneys.

The aim of this study was to test the hypothesis that the previously described (19) therapeutic effects of long-term oc-
treatment on structure and function of the TAL in cirrhotic rats are mediated by a direct renal effect of the compound. For that purpose, rats with liver cirrhosis induced by CBL, were treated intrarenally with octreotide in a long-acting release formulation (octreotide-LAR). With this formulation, octreotide is incorporated into microspheres of a biodegradable polymer and constant plasma concentrations can be obtained for >4 wk after a single subcutaneous (sc) injection (10). CBL rats and sham-operated normal rats were given a single intrarenal low-dose injection (100 μg/kg body wt). Control rats received the same sc dose, which demonstrated that this dose had no systemic hemodynamic effects.

The effects of intrarenal octreotide treatment on overall sodium balance were studied by conducting sodium balance studies in metabolic cages 4 wk after the induction of liver cirrhosis by CBL. Renal function studies were conducted in conscious, chronically instrumented rats, where the natriuretic efficacy of furosemide was used as an indirect measure of TAL sodium handling. The effect of intrarenal octreotide treatment on outer medullary hypertrophy was evaluated by stereological examination, and finally we conducted Western blotting analyses to measure the protein level of furosemide-sensitive Na-K-2Cl cotransporter (NKCC2) and the α-subunit of the Na-K-ATPase within the ISOM.

**EXPERIMENTAL PROCEDURES**

**Animal Preparation**

Barrier-bred and specific pathogen-free female Wistar rats (210–230 g) were obtained from the Department of Experimental Medicine, the Panum Institute, University of Copenhagen (Copenhagen, Denmark). The animals were housed in a temperature (22–24°C)- and moisture (40–70%)-controlled room with a 12:12-h light-dark cycle (light on from 6 AM to 6 PM). Animals were given free access to tap water and a diet with 140 mmol/kg of sodium, 275 mmol/kg potassium, and 23% protein (Altromin, Lage, Germany). All animal procedures followed the guidelines for the care and handling of laboratory animals established by the Danish Government. Cirrhosis was induced by CBL as described by Kountouras and co-workers (23).

Briefly, biliary obstruction induces portal inflammation and bile duct proliferation, which eventually results in the formation of cirrhosis. After 5 wk the livers have a firm consistence with a yellow, micronodular surface. Histologically, the livers are fibrotic, with regeneration nodules due to extensive periporal fibrosis along with pronounced proliferation of bile ducts. The residual hepatocytes show reactive changes with cytoplasmatic and nuclear enlargement (16, 23). Together, these changes are consistent with secondary biliary cirrhosis. Control rats were subjected to sham-CBL.

On the day of CBL or sham-CBL, rats were treated with octreotide-LAR (11) or microparticles without active substance (vehicle). The dose of octreotide-LAR (100 μg/kg body wt) was 100 times lower than the dose we previously used (19), and dose-response experiments showed that sc administration of this low dose of octreotide-LAR had no effect on renal hemodynamics, the plasma level of aldosterone, or daily sodium balance in CBL rats. One-half of the rats received intrarenal injections of octreotide-LAR (100 μg/kg body wt) given as 4 injections in each kidney in a total volume of 16 μl and sc injections of vehicle (80 μg/kg body wt). The other one-half of the rats received intrarenal injections of vehicle (4 injections in each kidney in a total volume of 16 μl) and sc injections of octreotide-LAR (100 μg/kg body wt). Injection of octreotide-LAR or vehicle (microparticles) was done by use of a 4-μl microsyringe where the needle was set at a 90° angle to the surface of the kidney. The injections were then given within the corticomedullary border. To avoid infection, all injections were made under aseptic conditions.

**Series I (Sodium Balance, Renal Function, Plasma Biochemistry, and Histology)**

Two weeks after CBL or sham-CBL, permanent, medical grade Tygon catheters were implanted into the femoral artery and vein and a permanent suprapubic bladder catheter was implanted into the urinary bladder as described previously (16–19, 21, 32). After instrumentation, the animals were housed individually.

**Experimental groups.** The experimental groups were as follows: control, sham-operated rats treated with intrarenal vehicle and octreotide-LAR sc; control-IR, sham-operated rats treated with intrarenal octreotide-LAR and vehicle sc; CBL, CBL rats treated with intrarenal vehicle and octreotide-LAR sc; and CBL-IR, CBL rats treated with intrarenal octreotide-LAR and vehicle sc.

**Metabolism studies.** During the last 5 days before the renal function study, rats were housed in metabolic cages (Tech-Cot, model 1700, Scandbur, Lellinge, Denmark) which allowed accurate determination of 24-h urine volume and food and water intake. During housing in the metabolic cages, the diet was changed to a granulated standard diet with the same sodium and potassium content as the pelleted diet given before the stay in the metabolic cages (140 mmol/kg of sodium, 275 mmol/kg potassium; catalog no. 1310, Altromin International), which has added lithium citrate (12 mmol of lithium/kg dry diet). This dose of lithium given in the diet produced plasma lithium concentrations in the range 0.1–0.2 mmol/l without influencing renal function (24). After 2 days of adaptation, daily 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. Daily sodium balance was calculated as sodium intake minus urinary sodium excretion: the average over 3 days was used to evaluate the degree of sodium retention. To optimize urinary recovery of sodium, the metabolic cage was rinsed with 40–50 ml of demineralized water after every urine collection.

**Renal clearance study.** The animals were transferred to a restraining cage 4 wk after CBL or sham-CBL. Renal function was examined by clearance techniques where [14C] 5-[ethylammonium] bromide clearance was used as a marker for the effective renal plasma flow (ERPF), [3H]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. Renal clearances (C) and fractional excretions (FE) were calculated by the standard formula

\[ C = \frac{U \times V}{P} \]

where \( U \) is concentration in urine, \( V \) is urine flow rate, and \( P \) is plasma concentration (for further details, see Refs. 16–19, 21).

After a 90-min equilibration period, urine was collected during two 30-min control periods. Then, furosemide (7.5 mg/kg body wt; Dumex, Copenhagen, Denmark) was given as an intravenous bolus injection (37.5 μg furosemide/s) and urine was collected in four periods of 10 min each. Arterial blood samples of 300 μl each were collected at the end of the equilibration period, at the end of the control periods, and at the end of the experiment. All blood samples were replaced immediately with heparinized blood from a normal donor rat. Mean arterial pressure and heart rate (HR) were measured continuously as described previously (16–19, 21). Urine volume was determined gravimetrically. Concentrations of sodium, potassium, and lithium in plasma and urine were determined by atomic absorption spectrophotometry. [3H]Inulin and [14C] 5-[ethylammonium] bromide in plasma and urine were determined by dual-label liquid scintillation counting. The concentration of furosemide in urine was determined by HPLC (2; for further details, see Refs. 16–19, 21).

**Plasma biochemistry.** The plasma concentration of aldosterone was measured by radioimmunoassay using a commercial kit (Coat-A-Count Aldosterone, DPC, Los Angeles, CA). Vasopressin was extracted from plasma in C18 SEP-Pak cartridges and measured by a radioimmunoassay as described earlier (22). All blood samples were
taken from the arterial catheter and replaced immediately with heparinized blood from a normal donor rat.

Histological examinations. Three days after the renal clearance study the left kidney was perfused in vivo for 3 min with 1.5% glutaraldehyde in Tyrode’s solution with added 2.25% dextran T-40 (perfusion pressure 150 mmHg) and postfixed in perfusion fluid. Perfusion-fixed kidneys were then cut into slices with alternating thicknesses of 1 and 2 mm. The cutting was done at a 90° angle on the longitudinal axis of the kidney. The 2-mm-thick slices were embedded in paraffin and 3- to 4-μm-thick sections were cut and stained with hematoxylin-eosin or Masson-trichrome. Data from all 2-mm-thick slices were included in the material, which means that about six to seven sections from each kidney were examined. The Masson-trichrome-stained sections were used for estimation of the volume fraction of the different zones in the rat kidney by use of computerized point counting. The total number of points hiding each kidney slice was 200–300.

The absolute volumes of the renal zones were estimated by multiplying the volume fractions with the volume of the kidney (equal to the kidney weight assuming that the specific gravity of the kidney is 1 g/cm³).

Then, the volume fractions of the epithelium and the tubular lumen within the ISOM were estimated by count counting (a /H11003 concentration of 2 Rockford, IL). All samples were then diluted to a final protein (82 ng/ml buffer) with pH adjusted to 7.2 with 0.1 M HCl. Protein salt, protease inhibitors Pefabloc (0.1 mg/ml buffer) and leupeptin (4 taining 300 mM sucrose, 25 mM imidazole, 1 mM EDTA-disodium Ika, Staufen, Germany) in 9 ml ice-cold homogenizing buffer con-

and the right kidney was removed, and the outer medulla was isolated from the arterial catheter and replaced immediately with hepa-

then fixed in perfusion fluid. Glutaraldehyde in Tyrode’s solution with added 2.25% dextran T-40 (perfusion pressure 150 mmHg) and postfixed in perfusion fluid. Perfusion-fixed kidneys were then cut into slices with alternating thicknesses of 1 and 2 mm. The cutting was done at a 90° angle on the longitudinal axis of the kidney. The 2-mm-thick slices were embedded in paraffin and 3- to 4-μm-thick sections were cut and stained with hematoxylin-eosin or Masson-trichrome. Data from all 2-mm-thick slices were included in the material, which means that about six to seven sections from each kidney were examined. The Masson-trichrome-stained sections were used for estimation of the volume fraction of the different zones in the rat kidney by use of computerized point counting. The total number of points hiding each kidney slice was 200–300.

The absolute volumes of the renal zones were estimated by multiply-

ing the volume fractions with the volume of the kidney (equal to the kidney weight assuming that the specific gravity of the kidney is 1 g/cm³).

Then, the volume fractions of the epithelium and the tubular lumen within the ISOM were estimated by count counting (a ×100 oil objective and a grid with 16 points were used). One-third of the points were circled and used for the estimation of reference space, whereas all other points were used for estimation of tubular epithelium and lumen.

The absolute volume of the TAL epithelium was calculated as

\[ V_{\text{TAL}} = V_{\text{V}}(\text{TAL}) \cdot V_{\text{V}}(\text{ISOM}) \cdot V(\text{kidney}) \]

where \( V_{\text{V}}(\text{TAL}) \) is the volume fraction of TAL epithelium in the ISOM, \( V_{\text{V}}(\text{ISOM}) \) is the volume fraction of ISOM, and \( V(\text{kidney}) \) is the kidney volume. The absolute volumes of the CD epithelium as well as tubular lumen of the TAL and CD were calculated accordingly (for further details, see Refs. 11, 12, 16, 19 and 21).

Series 2 (Western Blotting)

An additional set of animals (8 in each group) was used for Western blot analyses. Rats were anesthetized with isoflurane/N₂O, and the right kidney was removed, and the outer medulla was isolated and then homogenized using a tissue homogenizer (Ultra-Turrax T8, Ika, Staufen, Germany) in 9 ml ice-cold homogenizing buffer containing 300 mM sucrose, 25 mM imidazole, 1 mM EDTA-disodium salt, protease inhibitors Pefabloc (0.1 mg/ml buffer) and leupeptin (4 μg/ml buffer), and phosphatase inhibitors sodium orthovanadate (184 μg/ml buffer), sodium fluoride (1.05 mg/ml buffer), and okadaic acid (82 ng/ml buffer) with pH adjusted to 7.2 with 0.1 M HCl. Protein concentration in the supernatant was measured by use of a commercial kit (BCA Protein Assay Reagent Kit, catalog no. 23226, Pierce, Rockford, IL). All samples were then diluted to a final protein concentration of 2 μg/μl by adding sample buffer (in the final solution: 486 mM Tris-HCl, pH 6.8, 8.7% glycerol, 104 mM SDS, 0.0875 mM bromphenol blue), dithiothreitol (25 mM in the final solution), and homogenizing buffer. Finally, the samples were solubi-

lized at 60°C for 10 min.

Samples of homogenates were run on 7.5% polyacrylamide gels. The proteins were then transferred by electrophoresis from the gels to polyvinylidene difluoride membranes (90 min, 100 V, 200 mA). After blocking in 5% milk in PBS-T buffer for 60 min, membranes were probed overnight at 4°C with the appropriate primary antibody. For measurement of NKCC2, we used a rabbit polyclonal anti-NKCC2 antibody raised to a synthetic peptide corresponding to amino acids 33–55 of rat NKCC2 (9, 21). For measurement of Na-K-ATPase, we used a rabbit polyclonal anti-Na,K-ATPase antibody [P448 (rabbit) or P0449 (goat), diluted 1:3,000; Dako] using an enhanced chemiluminescence system (ECL+. Amersham). The broad ~165-kDa band corresponding to NKCC2 and the ~96-kDa band corresponding to Na-K-ATPase were scanned by use of a FluorX Multi Imager (Bio-Rad Laboratories). Densitometry of individual bands was quantified using the software program Quantity One, version 4.2.3 (Bio-Rad Laboratories). Every blot was run in duplicate, and protein labeling in samples from the different groups was ex-

pressed relative to the mean expression of the control material run on the same gel. Because we only were able to run eight samples on the same gel (loading volume 20 ml), we had to split the total number of samples on two separate blots.

Statistics

Data are presented as means ± SE. To evaluate the effects of intrarenal octreotide-LAR treatment, average values during the two 30-min control periods were used for comparisons among groups. 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 compar-

isons 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

Body and Organ Weights

In agreement with our previous findings (16–19, 21), CBL rats and control rats had the same average daily weight gain and similar body weights at the end of the study period. When the abdominal cavity was exposed, there were no signs of ascites in any animals. Kidney weight and kidney-to-body weight ratio were significantly increased in the CBL group relative to control animals. Intrarenal octreotide treatment prevented renal hypertrophy in cirrhotic rats (Table 1).

Plasma Biochemistry

The plasma aldosterone concentration was similar in all four groups, whereas the plasma vasopressin concentration was significantly increased in CBL rats. Plasma sodium and potassium concentrations and plasma osmolality were within the normal range in the CBL rats and similar to plasma levels found in control animals. All parameters were unaffected by the mode of octreotide administration (Table 2).

Effect of Octreotide on Renal Sodium Balance

Figure 1 shows daily sodium intake, sodium excretion, and sodium balance (intake minus urinary excretion) during the last 3 days before the renal clearance experiments. All rats were in positive sodium balance, which is in accordance with the

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>Kidney Weight-to-Body Weight Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>240 ± 3</td>
<td>2.38 ± 0.18</td>
<td>0.99 ± 0.08</td>
</tr>
<tr>
<td>Control-IR (n = 7)</td>
<td>242 ± 9</td>
<td>2.41 ± 0.11</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>CBL (n = 7)</td>
<td>255 ± 7</td>
<td>3.26 ± 0.03</td>
<td>1.29 ± 0.05</td>
</tr>
<tr>
<td>CBL-IR (n = 7)</td>
<td>254 ± 5</td>
<td>2.60 ± 0.14</td>
<td>1.02 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; IR, intrarenal octreotide administration; CBL, common bile duct ligation. *P < 0.01 vs. control. †P < 0.01 vs. CBL.
positive weight gain found in all four groups. The daily sodium intake was similar in all four groups, suggesting that neither CBL nor treatment with octreotide-LAR affected the rats’ appetite. However, daily renal sodium excretion was significantly lower in the CBL group than in controls. Thus the CBL group had sodium retention relative to controls. Intrarenal octreotide-LAR administration had no effect on sodium balance in control rats, but it prevented sodium retention in cirrhotic rats due to a significant increase in the daily renal sodium excretion (+32% relative to CBL). Daily water intake, urine flow rate, and urine osmolality were similar in all groups (data not shown).

Systemic and Renal Hemodynamics

Table 3 shows systemic and renal hemodynamics before and during the diuretic peak response to intravenous furosemide in the four groups. Liver cirrhosis was associated with a marked increase in ERPF (+34% compared with controls), a fall in effective filtration fraction (EFF; −24% compared with controls), whereas GFR was unchanged. In contrast to our previous study (19) showing that systemic octreotide treatment normalized both ERPF and EFF in cirrhotic rats, neither ERPF nor EFF was corrected by intrarenal octreotide administration. In all four groups, systemic and renal hemodynamic variables were unchanged during the furosemide-induced peak diuresis.

Renal Water and Electrolyte Handling

During baseline conditions, V, U_{NaV}, FE_{Na}, and FE_{Li} were similar in all groups¹ (data not shown). However, during the diuretic peak response (0–10 min after furosemide administration) (Fig. 2), the diuretic and the natriuretic responses to furosemide were significantly increased in the CBL group compared with controls. Thus the diuretic response to furosemide was increased by 63% (V: 183.5 ± 11.1 vs. 112.9 ± 6.0 μl·min⁻¹·100 g body wt⁻¹; P < 0.001) and the natriuretic response by 64% (U_{NaV}: 19.45 ± 1.32 vs. 11.88 ± 1.10 μmol·min⁻¹·100 g body wt⁻¹; P < 0.001). Intrarenal octreotide treatment did not modify the diuretic and natriuretic responses to furosemide in the control rats, but in CBL-IR group these responses were significantly attenuated (V: 132.5 ± 9.0 vs. 183.5 ± 11.1 μl·min⁻¹·100 g body wt⁻¹; P < 0.001 and U_{NaV}: 14.56 ± 0.81 vs. 19.45 ± 1.32 μmol·min⁻¹·100 g body wt⁻¹; P < 0.001). The lithiuretic response to furosemide was similar in all four groups.

¹ Renal clearance experiments were performed during the inactive period of the rat (i.e., during the daytime), when sodium- and water-retaining mechanisms are maximally activated. To get stable urine production during these conditions, all rats were slightly water loaded by infusion of a hypotonic glucose solution (2.5 ml/h). Therefore, as previously described, baseline levels of urine flow rate (V) were similar (i.e., clamped) in all 4 groups as previously demonstrated (16–19, 21). Furthermore, in accordance with previous studies performed during the daytime and with low sodium infusion (infusion rate 32.5 μmol Na/h) (16–19), renal sodium handling was similar in all 4 groups.
Furosemide Excretion Rate and Natriuretic Efficiency of Furosemide

Furosemide is secreted in the proximal tubules and is delivered to its site of action in the TAL by the tubule fluid. Because furosemide is not reabsorbed in nephron segments further downstream, the urinary furosemide excretion rate (UFurV) reflects the amount of furosemide delivered to the NKCC2 at the luminal site of the TAL (6). Thus the natriuretic efficiency of furosemide is calculated as

\[ \text{"Natriuretic Efficiency" = } \frac{UNaV}{UFurV} \]

This measure is considered the most accurate parameter for comparisons of the natriuretic response of furosemide among different treatment groups. UFurV was increased by 29% in the CBL group. Intrarenal octreotide treatment did not affect UFurV in cirrhotic or control rats. Natriuretic efficiency was increased by 53% in the CBL groups compared with the control group (0.69 ± 0.05 vs. 0.44 ± 0.04 μmol Na/μg furosemide; \( P < 0.001 \)), and this increase in efficiency was prevented by intrarenal octreotide administration [0.51 ± 0.02 μmol sodium/μg furosemide; \( P < 0.001 \) vs. CBL; not significant (NS) vs. control]. Thus intrarenal octreotide treatment normalized the natriuretic response to furosemide in CBL rats (Fig. 3).

Renal Histopathology

Measurement of the renal zones by use of point counting revealed that the CBL group, as previously shown (16, 19, 21), had a marked increase in the fractional volume of the ISOM with a compensatory reduction of the fractional volume of the other renal zones, which was most pronounced within the cortex (Table 4). Intrarenal octreotide treatment was associated with a normalization of the renal zones in CBL rats (Fig. 3).

Table 3. Systemic and renal hemodynamics before (control) and during the diuretic peak response 0–10 min after iv administration of furosemide (7.5 mg/kg body wt) 4 wk after CBL or sham-CBL (control) in rats chronically treated with intrarenal octreotide

<table>
<thead>
<tr>
<th>MAP, mmHg</th>
<th>ERPF, ml/min-1·100 g body wt-1</th>
<th>GFR, ml/min-1·100 g body wt-1</th>
<th>EFF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>117 ± 3</td>
<td>120 ± 3</td>
<td>3.91 ± 0.17</td>
</tr>
<tr>
<td>Control-IR (n = 7)</td>
<td>119 ± 3</td>
<td>118 ± 4</td>
<td>4.03 ± 0.14</td>
</tr>
<tr>
<td>CBL (n = 7)</td>
<td>113 ± 3</td>
<td>116 ± 3</td>
<td>5.23 ± 0.28*</td>
</tr>
<tr>
<td>CBL-IR (n = 7)</td>
<td>115 ± 2</td>
<td>114 ± 4</td>
<td>4.91 ± 0.25†</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; Fur, furosemide; MAP, mean arterial pressure; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; EFF, effective filtration fraction. *\( P < 0.01 \) vs. control. †\( P < 0.01 \) vs. CBL.

Fig. 2. Furosemide was administered in a bolus injection (7.5 mg/kg body wt iv) to conscious, chronically instrumented rats subjected to CBL or sham-CBL (control) 4 wk earlier and chronically treated with intrarenal octreotide. Values are means ± SE; n = 7/group. V, urine flow rate; UNaV, urinary sodium excretion rate; FENa and FELi, fractional excretion of sodium and lithium, respectively. *\( P < 0.001 \) vs. control. #\( P < 0.01 \) vs. CBL.
Furosemide (Fur) excretion rate (U_{Fur}V) and natriuretic efficiency of furosemide (U_{NaV}/U_{Fur}V) during furosemide peak diuresis. Furosemide was administered in a bolus injection (7.5 mg/kg body wt iv) to conscious, chronically treated with intrarenal octreotide. Values are means ± SE; n = 7/group. *P < 0.05 vs. control within period. †P < 0.05 vs. CBL.

with a normalization of the volume of the ISOM and thereby also a normalization of the fractional volume of the other renal zones including the cortex (Table 4).

The absolute volumes of the renal zones are shown in Fig. 4. The absolute volume of the ISOM was significantly increased in the CBL group compared with controls (absolute volume: 249 ± 12 vs. 160 ± 11 mm³; P < 0.001). This selective hypertrophy of the ISOM in kidneys from cirrhotic rats was prevented by intrarenal octreotide administration (absolute volume: 131 ± 12 vs. 249 ± 12 mm³; P < 0.001). Intrarenal octreotide treatment did not affect the volume distribution of the renal zones in control rats (Table 4 and Fig. 4). Compared with controls, the CBL group had a 11% increase in fractional and a 52% increase in absolute volume of TAL epithelium (absolute volume: 78.4 ± 4.8 vs. 51.6 ± 3.8 mm³; P < 0.001). This cellular hypertrophy in CBL rats was prevented by intrarenal octreotide administration (absolute volume: 55.4 ± 1.2 vs. 78.4 ± 4.8 mm³; P < 0.001).

Whereas the fractional volume of the CD epithelium was unchanged, the absolute volume was increased by 61% in the CBL group relative to controls (23.3 ± 1.2 vs. 14.5 ± 1.4 mm³; P < 0.001). This increase was also prevented by intrarenal octreotide treatment. Intrarenal octreotide treatment had no significant effects on tubular epithelial volumes in control rats. The absolute volumes of the tubular lumen in both TAL and CD epithelium were similar in all four groups (data not shown).

**Western Blot Analyses of Sodium Transporters in Renal Outer Medulla**

Figure 5 shows Western blots of membrane fractions (40 μg protein/lane) from renal outer medullary preparations. As previously shown (21), densitometric analyses revealed increased protein levels of NKCC2 in CBL rats compared with sham-operated controls (control: 100 ± 14% vs. CBL: 243 ± 38%; P < 0.05). Octreotide treatment did not affect the protein levels of NKCC2 in either the sham-operated rats or in the rats with CBL-induced liver cirrhosis (control: 100 ± 11% vs. Sham: 99 ± 22%, NS; CBL: 100 ± 2% vs. CBL-IR: 96 ± 21%, NS). Neither cirrhosis nor intrarenal octreotide treatment affected the abundance of the α₁-subunit of the Na-K-ATPase within the outer medulla (control: 100 ± 20% vs. CBL: 131 ± 16%, NS; Sham: 100 ± 11% vs. Sham-IR: 96 ± 10%, NS; CBL: 100 ± 9% vs. CBL-IR: 110 ± 18%, NS). Together with the histological findings, showing that intrarenal octreotide treatment protected against the development of TAL hypertrophy within the outer medulla, the results indicate that the total number of transporters was reduced but at the single-cell level the number of transporters was unaffected.

**DISCUSSION**

A number of studies in humans and experimental animal models have shown that treatment with the somatostatin analog octreotide reverses systemic vasodilatation in conditions with portal hypertension due to portal vein stenosis or cirrhotic liver disease (1, 17, 29, 31, 43, 45). In agreement with this, we have previously shown that long-time treatment with octreotide in a 100-fold higher dose than used in the present study (19) prevented renal vasodilatation in cirrhotic rats without affecting mean arterial pressure in rats with CBL-induced liver cirrhosis. In accordance with the peripheral arterial vasodilatation hypothesis by Schrier et al. (36), the prevention of

**Table 4. Effects of chronic intrarenal octreotide administration on fractional volumes of the renal zones and fractional volumes of the thick ascending limb of Henle’s loop and collecting ducts in the inner stripe of the outer medulla 1 mo after CBL or sham-CBL (control) in rats**

<table>
<thead>
<tr>
<th></th>
<th>Cortex, %</th>
<th>Outer Stripe of Outer Medulla, %</th>
<th>Inner Stripe of Outer Medulla, %</th>
<th>Inner Medulla, %</th>
<th>TAL in the Inner Stripe of Outer Medulla, %</th>
<th>CD in the Inner Stripe of Outer Medulla, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 7)</td>
<td>66.3 ± 1.1</td>
<td>17.9 ± 0.8</td>
<td>12.2 ± 0.5</td>
<td>3.6 ± 0.2</td>
<td>30.5 ± 1.1</td>
<td>8.5 ± 0.5</td>
</tr>
<tr>
<td>Control-IR (n = 7)</td>
<td>66.7 ± 1.3</td>
<td>17.3 ± 0.8</td>
<td>11.6 ± 0.8</td>
<td>4.4 ± 0.5</td>
<td>30.2 ± 1.2</td>
<td>9.4 ± 0.7</td>
</tr>
<tr>
<td>CBL (n = 7)</td>
<td>63.5 ± 1.0*</td>
<td>16.3 ± 0.6</td>
<td>16.5 ± 0.5*</td>
<td>3.7 ± 0.4</td>
<td>33.9 ± 0.8*</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td>CBL-IR (n = 7)</td>
<td>67.3 ± 1.6†</td>
<td>18.1 ± 1.2</td>
<td>10.7 ± 1.2†</td>
<td>3.8 ± 0.6</td>
<td>28.8 ± 1.7†</td>
<td>9.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. TAL, thick ascending limb of Henle’s loop; CD, collecting ducts. *P < 0.05 vs. control. †P < 0.05 vs. CBL.
vasodilatation was associated with a marked attenuation in the degree of sodium retention in the cirrhotic rats. However, based on a few studies indicating that somatostatin might have direct renal effects (14, 28, 29, 40), together with a recent study showing the presence of somatostatin receptors within the renal tubules (3), we hypothesized that octreotide might have direct tubular effects. We therefore examined the effect of long-time local intrarenal octreotide treatment in CBL rats. The finding in the present study can be summarized as follows: intrarenal octreotide treatment 1) prevented sodium retention, 2) prevented the development of increased furosemide-sensitive sodium reabsorption in the TAL, and 3) prevented hypertrophy of the TAL epithelium in the ISOM in the liver cirrhotic rats. Furthermore, intrarenal octreotide treatment had no effect on renal plasma flow and GFR and it did not affect the plasma levels of aldosterone and vasopressin. Together, these results suggest that intrarenal octreotide treatment prevents sodium retention in cirrhotic rats partly by inhibition of increased sodium reabsorption in the TAL. The lack of changes in renal hemodynamics and in plasma endocrinology suggests that octreotide may have a direct tubular action.

Within the outer medulla, octreotide prevented TAL hypertrophy and the development of increased efficacy of furosemide previously found not only in CBL-induced cirrhosis but also in carbon tetrachloride-induced cirrhosis (20). This increased furosemide efficacy most probably reflects increased sodium reabsorption within the TAL in experimental cirrhosis. The present study as well as a recent study from our laboratory (21) further point to the fact that the abundance of NKCC2 is markedly increased within this tubular segment in cirrhotic rats. Interestingly, octreotide treatment did not alter the relative abundance of the NKCC2 or the α-subunit of the Na-K-ATPase within the outer medulla. This suggests that octreotide has an antihypertrophic effect but not a direct effect on the sodium reabsorption capacity of individual cells within the TAL.

The antihypertrophic effect of octreotide within the kidneys in diabetes has been investigated intensively by Flyvbjerg and co-workers (33), who suggest that octreotide primarily acts through inhibition of growth hormone (GH) hypersecretion and inhibition of IGF1. In our previous study (19) where we gave octreotide systemically in a 100-fold higher concentration than in the present study, we could not show any changes in either GH or IGF1, which, on the other hand, were not increased in our vehicle-treated cirrhotic rats. This finding makes it rather unlikely that octreotide in the previous as well as in the present study inserts its antihypertrophic effects through interaction

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**Fig. 4.** Absolute volumes of the renal zones, i.e., cortex, outer stripe of the outer medulla (OSOM), the outer medulla (ISOM), and the inner medulla, as well as the absolute volumes of the epithelium from thick ascending limb of Henle’s loop (TAL) and collecting ducts (CD) in the ISOM from rats subjected to CBL or sham operation (control) 4 wk earlier and chronically treated with intrarenal octreotide. Values are means ± SE; n = 7/group. *P < 0.01 vs. control. #P < 0.01 vs. CBL.

**Fig. 5.** Immunoblots of membrane fractions (40 μg protein/lane) from a renal outer medulla preparation. The rats were subjected to CBL or sham operation (Sham) 4 wk earlier and chronically treated with intrarenal octreotide. The immunoblots were reacted with affinity-purified anti-Na-K-2Cl cotransporter (NKCC2; revealing a band at ~165 kDa; A) or affinity-purified anti-α-subunit of the Na-K-ATPase (revealing a band at ~96 kDa; B). LAR, long-acting release formulation. *P < 0.05 vs. Sham.
with the GH axis. In fact, the recent finding of ssr2 within the TAL (3) rather points to a direct effect of octreotide within this tubular segment.

ssr2 is Gq coupled, which means that stimulation of the receptor inhibits cAMP accumulation, and it has previously been shown that somatostatin has the ability to inhibit AVP-mediated cAMP accumulation within both the TAL and the CDs (14, 40). It is well known that AVP mediates its effect within the TAL through the Gs-coupled V2 receptor and AVP treatment of homozygous Brattleboro rats (characterized by central diabetes insipidus) induces marked hypertrophy within the ISOM (4). Similarly, we have previously shown the absence of TAL and thereby ISOM hypertrophy in Brattleboro rats with CBL-induced cirrhosis (16). Together, this points to the possibility that octreotide induces its antihypertrophic effects in the outer medullary TAL through inhibitory effects on the V2-signaling pathway.

If that is the mechanism responsible for the antihypertrophic effect of octreotide, then it could be questioned whether ssr2 stimulation also could interact with other Gs-coupled signal pathways within the TAL. We have recently shown that bilateral renal denervation, like octreotide treatment, has the ability to normalize renal sodium handling in rats with CBL-induced cirrhosis (21). Renal denervation not only had an effect on sodium balance, but it normalized the natriuretic response to furosemide and markedly reduced the abundance of NKCC2 within the outer medulla in CBL rats. A mechanism that most probably reflects that renal sympathetic nerves, through β-receptor stimulation, has marked stimulatory effects on sodium reabsorption within the TAL. However, renal denervation had no effect on the hypertrophy of the ISOM in these cirrhotic rats. It therefore seems unlikely that octreotide primarily acts through inhibition of β-adrenergic transmission within the TAL.

It has also been shown that glucagon has the ability to stimulate TAL sodium reabsorption within the outer medullary TAL (8), and we and others have shown increased fasting levels of glucagon in cirrhotic liver disease (19, 26). Furthermore, it has been described that high protein intake, which is known to increase fasting plasma glucagon, induces marked and selective hypertrophy of the outer medullary TAL (5). Together, this indicates that glucagon could contribute to the increased NaCl reabsorption and the epithelial hypertrophy in TAL in rats with liver cirrhosis. Further studies aimed to investigate the role of glucagon in the development of renal dysfunction in liver cirrhosis are warranted. Moreover, it has to be pointed out that the present study, which was focused on effects within the outer medullary TAL, does not exclude that octreotide has effects on other tubular segments, including the CDs.

A number of studies have examined the effect of octreotide in liver cirrhotic patients; however, most studies have focused on a potential beneficial effect on variceal bleeding and/or hepatorenal syndrome, whereas only a few studies specifically have focused on renal function. Mountokalakis and co-workers (30) reported in 1988 that acute intravenous treatment with octreotide had the ability to increase creatinine clearance and decrease urine osmolality in a small group of cirrhotic patients with ascites. In contrast to this, a number of studies examining the effect of continuous octreotide treatment have been unable to show significant effects on renal function. Sabat and co-workers (35) treated cirrhotic patients with ascites for 5 days with octreotide or vehicle and found that the treatment significantly reduced plasma renin, aldosterone, and glucagon, but despite these beneficial effects they were unable to show any effect on renal function. Similarly, Malesci and co-workers (26) were unable to show any improvement in renal function in 12 cirrhotic patients treated with octreotide for 2 wk and, finally, Ottesen and co-workers (31) have examined renal function before and after a single sc injection of octreotide-LAR. The treatment induced a plasma concentration of octreotide comparable to the plasma concentration found in our previous study in rats. Despite that, the authors were unable to see any changes in plasma concentration of a number of hormones including GH, IGF-1, renin, and aldosterone. Furthermore, they were unable to show any effect on renal function including renal plasma flow, GFR, and sodium excretion. Together, these studies indicate a lack of effect of octreotide in liver cirrhotic patients. However, none of the studies have looked at overall sodium balance doing the treatment period, and the setup can be questioned where renal function is examined during a relatively short renal clearance study during conditions that are comparable to the baseline conditions during the clearance experiment in the present study. In fact, renal clearance studies most often are designed to get stable urine production, and experiments are therefore most often conducted during conditions with relatively high infusion rates of water and sodium. This makes it very difficult to identify small changes in “real” excretion of sodium and water. This is the case in the present study, and this is most probably the case in most human studies. In fact, if the conclusions in the present study were drawn from baseline conditions alone (i.e., before furosemide infusion), where the handling of both renal water and sodium was similar in all groups due to a slight fluid overload, it would have been concluded that octreotide treatment was without effect on renal function. However, the metabolic cage studies show marked differences in 24-h sodium handling, and renal function after the test dose of furosemide also shows marked differences among the groups. Detailed sodium balance studies as well as renal clearance in liver cirrhotic patients aimed to investigate the effect of a tubular challenge, which could be furosemide infusion or extracellular volume expansion with isotonic saline, are therefore warranted to make final conclusions on the potential renal effect of octreotide treatment in liver cirrhotic patients.

In summary, the present study demonstrated that long-term intrarenal octreotide treatment prevented sodium retention associated with increased furosemide-sensitive sodium reabsorption and epithelial hypertrophy in the TAL in rats with CBL-induced liver cirrhosis. Furthermore, octreotide normalized tubular function in the absence of changes in renal hemodynamics or in the plasma levels of aldosterone and vasopressin. Together, the results suggest that octreotide might have direct tubular action on sodium reabsorption in the TAL in cirrhotic rats.

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References


