Losartan treatment normalizes renal sodium and water handling in rats with mild congestive heart failure

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Received 27 April 2001; accepted in final form 4 September 2001

Staailtoft, Dennis, Søren Nielsen, Nadeem R. Janjua, Sten Christensen, Ole Skøtt, Niels Marcussen, and Thomas E. N. Jonassen. Losartan treatment normalizes renal sodium and water handling in rats with mild congestive heart failure. Am J Physiol Renal Physiol 282: F307–F315, 2002; 10.1152/ajprenal.00132.2001.—This study was designed to examine the effect of losartan treatment on renal tubular function in rats with mild congestive heart failure (CHF) induced by ligation of the left anterior descending artery. In rats with CHF, there was a significant decrease in daily sodium excretion, which caused sodium retention relative to control rats. Renal function studies revealed that glomerular filtration rate and proximal tubular sodium handling were normal. However, expression of the Na+/K+/2Cl− cotransporter (NKCC2) in the thick ascending limb of Henle’s loop was increased. Moreover, vasopressin-mediated renal water reabsorption, as evaluated by the aquaretic response to selective V2-receptor blockade, was significantly increased. Losartan treatment normalized expression of NKCC2 and decreased expression of the vasopressin-regulated water channel aquaporin-2. This was associated with normalization of daily sodium excretion and normalization of the aquaretic response to V2-receptor blockade. Together, these results indicate that, in rats with CHF, losartan treatment inhibits increased sodium reabsorption through NKCC2 in the thick ascending limb of Henle’s loop and water reabsorption through aquaporin-2 in the collecting ducts, which may be involved in improving renal function in losartan-treated CHF rats.

aquaporin-2; sodium-potassium-2 chloride cotransporter; vasopressin; thick ascending limb; collecting ducts

Retention of sodium and water is a common and clinically important complication of congestive heart failure (CHF). The progressive decrease in left ventricular function results in activation of several neurohormonal compensatory systems, including the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). These compensatory mechanisms serve to maintain perfusion of vital organs but have several deleterious consequences. Renal perfusion and glomerular filtration rate (GFR) decrease and tubular reabsorption of sodium and water increases, resulting in extracellular volume expansion and, eventually, formation of edema. At terminal stages of the disease, the condition is complicated by increased vasopressin (AVP) levels, which result in excessive water retention and hyponatremia. In patients with early mild CHF and normal plasma levels of renin and aldosterone, the ability to excrete an acute sodium load is impaired, and when daily sodium intake is increased, these patients develop sodium retention (35, 36). The mechanisms behind this impairment in sodium handling in mild CHF are not fully clarified, but it has been suggested that renal sodium retention in mild CHF is caused by an increase in proximal tubular sodium reabsorption (21, 36). However, in another study using lithium clearance (CLi) as a marker for delivery of fluid out of the proximal tubules, Eiskjaer and co-workers (9) showed that proximal tubular function was normal in patients with CHF and increased plasma levels of renin and aldosterone.

Recent experimental studies from our laboratory suggest that functional changes in the thick ascending limb of Henle’s loop (TAL) may play an important role in conditions with early sodium retention. We previously showed increased sodium reabsorption in the TAL of liver cirrhotic rats with sodium retention but normal plasma aldosterone concentration (13, 14, 17). Moreover, it has recently been reported that expression of the furosemide-sensitive Na+/K+/2Cl− cotransporter (NKCC2) in the TAL is significantly increased in rats with CHF (23, 28). The changes were present in rats with mild to moderate CHF (28) and in rats with more severe CHF (23).

The rate of water reabsorption in the collecting ducts (CDs) is determined by transepithelial water permeability, which is regulated by AVP. However, sodium reabsorption in the TAL plays a major role in generation of the corticomедullary osmotic gradient, which is the driving force for the AVP-regulated CD water re-
absorption. A secondary effect of increased sodium reabsorption in the TAL would therefore be increased CD water reabsorption, which eventually could result in water retention. AVP regulates water permeability in CDs by increasing the expression and plasma membrane targeting of the membrane-bound water channel aquaporin-2 (AQP2) (for review see Ref. 26).

Treatment with the angiotensin-converting enzyme (ACE) inhibitor enalapril or with the ANG II type 1 (AT1)-receptor antagonist losartan normalized tubular function in patients with mild CHF and normal plasma levels of renin and aldosterone (21, 36). The present study was therefore designed to examine the effect of treatment with losartan on daily sodium balance and tubular function, including the expression of NKCC2 and AQP2 [total AQP2 and phosphorylated and, thereby, activated AQP2 (pAQP2) (4)] in rats with mild CHF induced by ligation of the left anterior descending coronary artery (LAD). CHF rats were characterized by increased left ventricular end-diastolic pressure (LVEDP) but normal plasma levels of renin, aldosterone, and AVP. Renal function was examined in chronically instrumented rats under control conditions and during acute administration of the AVP V2-receptor antagonist OPC-31260. The aquaretic response to acute V2-receptor blockade was, as previously shown (15, 16), used as an estimate of the AVP-mediated renal water reabsorption in vivo. [Within minutes, blockade of the V2 receptor causes an almost complete disappearance of AQP2 from the apical membrane of CD principal cells associated with a marked increase in solute-free urine production (3).]

**METHODS**

**Animal Preparation**

Female Wistar rats (230–260 g body wt; Charles River, Hannover, Germany) were given free access to tap water and a diet with 140 mmol/kg sodium, 275 mmol/kg potassium, and 23% protein. All animal procedures followed the guidelines for the care and handling of laboratory animals established by the Danish government. CHF was induced by ligation of the LAD. Brieﬂy, the rats were anesthetized with halothane-nitrous oxide and artificially ventilated with 100% oxygen after endotracheal intubation. A left thoracotomy was performed, and the LAD was ligated using a 6-0 silk suture. The thorax was closed in layers, and a negative pressure was induced in the left pleural cavity to unfold the lungs. Sham-operated animals underwent the same procedure without ligation of the LAD. Buprenorphine (0.05 mg/kg sc) was administered to relieve postoperative pain. At 2 wk after LAD or sham ligation, the rats were anesthetized with fentanyl-fluanisone and midazolam, and permanent Tygon catheters were implanted into the abdominal aorta and inferior caval vein via a femoral artery and vein. A permanent suprapubic bladder catheter was implanted into the urinary bladder. After instrumentation, the rats were housed individually (for details see Refs. 13, 14, 17, and 29).

**Experimental Protocol**

The study design is outlined in Fig. 1. The rats were anesthetized with halothane-nitrous oxide 3 wk after LAD ligation, and LVEDP was measured using a catheter inserted into the left ventricle via the right carotid artery. Halothane concentration was adjusted to stabilize mean arterial pressure (MAP) at the level in the awake animal before anesthesia. After the LVEDP measurement, the right carotid artery was tied off so that all the rats only had one functional carotid artery throughout the rest of the study. (Whether unilateral carotid artery occlusion itself may have an influence on the interpretation of the study is unknown. However, the right carotid artery of all the rats, including the sham-operated rats, was tied off.) Then half of the rats were randomized to losartan treatment for 10 days (10 mg·kg⁻¹·day⁻¹ ip) using implanted osmotic minipumps (Alzet 2ML4). On day 26 the rats were placed in metabolic cages for accurate determination of daily food and water intake. After 2 days of adaptation, sodium balance was measured daily for 3 days, and the average of the three values was used. Briefly, 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. Sodium balance was then calculated as the difference between sodium intake and sodium excretion (for details see Refs. 13, 14, and 17). Blood samples for determination of plasma levels of renin and AVP were drawn on the day of the renal clearance studies. Blood samples for aldosterone were drawn at the termination of the experiment.

The animals were divided into groups as follows: untreated sham-operated control rats (n = 10), losartan-treated sham-operated control rats (n = 11), untreated rats with CHF induced by LAD (n = 8), and losartan-treated rats with CHF induced by LAD (n = 8).
Renal Clearance Studies

Renal function was examined 31 days after LAD ligation or sham operation. The rats were placed in restraining cages, and renal function was examined by clearance techniques (13, 14, 17). Briefly, 14C-labeled tetraethylammonium bromide clearance was used as a marker for the effective renal plasma flow, [3H]inulin clearance as a marker for GFR, and C14 as a marker for the delivery of fluid from the proximal tubule (34). Renal clearances (C) and fractional excretions (FE) were calculated by the standard formula

\[ C = U \cdot V/P \]

\[ FE = C/GFR \]

where \( U \) is concentration in urine, \( V \) is urine flow rate, and \( P \) is plasma concentration.

Absolute proximal sodium reabsorption rate (APRNa) was calculated as follows: \( APR_{Na} = (GFR - C_{Li}) \cdot P_{Na} \). Fractional distal water reabsorption was calculated as \( V/C_{Li} \). MAP was measured throughout the study. After a 90-min equilibration period, urine was collected during two 30-min control periods. Then intravenous infusion of the selective \( V_2 \)-receptor antagonist OPC-31260 (prime 800 \( \mu \)g/kg body wt and 400 \( \mu \)g/kg \( \cdot \)h \( ^{-1} \)) was started. After a 60-min equilibration period, urine was collected in three 30-min periods. Total body water content was kept constant during \( V_2 \)-receptor blockade by intravenous replacement of urine losses with 150 mM glucose using a computer-driven servo-control system (for further details, including analytic procedures, see Refs. 13–17).

Measurement of NKCC2, AQP2, and pAQP2 by Semiquantitative Immunoblotting

The rats were anesthetized with halothane-nitrous oxide 4 days after the clearance experiment (e.g., 5 wk after CHF or sham operation), and the right kidney was removed and cut into 3- to 4-μm-thick sections. The heart were cut into 2-mm-thick slices, which were embedded in paraffin and cut into 3- to 4-μm-thick sections. The sections were stained with hematoxylin-eosin. The volume fraction of connective tissue was measured by point counting (for details see Ref. 11).

Statistics

Values are means ± SE. To evaluate the effects of \( V_2 \)-receptor blockade, the average value during the two 30-min control periods was compared with the average value during the last two 30-min periods during OPC-31260-induced diuresis. Comparisons were performed by two-way analysis of variance followed by Fisher’s least significant difference test. Differences were considered significant at the 0.05 level.

RESULTS

LVEDP and the amount of connective tissue in the heart were significantly increased in CHF rats (Table 1). Plasma levels of aldosterone, renin, and AVP were normal in CHF rats (Table 1). As expected, losartan treatment significantly increased the plasma level of renin in CHF and sham-operated rats. Plasma AVP levels were unchanged in the losartan-treated animals.
compared with the respective control groups. The plasma aldosterone level was significantly reduced in losartan-treated CHF rats compared with untreated CHF rats. This effect of losartan was not found in the sham-operated control rats (Table 1). Daily sodium intake was similar in all groups, but daily sodium excretion significantly decreased in CHF rats, which indicated sodium retention relative to control animals (Table 2). Losartan treatment had no effect on sodium handling in the sham-operated control rats. However, losartan treatment significantly increased daily sodium excretion in the CHF rats, with the result that sodium handling was normalized in these animals. Losartan treatment tended to increase daily urine excretion in CHF and sham-operated rats; however, the increase was not statistically significant (Table 2).

**Renal Function Studies**

**Baseline values.** Losartan caused a significant decrease in MAP in sham-operated and CHF rats. Losartan treatment did not affect effective renal plasma flow, GFR, C1,F, FE1, or APBNa, nor did these variables differ between CHF rats and sham-operated controls. Baseline values for V and sodium excretion rate were similar in all groups1 (Table 3).

**Effect of V2-receptor blockade on renal water handling.** As previously shown in normal and cirrhotic rats (12, 15, 16), MAP, renal plasma flow, GFR, C1,F, and FE1Na were unchanged during treatment with OPC-31260 (data not shown), indicating that V2-receptor blockade did not affect systemic and renal hemodynamics or the renal handling of sodium. Renal sodium handling was similar in all groups. V2-receptor blockade increased V, free water clearance, and fractional distal water excretion (V/C1,F) in all groups. As shown in Fig. 2, the aquaretic response to V2-receptor blockade was significantly increased in the CHF group compared with sham-operated controls. Losartan treatment normalized the aquaretic response to V2-receptor blockade in CHF rats.

**Renal Expression of NKCC2**

Figure 3, A and C, shows immunoblots of membrane fractions (20 μg protein/lane) from whole kidney preparations. As previously shown (8, 15), the affinity-purified anti-NKCC2 protein antibody recognizes the 161-kDa band. Densitometry of all samples (Fig. 3B) from CHF rats revealed a 33% increase in NKCC2 expression compared with sham-operated control rats: 133 ± 8 vs. 100 ± 5% (P < 0.05). Losartan treatment significantly decreased expression of NKCC2 in CHF rats: 77 ± 7 and 100 ± 12% (P = 0.05) in losartan-treated and untreated CHF rats, respectively (Fig. 3D). Losartan had no effect on NKCC2 expression in sham-operated rats: 100 ± 4 and 104 ± 7% in untreated and losartan-treated sham-operated rats, respectively (not significant; blot not shown).

**Renal Expression of AQP2 and pAQP2**

Figure 4, A and C, shows immunoblots of membrane fractions (20 μg protein/lane) from whole kidney preparations. As previously shown (7, 22, 27), the affinity-purified anti-AQP2 protein antibody recognizes the 29- and 35- to 50-kDa bands, corresponding to nonglycosylated and glycosylated AQP2 protein, respectively. Densitometry of all samples (Fig. 4B) revealed that AQP2 expression was unchanged in CHF rats. However, losartan treatment significantly decreased AQP2 expression in rats with CHF: 83 ± 6 and 100 ± 5% in losartan-treated and untreated CHF rats, respectively (P = 0.05; Fig. 4D). Losartan had no effect on AQP2 expression in sham-operated rats: 100 ± 6 and 109 ± 5% in untreated and losartan-treated sham-operated rats, respectively (not significant; blot not shown).

Similarly, Fig. 5, A and C, shows immunoblots of membrane fractions from whole kidney preparations. As previously shown (4), the affinity-purified anti-pAQP2 protein antibody recognizes the 29- and 35- to 50-kDa bands, corresponding to nonglycosylated and glycosylated pAQP2 protein, respectively. Densitometry (Fig. 5B) showed that pAQP2 expression was unchanged in the CHF rats. Losartan treatment significantly decreased pAQP2 expression in losartan-treated CHF rats: 80 ± 7 and 100 ± 4% in losartan-

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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 obtain a stable urine production under these conditions, all rats were slightly water loaded by infusion of a hypotonic glucose solution (2.5 ml/h), as previously described (15, 16). Therefore, baseline levels of V were similar (i.e., clamped) in all four groups, as previously demonstrated (15, 16). Furthermore, in accordance with previous studies performed during daytime and with a low sodium infusion rate (32.5 μmol/h) (15, 16), the renal sodium handling was similar in all four groups. Plasma osmolality was similar in all groups and was unchanged throughout the renal clearance experiments, which indicates that the servo-controlled intravenous volume replacement was effective (data not shown).

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Table 2. Daily urine production, sodium intake, sodium excretion, and sodium balance in rats

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 10)</th>
<th>Sham-LOS (n = 12)</th>
<th>CHF (n = 8)</th>
<th>CHF-LOS (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine production, ml·day⁻¹·100 g body wt⁻¹</td>
<td>9.5 ± 1.3</td>
<td>11.0 ± 1.3</td>
<td>9.5 ± 0.5</td>
<td>11.2 ± 1.2</td>
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<tr>
<td>Sodium intake, μmol/day</td>
<td>2232 ± 219</td>
<td>2377 ± 116</td>
<td>2289 ± 214</td>
<td>2372 ± 70</td>
</tr>
<tr>
<td>Sodium excretion, μmol/day</td>
<td>2194 ± 148</td>
<td>1890 ± 208*</td>
<td>2132 ± 108†</td>
<td></td>
</tr>
<tr>
<td>Sodium balance, μmol/day</td>
<td>87 ± 97</td>
<td>177 ± 65</td>
<td>438 ± 88*</td>
<td>240 ± 85†</td>
</tr>
</tbody>
</table>

Values represent average of 3-day collections and are expressed as means ± SE. Sodium balance = sodium intake − sodium excretion. *P < 0.05 vs. sham; †P < 0.05 vs. CHF.
Table 3. Effects of losartan treatment on MAP, ERPF, GFR, $\dot{V}$, $U_{\text{Na}V}$, $C_{\text{Li}}$, $F_{\text{ELi}}$, and $\text{APR}_{\text{Na}}$ in rats

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 10)</th>
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<th>CHF (n = 8)</th>
<th>CHF-LOS (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>114 ± 4</td>
<td>98 ± 2*</td>
<td>107 ± 4</td>
<td>94 ± 4*</td>
</tr>
<tr>
<td>ERPF, ml·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>3.78 ± 0.17</td>
<td>3.86 ± 0.21</td>
<td>3.69 ± 0.2</td>
<td>3.27 ± 0.25</td>
</tr>
<tr>
<td>GFR, ml·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>1.05 ± 0.04</td>
<td>1.11 ± 0.04</td>
<td>1.08 ± 0.06</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>$V_{\text{u}}$, µl·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>23.3 ± 2.8</td>
<td>23.3 ± 2.5</td>
<td>19.4 ± 2.4</td>
<td>19.5 ± 2.6</td>
</tr>
<tr>
<td>$U_{\text{Na}V}$, nmol·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>423 ± 151</td>
<td>354 ± 79</td>
<td>246 ± 154</td>
<td>376 ± 58</td>
</tr>
<tr>
<td>$C_{\text{Li}}$, ml·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>0.286 ± 0.031</td>
<td>0.333 ± 0.011</td>
<td>0.308 ± 0.035</td>
<td>0.287 ± 0.025</td>
</tr>
<tr>
<td>$F_{\text{ELi}}$, %</td>
<td>26.8 ± 2.2</td>
<td>30.6 ± 1.5</td>
<td>28.3 ± 1.8</td>
<td>29.1 ± 1.2</td>
</tr>
<tr>
<td>$\text{APR}_{\text{Na}}$, mmol·min$^{-1}$·100 g body wt$^{-1}$</td>
<td>0.109 ± 0.003</td>
<td>0.108 ± 0.007</td>
<td>0.109 ± 0.006</td>
<td>0.103 ± 0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; $V_{\text{u}}$, urine flow rate; $U_{\text{Na}V}$, urinary sodium excretion; $C_{\text{Li}}$, lithium clearance; $F_{\text{ELi}}$, fractional lithium excretion; $\text{APR}_{\text{Na}}$, absolute proximal sodium reabsorption. *P < 0.05 vs. sham; †P < 0.05 vs. CHF.

treated and untreated CHF rats, respectively ($P = 0.05$; Fig. 5D). Losartan had no effect on pAQP2 expression in sham-operated rats: $100 ± 8$ and $105 ± 5\%$ in untreated and losartan-treated sham-operated rats, respectively (not significant; blot not shown).

**DISCUSSION**

The major finding of the present study is that losartan treatment significantly improves renal function in rats with mild CHF by a mechanism that probably involves inhibition of increased sodium reabsorption in the TAL.

Sodium balance studies revealed sodium retention in rats with mild CHF (normal circulating levels of renin and aldosterone) relative to control rats. Renal function studies showed normal proximal tubular function. However, expression of NKCC2 in the TAL was increased, and the AVP-mediated renal water reabsorption, as evaluated by the aquaretic response to selective $V_{2}$-receptor blockade, was significantly increased in these rats. Losartan treatment normalized expression of NKCC2 and decreased expression of the AVP-regulated water channel AQP2. This was associated with normalization of the sodium balance and the aquaretic response to $V_{2}$-receptor blockade.

**Sodium Retention in Mild CHF**

CHF is characterized by a decrease in left ventricular function and cardiac output, which results in compensatory activation of several neurohormonal systems. Along with the activation of the RAAS and the sympathetic nervous system, renal perfusion and GFR will decrease and tubular sodium reabsorption will increase. However, recent studies in patients with mild CHF (35, 36) as well as the present study show that renal sodium retention can occur without increased plasma levels of renin and aldosterone. The mechanism that initiates sodium retention in mild CHF is uncertain. Volpe and co-workers (35, 36) suggested that the tubular dysfunction in sodium handling in patients with mild CHF is localized to the proximal tubules. This assumption was based on data from clearance experiments during water loading, where they found that fractional free water clearance and $F_{\text{Ei}}$ were significantly decreased in patients with mild CHF, suggesting decreased distal tubular delivery of sodium in these patients. Eiskjaer and co-workers (9) used the $C_{\text{Li}}$ technique to evaluate proximal tubular function. They found that $F_{\text{ELi}}$ was normal in patients with CHF and increased plasma levels of renin and aldosterone. This suggests that delivery of fluid out of the proximal tubules was normal in these CHF patients. In the present study, $\text{APR}_{\text{Na}}$ was unchanged in rats with mild CHF. However, functional data from the clearance experiments (see below) and immunoblot data suggest that TAL sodium reabsorption was increased in these rats. It is notable that increased sodium reabsorption in TAL would also decrease distal sodium delivery, thereby decreasing $F_{\text{Ei}}$ and fractional free water clearance during maximal water diuresis; therefore, it cannot be excluded that the patients examined by Magri et al. (21) and Volpe and co-workers (35) actually had increased sodium reabsorption in the TAL. Interestingly, we recently showed that rats with sodium retention due to liver cirrhosis, with or without increased plasma levels of aldosterone, show evidence of increased sodium reabsorption in the TAL (13, 14, 17). Studies to directly measure transepithelial sodium transport in isolated perfused TAL are warranted to examine the potential role of increased TAL sodium reabsorption under conditions of extracellular volume expansion, as in CHF and cirrhosis.

**Effects of Losartan on Renal Sodium Handling in CHF Rats**

Immunohistochemical studies in rats have revealed that AT$_{1}$ receptors are present throughout the kidneys, including the proximal tubules, and the cortical and outer medullary TAL and CDs (24). The ANG II concentration in the proximal tubules greatly exceeds that in plasma, suggesting that the proximal tubules secrete ANG II or precursors of ANG II into the tubular fluid, where it may activate luminal AT$_{1}$ receptors (25). Micropuncture studies in rats and studies on isolated perfused proximal tubules from rabbits support the hypothesis that luminal ANG II stimulates proximal tubular sodium reabsorption (20, 31). In addition to the effects of ANG II on the proximal tubules, ANG II has also been shown to stimulate NH$_{4}^{+}$ transport through the NKCC2 transporter in isolated TALs from rats (1).
The present study showed that treatment with the AT1-receptor antagonist losartan reversed sodium retention in CHF rats and significantly decreased expression of NKCC2. This finding suggests that the effects of losartan in CHF rats, in addition to a possible effect on the proximal tubules, also include an effect on sodium reabsorption in the TAL. Interestingly, it was recently shown that treatment with the ACE inhibitor captopril was unable to normalize NKCC2 mRNA levels in rats with moderate CHF due to ligation of the LAD (28).
possible explanation for the lack of effect could be that captopril treatment was unable to inhibit intrarenal formation of ANG II in the rats. Recently, it was shown that treatment with an ACE inhibitor that completely blocked the formation of ANG II in plasma was unable to block intrarenal ANG II formation (Dr. G. Navar, personal communication). The mechanism behind the finding is unknown. However, it is well known that ACE inhibition significantly increases bradykinin levels. Bradykinin has been shown to inhibit sodium reabsorption in isolated TALs through a bradykinin type 2-receptor-mediated pathway (10). Further studies are therefore warranted to examine potential differences.

Fig. 4. Immunoblots of membrane fractions from whole kidney homogenates prepared from female Wistar CHF and Sham rats. Rats were untreated or treated for 10 days with losartan (10 mg·kg⁻¹·day⁻¹ ip). A: immunoblot from Sham and CHF rats. Immunoblot was reacted with affinity-purified anti-aquaporin-2 (AQP2) and reveals 29- and 35- to 50-kDa AQP2 bands. B: results from densitometry performed on all rats. C: immunoblots from CHF and CHF-LOS rats. Immunoblot was reacted with affinity-purified anti-AQP2 and reveals 29-kDa and 35- to 50-kDa AQP2 bands. D: results from densitometry performed on all rats. *P < 0.05 vs. CHF.

Fig. 5. Immunoblots of membrane fractions from whole kidney homogenates prepared from female Wistar CHF and Sham rats. Rats were untreated or treated for 10 days with losartan (10 mg·kg⁻¹·day⁻¹ ip). A: immunoblot from Sham and CHF rats. Immunoblot was reacted with affinity-purified anti-pAQP2 and reveals 29- and 35- to 50-kDa pAQP2 bands. B: results from densitometry performed on all rats. C: immunoblots from CHF and CHF-LOS rats. Immunoblot was reacted with affinity-purified anti-pAQP2 and reveals 29- and 35- to 50-kDa pAQP2 bands. D: results from densitometry performed on all rats. *P < 0.05 vs. CHF.
in ACE inhibition and selective AT1-receptor blockade on TAL function.

It is unknown whether all the effects of losartan are direct tubular effects or whether secondary systemic effects of the drug could have an effect as well. DiBona and co-workers (5) recently found that losartan treatment of CHF rats significantly improved cardiac baroreflex regulation of renal sympathetic nerve activity, which was associated with improved ability to excrete acute and chronic sodium loads. Moreover, a significantly decreased expression of NKCC2 has recently been reported in renal-denervated rats fed a low-sodium diet (33). Autoradiographic studies have shown intense norepinephrine labeling throughout the tubules, including the proximal tubules and the cortical TAL (2). Thus renal nerve activity may be involved in the long-term regulation of sodium reabsorption in the TAL. Furthermore, it has been suggested that ANG II facilitates renal norepinephrine release (6), and losartan treatment would thus attenuate norepinephrine release in the kidneys. Therefore, it is possible that the effect of losartan on renal sodium handling in the present study is mediated, at least in part, by the renal nerves. This hypothesis will be examined further in the near future, when the role of renal nerves in TAL sodium handling in rats with CHF will be examined.

AVP-Regulated Water Reabsorption in Rats With Mild CHF

As described by Christensen et al. (3), within minutes, acute V2-receptor blockade causes an almost complete disappearance of AQP2 from the apical membrane of CD principal cells associated with a marked increase in solute-free urine production. An aquaretic response to V2-receptor blockade under conditions where total body water content is kept constant can therefore be used as an estimate of the AVP-mediated renal tubular water reabsorption in vivo.

In the present study, the aquaretic response to selective V2-receptor blockade was significantly increased in the CHF rats. This suggests that the AVP-mediated water reabsorption was increased in these rats. AVP regulates water permeability in the renal collecting ducts by increasing the expression and plasma membrane targeting of the membrane-bound water channel AQP2. In addition to its actions on CD water permeability, AVP also stimulates expression of NKCC2 and the tubular sodium reabsorption in the TAL by a V2-receptor-mediated mechanism (19, 32). AVP-regulated CD water reabsorption will therefore depend on 1) AQP2 in the luminal membrane of the CD principal cells and 2) the magnitude of the corticomedullary osmotic gradient generated by sodium reabsorption in the TAL. In the present study, expression of AQP2 in CHF rats was normal. Furthermore, measurements of pAQP2 did not indicate increased luminal membrane targeting of AQP2 in the CHF rats. Therefore, we hypothesize that the CHF rats have increased transepithelial water reabsorption in the CDs due to an increased corticomedullary osmotic gradient secondary to increased sodium reabsorption in the TAL.

We recently showed an increased corticomedullary osmotic gradient due to increased sodium reabsorption in the TAL in liver cirrhotic rats with early sodium retention (15), but in contrast to the present findings in CHF, AQP2 expression was significantly decreased in the cirrhotic rats. We have therefore suggested that the downregulation of AQP2 could be a compensatory “escape” mechanism aimed to limit excessive water reabsorption. The mechanisms behind such a compensatory escape from AVP stimulation in the CDs of cirrhotic rats are unknown. Also, it remains to be explained why this escape mechanism is not activated in rats with CHF.

Losartan treatment significantly decreased expression of AQP2 in CHF rats. The mechanism behind this effect of losartan is unclear. It is well described that ANG II stimulates the release of AVP from the posterior pituitary by an AT1-receptor-regulated mechanism (30). However, in the present study, plasma AVP levels were unchanged in the rats treated with losartan. AT1 receptors are present in the CDs, and recently it has been reported that ANG II augments AVP-stimulated facilitated urea transport in rat inner medulla CDs (18). However, that ANG II should have a direct tonic effect on the CD principal cells and, thereby, AQP2 expression in CHF rats is not very likely, inasmuch as expression of AQP2 was similar in CHF and sham rats. We recently showed that chronic aldosterone-receptor blockade significantly reduced expression of AQP2 in normal rats (17). In the present study, losartan treatment reduced plasma aldosterone in CHF, but not in sham, rats. Further studies are warranted to examine the role of the RAAS system in AQP2 expression and CD water reabsorption.

In summary, we have shown that, similar to patients with mild CHF, rats with mild CHF have abnormal renal sodium and water handling. Our findings suggest that this could be explained by increased sodium reabsorption in the TAL. Treatment with losartan normalized renal sodium and water handling.

We acknowledge the technical assistance of A. Nielsen, I. Nielsen, B. Sandborg, B. Seider, H. Holmegaard, and H. Hoye. We are grateful to Dr. J. Warberg for performing the plasma vasopressin analyses.

This work received financial support from the Danish Medical Research Council, Danish Heart Foundation, Novo Nordic Foundation, Eva and Robert Voss Hansen Foundation, Ruth König-Petersen Foundation, Helen and Ejnar Bjørnow Foundation, Karen Elise Jensen Foundation, University of Aarhus Foundation, and European Union Commission (EU-Biotech and EU-TMR Programs).

REFERENCES

3. Christensen BM, Marples D, Jensen UB, Frokiaer J, Sheikh-Hamad D, Knepper M, and Nielsen S. Acute effects

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