Effects of sympathetic nerves and angiotensin II on renal sodium and water handling in rats with common bile duct ligature

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Veelken, Roland, Karl F. Hilgers, Markus Porst, Holger Krause, Andrea Hartner, and Roland E. Schmieder. Effects of sympathetic nerves and angiotensin II on renal sodium and water handling in rats with common bile duct ligature. Am J Physiol Renal Physiol 288: F1267–F1275, 2005. First published February 8, 2005; doi:10.1152/ajprenal.00069.2003.—We tested the hypothesis that angiotensin II is likely to be mandatory for the neurogenic systemic and volume retention in cirrhotic rats with common bile duct ligature (BDL) following an acute volume load. To assess the neural control of volume homeostasis, 21 days after common BDL rats underwent volume expansion (0.9% NaCl; 10% body wt over 30 min) to decrease renal sympathetic nerve activity. Untreated animals, rats with renal denervation or pretreated with a nonhypotensive dose of an angiotensin II type 1 receptor antagonist were studied. The renal renin-angiotensin system was assessed by immunohistochemistry and RT-PCR. Rats with BDL excreted only 71 ± 4% of the administered volume load. In cirrhotic rats pretreated with an angiotensin II AT1 inhibitor or after renal denervation, these values ranged significantly higher from 98 to 103% (P < 0.05 for all comparisons). Renal sympathetic nerve activity decreases by volume expansion were impaired in BDL rats (P < 0.05) but unaffected by angiotensin II receptor inhibition. In kidneys of BDL animals, renin mRNA was increased, and immunohistochemistry revealed increased staining for peritubular angiotensin II. Renal denervation in BDL animals reduced renin expression within 5 days to control levels. In conclusion, the impaired excretion of an acute volume load in rats with liver cirrhosis is due to effects of an increased renal sympathetic nerve activity that are likely to be dependent on intrarenal angiotensin II and renin. We speculate that similar changes may contribute to long-term volume retention in liver cirrhosis.

SODIUM AND VOLUME RETENTION is a common problem in patients with liver cirrhosis (33, 34). Neurohumoral vasoconstrictors but also the production of a vasodilator like nitric oxide are considerably activated (16, 21, 22, 27, 29, 31, 42).

Inhibition of the sympathetic nervous system (1, 35) or angiotensin II in patients (30, 38) or animal models (10, 14, 20) could improve sodium and water excretion transiently, although possible decreases in blood pressure or renal perfusion could eventually worsen volume homeostasis instead of improving it (1, 8, 9).

We could demonstrate in rats that the effects of increased renal sympathetic nerve activity (RSNA) on renal sodium and water excretion could be inhibited by very low doses of an angiotensin II AT1 receptor inhibitor not interfering with systemic angiotensin II effects or renal hemodynamics (39). These results suggested a tonic, indispensable influence of angiotensin II on the intrarenal effects of increased sympathetic drive. With respect to liver cirrhosis, these findings suggest the possibility that renal sodium and water handling could be improved by inducing a functional renal sympathetic inhibition with angiotensin II AT1 receptor inhibitors. Recent reports could be seen to support this concept as renal nerve stimulation augmented the effect of intraluminal angiotensin II on proximal tubule transport in rats, an effect that was attenuated by an ACE-inhibitor (32).

Hence, we wanted to test the hypothesis that in rats with liver cirrhosis after common bile duct ligature (BDL) the application of nonhypotensive doses of an angiotensin II AT1 receptor antagonist improves the excretion of an infused saline load significantly. In addition, we investigated whether the intrarenal renin-angiotensin system and renal sympathetic outflow are increased after common BDL, compared with control animals.

MATERIALS AND METHODS

Preparation of Animals

Male Sprague-Dawley rats, weighing 250–300 g (Charles River Wiga, Sulzfeld, FRG), were kept in a room at 24 ± 2°C, 60–80% humidity, and a 12:12-h light-dark cycle. Rats were fed a normal diet containing 0.2% sodium (C-1000, Altromin, Lage, Germany) with free access to tap water. All procedures in animals were performed according to the guidelines of the American Physiological Society and were approved by the local government’s committee on animal research ethics.

Common BDL. Rats were anesthetized with methohexital sodium (Brevimytal, Lilly, Bad Homburg, Germany) intraperitoneally. In 25 rats we performed a common BDL: additional 25 rats underwent sham operations. Abdominal midline incisions were made, the common bile duct was mobilized, ligated with a suture close to the liver and cut (14). All of the BDL rats were found to have ascites as evidenced by visible pools of fluid in the lateral abdominal gutters after being killed following the experiments 3 wk later.

Renal denervation. Sixteen days after BDL or sham operation, rats were again anesthetized as described above. One group of 10 rats of either the liver cirrhotic or the control animals was bilaterally denervated, and the others underwent sham operations. Bilateral flank incisions were made and renal denervation was performed by surgically stripping the renal arteries and veins of adventitia, cutting all visible renal nerve bundles under a dissection microscope (>25), and coating the vessels with a solution of 10% phenol in 95% ethanol as described previously (40). This renal denervation procedure prevents the renal vasoconstrictor response to suprarenal lumbar sympathetic nerve stimulation and prevents the antinatriuretic response to envi-
ronmental stress. It can be demonstrated that this procedure reduces renal catecholamine histofluorescence to nondetectable levels and reduces renal tissue norepinephrine concentration to <5% of control (23, 40).

Test for efficacy of renal denervation. Kidneys assayed after animals were killed from all groups (renally innervated and denervated) were stored at −80°C and later processed as follows (4, 7). The tissue samples used to determine norepinephrine content were first extracted in 40 vol of ice-cold 0.1 M HClO₄. After extraction, the samples were centrifugated and 20 μl of the clear supernatant were transferred to a respective HPLC column. The peak heights obtained from the tissue samples were compared with those from known standards and then converted to nanograms of norepinephrine per gram of tissue.

Preparation for experiments. Three weeks after BDL, rats were again anesthetized with methohexital, and polyethylene catheters were inserted in a femoral vein and a femoral artery led out at the neck of the rat. In five rats with BDL and five control rats, blood was drawn from the arterial line 6 h after anesthesia in tubes containing 50 μl of ortho-phenanthroline (26 mmol/l) with Na-EDTA (125 mmol/l) for later measurement of angiotensin II. These rats were then anesthetized with thiobarbital, killed, and the kidneys were snap-frozen for RNA extraction or placed in methyl-carnoy solution for fixation. In the remaining rats, during methohexital anesthesia a fistula (polyethylene tubing) was inserted in the bladder and led out through the abdominal wall. A bipolar electrode (0.2-mm stainless steel wire, Science Products, Frankfurt, Germany) was placed on the left renal sympathetic nerve by a flank incision for direct RSNA recordings as previously described (9, 35, 36). This technique only allows for the recording of efferent nerve activity, as the activity of afferent nerve fibers is dependent on efferent nerve activity and too low to be assessed in the setting used (10). RSNA was amplified using a Grass P511 band-pass amplifier with a Grass HIP511 high-impedance probe (Grass Instruments, Quincy, MA) and then channeled to a rectifying voltage integrator (Grass model 7P10). The integrated voltage signals were displayed on the polygraph. The recording electrode was fixed to the nerve bundle with a silicone adhesive (Wacker Sil-Gel 604, Wacker-Chemie, Munich, Germany), and the electrode was tunneled to the back of the neck and exteriorized. Integrated RSNA was expressed as millivolts per second per 1-s interval. The data are mainly expressed as percent change from control values according to the conventional expansion of multifiber recordings (39). Besides presenting the RSNA data as line/scatter plots, we also calculated the “area under the curve” in some experiments by increasing the number of evaluated data points to 50 per RSNA recordings in each experimental group. As area under the curve, we defined the area between the 100% line and the curves represented in the line scatter plots. The area under the curve was calculated with the help of a commercially available graphing and analyzing program (Sigmaplot 2001; Version 7.0).

Experimental Procedures

Assessment of the effects of the dose of the angiotensin II AT₁ receptor antagonist ZD-7155. We administered a nondepressor doses of the angiotensin II receptor antagonist ZD-7155 (6 μg iv) to putatively inhibit the renal effects of angiotensin II in BDL and control animals. To prove this assumption, 6 μg of ZD-7155 or vehicle (0.9% NaCl) were injected intravenously at the beginning of a series of experiments after having achieved a “steady-state” situation (saline input = urine output) following the experimental procedures. After two 10-min control periods, nonpressor infusions of angiotensin II (either 6, 12, or 24 ng/min) were begun for additional 10 min. Then two recovery periods of 10 min each followed.

Assessment of different amounts of volume loads on RSNA. Volume expansions of 5 and 10% of the animals body weights were administered to cirrhotic and healthy rats, and the respective responses of renal nerve activity were assessed. Eventually, we infused an amount of saline of 10% body wt in all further experiments as we wanted to achieve a more severe challenge of sodium and water handling by the kidneys with this higher degree of volume expansion.

Angiotensin II AT₁ receptor blockade in rats with BDL. Following surgical preparation, rats were placed in rat holders to permit steady-state urine collection. Urine was collected in 15- or 30-min fractions. Physiological saline was infused at a rate of 60 μl/min from the end of anesthesia until the end of all experimental maneuvers containing sufficient quantities of inulin and para-aminohippurate (PAH) for determination of inulin and PAH clearances (3, 17). Rats were allowed an equilibration period for at least 6 h after the end of the anesthesia. The recovery period ranged from 6 to 15 h with a median of 11 h. The recovery time was judged to be sufficient when the animals had gained a steady-state situation. Steady state meant that the background infusion of 60 μl/min equaled the urinary excretion per minute. This was used as a parameter to judge the recovery of the kidney function from surgery. This procedure allowed us to minimize the influence of postsurgical stress on our experimental outcome on the one hand, but to extend the recovery time not unnecessarily as to prevent a declining quality of renal nerve activity on the other. Hence, experimental protocols were started after urine output equaled saline input for at least four 15-min periods. Values for urine flow and sodium excretion of the last of these sampling periods were taken as data points at 0 min at the beginning of the first control period of the experimental protocol.

After having achieved the steady-state situation, the angiotensin II AT₁ receptor antagonist ZD-7155 (6 μg iv) or vehicle (0.9% NaCl) was given as a bolus injection (30 μl/vol iv) in these experiments. The dosage of the AT₁ receptor antagonist ZD-7155 was previously proven to be only intrarenally effective (39). After two 15-min control periods, all rats were infused for 30 min with saline (10% body wt). Thereafter, three further periods of 30 min each were allowed for recovery. Blood samples of 150 μl were taken at the midpoint of each period for the assessment of glomerular filtration rate and PAH. At the end of the experiments, 3 mg of the ganglion blocking agent tetramethylammonium (Hoffmann-LaRoche, Basel, Switzerland) were injected to shut off postsynaptic RSNA. The background activity remaining thereafter was subtracted from the activity recorded throughout the experiment. Finally, the rats were killed and both kidneys and the liver were excised and weighed. The experimental protocol was repeated in bilaterally denervated cirrhotic rats and controls or in animals without renal sympathetic innervation and additional pretreatment with AT₁ receptor antagonist ZD-7155.

Urine Analysis

Urine volume was determined gravimetrically. Urine and plasma sodium concentrations were measured by flame photometry. Values for urinary volume were expressed per gram kidney weight (g/KW). Urine and plasma inulin and PAH concentrations were determined by the anthrone and ethylenediamine methods to assess inulin and PAH clearances.

Angiotensin II Measurements

Blood samples obtained as described above were immediately centrifuged, and plasma was stored at −26°C until analysis. Angiotensin II was measured by radioimmunoassay as described previously (25, 26). The angiotensin II antiserum “Celine III” (26) exhibited 1% cross-reactivity with angiotensin I and 100% cross-reactivity with angiotensin III and IV, respectively.

Real-Time PCR of Renin

Four rats of each group (control, DNx-control, cirrhosis, DNx cirrhosis) were used to assess whether the induction of cirrhosis 21 days and/or renal denervation 5 days before the actual experiments could alter renal renin expression.
Renal tissue was extracted with TriFast reagent (Peqlab, Erlangen, Germany) by the method of Chomczynski (5).

First-strand cDNA was synthesized with TaqMan reverse transcription reagents (Applied Biosystems, Darmstadt, Germany) using random hexamers as primers. Final RNA concentration in the reaction mixture was adjusted to 0.1 ng/µl. Reactions without Multiscribe reverse transcriptase were used as negative controls for genomic DNA contamination.

PCR was performed with an ABI PRISM 7000 Sequence Detector System and TaqMan Universal PCR Master Mix (Applied Biosystems) according to the manufacturer’s instructions. For amplification of the rat renin cDNA, the forward primer was 5’-GCTACATGGAGAATGGGACTGAA-3’ and the reverse primer was 5’-ACCACTCTTGGCTGAGGAAAC-3’ and the FAM-labeled probe was 5’-CCATCCACTATGGATCAGGGAAGGTCAA-3’. The relative amount of the specific RNA was normalized with respect to 18S rRNA. Primers used for amplification of 18S cDNA were forward 5’-CCATCCACTATGGATCAGGGAAGGTCAA-3’ and probe 5’-CGGCCGTGCTACTACCGAT-TGG-3’. All samples were run in triplicate.

Immunohistochemistry

After fixation in methyl-carnoy solution and embedding in paraffin, 2-µm sections were cut with a Leitz SM 2000 R microtome (Leica Instruments, Nussloch, Germany). Immunohistochemistry was performed (19) using a peroxidase reaction for detection of staining (Vectastain DAB kit, Vector Lab, Burlingame, CA). The antiserum used to detect rat renin (W. Fischli, Hofmann-LaRoche, Basel, Switzerland) was used at a dilution of 1:2,000 (18). For detection of angiotensin II, the polyclonal rabbit antiserum Celine III (26) was used at a dilution of 1:500.

In five cirrhotic and five control kidneys, quantification of juxtaglomerular renin or angiotensin II staining was performed by counting the number of glomeruli with an adjacent juxtaglomerular apparatus with positive staining; 250–450 glomeruli per kidney were counted, and the number of positive glomeruli was expressed as a percentage of the total number of glomeruli counted. Medullary interstitial peritubular angiotensin staining was graded by two blinded observers as 0 (no staining), 1 (occasional patchy staining of peritubular interstitium), or 3 (widespread medullary interstitial peritubular staining).

Statistics

Repeated-measures ANOVA was used to assess significance of differences within groups. Two-way ANOVA was used for comparisons between groups. A Newman-Keuls test was employed for post hoc testing. Only a priori defined hypotheses were tested (41). A P value <0.05 was considered significant. Results are expressed as means ± SE.

RESULTS

Mean body weight of cirrhotic animals (259 ± 9 g) was not different from controls (262 ± 10 g, n = 20, respectively). Rats with common BDL exhibited a visually detectable hyperbilirubinemia at the day of the experiment (cirrhotic rats 7.1 ± 0.6 mg/dl vs. controls 0.30 ± 0.08 mg/dl). Liver weight was significantly higher in cirrhotic rats (24 ± 3 g) compared with controls (11 ± 2 g). No differences were detectable in kidney weights (cirrhotic rats: 2.54 ± 0.11 g; controls: 2.42 ± 0.13 g).

The assessment of our renal denervation surgery showed the following results: noradrenaline content in renally innervated animals was 160.3 ± 18.7 ng/g wet tissue (n = 12). In renally denervated animals, it decreased to 2.53 ± 0.7 ng/g wet tissue (n = 12), suggesting successful procedures (4).

Assessment of Renal Effect of Dose of Angiotensin II AT1 Receptor Antagonist ZD-7155 Used

The low dose of ZD-7155 administered did not induce any hemodynamic responses. It was, however, effective in the kidney as two of the administered three nonpressor doses of ANG II (12 and 24 ng/min) induced water and sodium retention, which was significantly impaired after pretreatment with 6 µg of ZD-7155. (Fig. 1).

Assessment of Different Amounts of Volume Loads on RSNA

RSNA due to a volume load of 5% body wt was decreased during the time of infusion and returned gradually toward baseline levels during the following 1.5-h recovery period in controls and BDL animals (Fig. 2). However, the decreases were more pronounced in control animals compared with BDL rats.

The responses to a volume of 10% body wt were more complex. In controls, a pronounced decrease of RSNA during the infusion of the volume load was followed by a long-lasting depression of RSNA that did not recover after 1.5 h but had returned to baseline after 3 h. The nominal percent decrease in

Fig. 1. Effects of a nonpressor infusions of ANG II (6, 12, 24 ng/min for 10 min) on urine volume (UV) and sodium excretion rate (UNaV) in conscious rats. One group of rats received saline with respective doses of ANG II, whereas another group of intact rats was pretreated with 6 µg of the Ang II AT1 receptor antagonist ZD-7155 intravenously (bolus injection). Data represent means ± SE. UV and UNaV dropped significantly (P < 0.05) during infusions with the 2 higher doses of ANG II infusion in control rats but not in ZD-7155-treated animals. *Significant differences from control levels. KW, kidney weight.
RSNA was similar in BDL rats compared with controls during the infusion period. However, it returned rapidly thereafter toward control levels (Fig. 2).

The area under the curve in the experiments with volume expansion of 5% body wt were 1,152 ± 110 AU (arbitrary units) in cirrhotic vs. 2,696 ± 215 AU in control animals (ratio 1: 2, 3) (P < 0.05 vs. intact controls). The respective values in the experiments with volume expansion of 10% body wt were 2,177 ± 196 in cirrhotic vs. 5,499 ± 298 in control rats [ratio 1: 2, 5 (P < 0.05 vs. intact controls)].

Angiotensin II AT1 Receptor Blockade in Rats with BDL

Rats with common BDL excreted only 71.5 ± 4% of the administered volume load by 90 min after completing volume expansion. In cirrhotic rats pretreated with an angiotensin II AT1 inhibitor or after renal denervation (without or with additional AT1 inhibitor pretreatment) as well as in all control groups, the respective value ranged from 92 to 103% in 90 min. They were not different from one another but significantly greater than the excretion rate of the untreated cirrhotic animals (P < 0.05). As displayed in Figs. 3 and 4, absolute urinary flow rates (V) and urinary Na excretion (UNaV) increased to the highest level during the volume expansion period and were back to control values during the third 30-min period of recovery in cirrhocic animals and controls. This pattern was similar for the four groups of animals suffering from liver cirrhosis and for the four groups of control rats with no significant differences among them. However, animals with BDL and no further treatment exhibited a significantly smaller increase in urinary V and UNaV compared with all other groups of animals either suffering from liver cirrhosis or belonging to the healthy control groups (P < 0.05). In none of

Fig. 2. Responses of renal sympathetic nerve activity (RSNA) in cirrhotic and control animals following volume expansions with saline in an amount equivalent to 5 and 10% body wt. The infusions were administered in separate groups. In all experiments, the infusion time lasted for 30 min (15 to 45 min on the x-axis), the recovery time thereafter for 90 min (45 to 135 min on the x-axis). Data represent means ± SE. The dark gray area represents the mean withdrawal of RSNA seen in control and bile duct ligation (BDL) rats alike. The light gray area encodes the additional amount of sympathetic withdrawal seen in intact control rats.

Fig. 3. Effects of a volume expansion (saline 10% body wt) on mean arterial blood pressure (BP), heart rate (HR), urine volume (UV), and sodium excretion rate (UNaV) in conscious, restrained rats with BDL to induce liver cirrhosis. The abscissa indicates the time (min). Besides the experimental group with BDL, 3 further groups with BDL were investigated: renally denervated animals (DNX), animals pretreated with 6 μg of the ANG II AT1 receptor antagonist ZD-7155 (ANG inhibitor), and animals reviving ZD-7155 in addition to the renal denervation. Data represent means ± SE of 6 rats each. There were no significant changes in BP and HR in any group of animals. However, the excretion of the infused-saline load was significantly impaired in the untreated BDL animals as seen by the volume excretion (UV) and sodium excretion (UNaV) during recovery (P < 0.05). *Significant differences from control levels. #Significant differences between BDL animals and controls.
the experimental groups was blood pressure or heart rate affected by the experimental procedures (see Figs. 2 and 3). In Fig. 5 glomerular filtration rates and renal plasma flows for all groups are displayed. No significant differences occurred. Mean changes of RSNA in rats with BDL and control animals during volume expansion and recovery are displayed in Fig. 6, respectively. Given the settings of our Grass recording equipment, the basal absolute RSNA levels were for the cirrhotic groups $510 \pm 39 \mu V$ and for controls $360 \pm 34 \mu V$. In rats with BDL, the maximal decrease in RSNA occurred 20 min and in control animals 15 min after onset of volume expansion; there was no significant difference in the maximum depression of RSNA within the groups of cirrhotic rats and controls during the actual time of the volume expansion. However, in the first 30 min after cessation of volume expansion, RSNA returned to control values in rats with BDL rats and remained there for the duration of the recovery period. In control animals on the other hand, RSNA remained depressed and did not exhibit any recovery toward control values throughout the 1.5-h recovery period. In consequence, for the entire 1.5-h recovery period, RSNA was significantly different between rats with BDL whose RSNA was rapidly restored to baseline levels, and control animals, whose RSNA did recover to control levels after 3 h ($P < 0.05$).

This pattern was not influenced by the pretreatment with the angiotensin II AT1 inhibitor ZD-7155.

**Assessment of Angiotensin II and Renin**

Systemic angiotensin II level were not different between cirrhotic animals and controls ($78 \pm 14$ vs. $64 \pm 16$ fmol/ml; $n = 5$). RT PCR for renal renin expression (Fig. 7) in cirrhotic animals compared with controls, but angiotensinogen and angiotensin-converting enzyme mRNA levels were unaltered (data not shown). However, renal denervation 5 days before our experiments reduced renin expression in cirrhotic animals to levels seen in controls. Renal denervation did not influence renin expression in healthy controls (Fig. 7). Immunostaining for renin was confined to juxtaglomerular areas in cirrhotic and control rats. The number of glomeruli with adjacent renin staining was similar in cirrhotic rats (10.7 ± 1.3%) and controls (14.8 ± 1.8%). Similarly, the number of glomeruli with adjacent staining for angiotensin II was also not different between cirrhotic (18.7 ± 2.0%) and control (17.7 ± 5.1%) rats.

In contrast, a marked medullary interstitial peritubular staining for angiotensin II was detected in cirrhotic animals (Fig. 8). The staining was most prominent at the border between outer and inner medulla (Fig. 8). Staining was absent in most control rats (mean score, 0.20; range, 0–1) but present in all cirrhotic rat kidneys (mean score, 1.83; range, 1.5–2).

**DISCUSSION**

We demonstrated that in rats with liver cirrhosis after common BDL a low dose of an angiotensin II AT1 receptor inhibitor was able to restore the impaired excretion of an acutely infused saline load. The injected dose of the AT1 receptor inhibitor had no hemodynamic effects in rats with liver cirrhosis or controls. The acute administration of the saline load decreased RSNA in control animals, whereas RSNA quickly recovered in rats with liver cirrhosis after cessation of the volume infusion. This response of RSNA in cirrhotic rats during an acute volume expansion suggests a permanent altered control and/or increase of RSNA whose functional consequences, the impaired ability to excrete an acute expansion of the body volume, could be overridden by a low dose of an angiotensin II AT1 receptor inhibitor. Hence, the effect of RSNA on renal salt and water excretion might be dependent on the tonic influence of intrarenal angiotensin II levels.

One could argue that we only demonstrated that renal denervation and AT1 receptor inhibition relieved the volume retention in cirrhotic rats exposed to an acute volume load to the same extent but by different mechanisms. Hence, the AT1 receptor inhibition was able to produce a sort of “functional...
denervation” by chance. We cannot rule out this possibility completely. However, the fact that denervation and AT$_1$ receptor blockade did not lead to an exaggerated sodium and volume excretion is not in favor of this assumption: if sympathetic innervation and angiotensin II were to account for sodium and water excretion in our cirrhotic animals completely independent from one another, it is not easy to explain why the amount of salt and water excreted was again the same as with denervation alone under these circumstances. If low amounts of angiotensin (inhibited by small amounts of an AT$_1$ inhibitor) are mandatory for the tubular effect of sympathetic impulses, it is conceivable that angiotensin under these circumstances acts for example on pre- and postsynaptic receptors without any further effect on salt and water reabsorption. Our previous data on rats subjected to air-jet stress support the idea of an interaction between RSNA and tubular angiotensin II in renal sodium and water handling (39). Furthermore, a recent paper demonstrated that renal nerve stimulation increased the effect of intraluminal angiotensin II on proximal tubule transport in rats, an effect that was attenuated by an ACE-inhibitor (32).

While using a saline load of 10% body wt, we were naturally able to initially decrease RSNA further than in reports using less amount of fluid (9, 10). Furthermore, we got more complex response patterns of RSNA. During the actual infusion of saline of 10% body wt, RSNA was decreased more pronounced in controls compared with the infusion of a load of 5% body wt. This might be due to a more pronounced stimulation of baroreceptor mechanisms involved in the downregulation of renal nerve activity.

In addition, the decreases in RSNA in BDL animals were nominally lowered to same percentage degree as in the respective controls during the infusion of the volume load. However, the similar reduction of RSNA in BDL animals during actual volume expansion does not necessarily mean that the absolute sympathetic nerve activity was reduced to the same levels as in controls. Given the settings of our Grass recording equipment, the basal absolute RSNA levels were for the cirrhotic groups 510 ± 39 µV and for controls 360 ± 34 µV. Furthermore, the effect of renal denervation and the blunted excretion of the infused saline load in cirrhotic animals rather argue against this assumption of a similar absolute depression of RSNA in cirrhotic animals and controls.

The higher amount of fluid infused was also followed in the recovery period by responses that were phenomenologically different from the ones seen in the animals infused with a lower load. The mechanisms involved in the downregulation of RSNA decrease were obviously so strongly stimulated that it

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Fig. 5. Glomerular filtration rate (GFR) and renal plasma flow (RPF) during control, volume expansion, and recovery in rats with BDL (intact) on left and control animals (control) on right. Neither were there significant differences between BDL animals (intact) or control nor did renal denervation (DNX), pretreatment with 6 µg of the ANG II AT$_1$ receptor antagonist ZD7155 (ANG Inh.) intravenously or both interventions simultaneously influence renal perfusion.
took up to 3 h for RSNA to recover. In BDL animals, the vigorous stimulation of RSNA controlling mechanism during VE was followed by a strong counterregulation that restored RSNA rapidly to control levels again. A response quite different from what we and others saw in the animals receiving a load of 5% body wt (9).

Does this mean that the different shape of the curves in animals receiving loads of 5 and 10% body wt also suggests a completely different ability to withdraw sympathetic activity to the kidneys to volume expansion in the 10% group?

One has to take into account the sympathetic withdrawal during actual volume expansion and recovery period as a whole to answer this question. One possibility to evaluate sympathetic withdrawal is to calculate the area under the curve (e.g., in our experiments the area between the RSNA curves and the 100% line). These calculations showed that in our experiments in controls the sympathetic withdrawals were always much more pronounced than in cirrhotic animals. Furthermore, it turned out that the relationship of sympathetic withdrawal between cirrhotic animals and controls was about the same in both groups if assessed by the areas under the curves (1:2.2 in the 5% group and 1:2.5 in the 10% group). Hence, the impaired possibility to withdraw sympathetic activity to the kidneys was comparable in cirrhotic animals whether they received a saline load of 5 or 10% body wt.

In control animals, no effect of AT1 receptor inhibition or renal denervation could be observed since RSNA was likely lowered to a very low level of activity in the first place during the volume expansion with saline (10). This is in contrast to the likely situation in cirrhotic animals when renal sympathetic activity is not properly suppressed and controlled.

In our experiments with background infusions of nonpressor doses of angiotensin II, which altered urinary volume and sodium excretion, the specific AT1 receptor antagonist ZD-7155 could abolish these angiotensin II-dependent responses, suggesting a specific intrarenal effect (30). The kidney is known to be very sensitive to the effects of angiotensin II (17, 23).

In addition to the likely inadequately high RSNA, we found evidence for a stimulated intrarenal renin-angiotensin system, in agreement with previous studies (37). Renin synthesis was stimulated, although there was no abnormality of the intrarenal distribution of immunoreactive renin. In contrast, immunoreactive angiotensin II was clearly increased in cirrhotic rat kidneys in a medullary localization in the vicinity of proximal tubules, where the sympathetic innervation is most abundant (10).

The functional response to the AT1 antagonist supports the notion that intrarenal angiotensin II was increased. Under these circumstances, AT1 blockade was equivalent to renal denervation in restoring the excretory responses to a volume load. Although we cannot exclude additive effects of AT1 and sympathetic outflow on volume retention, a specific interaction between AT1 and sympathetic nerves appears more likely. Such an interaction could occur at peritubular sympathetic varicosities by AT1-mediated presynaptic stimulation of neurotransmitter release (36, 39) or at the juxtaglomerular appara-
tus by nerve-mediated stimulation of renin release (2). The peritubular angiotensin II staining points to an interaction with the renal nerves at the peritubular level (39).

More than a decade ago, Solis-Herruzo et al. (35) could demonstrate that the acute inhibition of sympathetic drive to the kidney of patients with severe liver cirrhosis could improve and stabilize the renal excretory function in these patients. In this study, RSNA to the kidney was blocked with the help of an injection of a anesthetic drug into the lumbar plexus. We speculate that a prescription of low doses of an angiotensin II AT1 receptor inhibitors could be an alternative way to achieve a comparable effect in situations of acutely exacerbating volume retention in liver cirrhotic patients. Our study with an angiotensin II AT1 receptor inhibitor does not only provide information how a low-grade inhibition of the renal renin-angiotensin system might improve sodium and water handling by the kidney in liver cirrhosis but could also implicate an advantage of AT1 receptor antagonists over ACE inhibitors in this respect: in experimental settings, AT1 receptor inhibitors had less detrimental effects on glomerular perfusion pressure as the vasodilatory properties of bradykinin on the afferent arteriole of the glomeruli were preserved (24).

Our study demonstrates that in rats with liver cirrhosis after common BDL, low doses of an angiotensin II AT1 receptor antagonist without effects on blood pressure and heart rate or detectable alterations in glomerular filtration rate and renal plasma flow induced a functional renal sympathetic denervation of the kidney, which restored the normal handling of acute volume expansions in these animals. Interestingly, we found signs of an increased activity of the renin-angiotensin system in cirrhotic rat kidneys in a medullary localization in the vicinity of proximal tubules, where the sympathetic innervation is most abundant (10). In how far this observation is of importance for the treatment of volume and sodium retaining in patients with liver cirrhosis warrants further research.

Fig. 8. Immunohistochemistry for renin (A) and angiotensin II protein (B) in cryostat kidney sections. Left: cirrhotic animal. Right: sham-operated control. Immunostaining for renin was confined to juxtaglomerular areas in cirrhotic and control rats and not different between both groups (magnification: ×250). In contrast, a marked medullary interstitial peritubular staining for angiotensin II was detected in cirrhotic animals compared with control rats (magnification: ×250 top; ×600 bottom).
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