Inhibition of cGMP-specific phosphodiesterase type 5 reduces sodium excretion and arterial blood pressure in patients with NaCl retention and ascites

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AGT ATG-3', antisense 5' -CGT GCC TCC AAT TGT G 3' (1577–2203, 627 bp, GenBank accession no. nm133584). Samples of normal adjacent kidney tissue from kidneys removed due to renal cancer were obtained from patients undergoing radical nephrectomy at the Department of Urology, Odense University Hospital. Patients had given informed written consent to participate, and the study was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) and The Danish National Board of Health (no. 20010035). PDE5 expression was estimated by quantitative PCR and RNase protection assay as described previously (15). In brief, total RNA was isolated from human tissue samples with an RNeasy Midi Kit (Qiagen, Rødovre, Denmark). RT-PCR was used to generate a fragment of human PDE5A1 cDNA: sense 5’-CCC ACT GAT CTA ATG AAC-3’ antisense 5’-AAT CAG CAT CTT CTC CTG-3’ (covering bases 2711–2905, 195 bp, GenBank accession no. xn003559). The PCR fragment was subcloned in plasmid pSP73, sequenced, and used to generate a radiolabeled antisense RNA probe by in vitro transcription with SP6 polymerase in the presence of [1577–2905]GTP (Amersham). Five hundred thousand counts per minute of the RNA probe were hybridized with 5, 10, and 20 ng total RNA and yeast tRNA at 60°C overnight and then digested with RNase A/T1 (20°C, 30 min) and proteinase K (37°C, 30 min). Protected fragments were separated on an 8% polyacrylamide gel. Autoradiography was performed at -80°C after 24 h. For quantitative PCR, 1 ng total RNA was reverse transcribed with a cDNA synthesis kit (Bio-Rad), and 50 ng served as a template for PCR amplification using the SYBR-green reaction mix according to the manufacturer’s instruction (Bio-Rad). Serial dilution of PDE5-plasmid DNA was used as template for generation of a standard curve. PDE5 standards and kidney cDNA samples were amplified in duplicate in 96-well plates, and PCR was performed for 42 cycles consisting of denaturation for 30 s at 95°C followed by annealing and polymerization at 60°C for 1 min. Fluorescence was detected during the annealing/extension step in each cycle. Specificity was ensured by melting curve analysis and by agarose gel electrophoresis.

**Immunohistochemical analysis of PDE5 in human kidney.** Kidney tissue was immersion fixed, paraffin embedded, and sectioned. After rehydration, tissue was microwaved for 20 min in DAKO antigen retrieval buffer and then incubated with 5, 10, and 20 ng total RNA and yeast tRNA at 60°C overnight and then digested with RNase A/T1 (20°C, 30 min) and proteinase K (37°C, 30 min). Protected fragments were separated on an 8% polyacrylamide gel. Autoradiography was performed at -80°C after 24 h. For quantitative PCR, 1 ng total RNA was reverse transcribed with a cDNA synthesis kit (Bio-Rad), and 50 ng served as a template for PCR amplification using the SYBR-green reaction mix according to the manufacturer’s instruction (Bio-Rad). Serial dilution of PDE5-plasmid DNA was used as template for generation of a standard curve. PDE5 standards and kidney cDNA samples were amplified in duplicate in 96-well plates, and PCR was performed for 42 cycles consisting of denaturation for 30 s at 95°C followed by annealing and polymerization at 60°C for 1 min. Fluorescence was detected during the annealing/extension step in each cycle. Specificity was ensured by melting curve analysis and by agarose gel electrophoresis.

**Effect of PDE5 inhibition on renal and cardiovascular parameters in patients with ascites and liver cirrhosis.** The human intervention study was performed in accordance with the Declaration of Helsinki and approved by the IRB and The Danish National Board of Health (no. 20000223). Written informed consent was obtained from all participants after full explanation of the purpose, nature, and risk of all procedures. Inclusion criteria were the presence of cirrhosis in a liver biopsy or clinical and laboratory evidence of cirrhosis combined with years of excessive alcohol intake. All patients were being treated with diuretics and had a history of previous abdominal paracentesis or ultrasonic examination showing the presence of ascites. Excessive alcohol intake was the cause of liver disease in all patients. Child-Pugh scores were 7–11. In six patients, portal hypertension was indirectly ascertained by the presence of esophageal varices at endoscopic examination. Exclusion criteria were signs of cardiovascular disease, diabetes mellitus, proteinuria (>0.3 g/24 h), gastrointestinal bleeding, or septic episodes within the last 14 days. Twelve patients with decompensated cirrhosis were recruited from the outpatient clinic. Eight patients completed the diet regimen and the intervention on both study days and were included (7 men/1 woman). One patient was excluded due to upper gastrointestinal bleeding during the run-in period, two patients did not want to attend the second study session, and one patient was excluded on the first study session due to a severe drop in blood pressure after sildenafil administration.

**Experimental design.** The study was a double-blinded, placebo-controlled crossover study. Each patient was investigated on 2 days separated by an interval of 2–3 wk. Patients were randomized to start with either 50 mg of sildenafil or placebo taken orally. The dose of sildenafil was chosen based on data showing inhibition of PDE5 in cirrhotic patients with minor side effects (27). Five days before each study day, diuretics (spironolactone, n = 8 and furosemide, n = 5) were withdrawn, and patients received a sodium-fixed diet (100 mmol sodium and 60–80 mmol potassium). On the day 6, after a 24-h urine collection for determination of sodium and potassium, and an overnight fast, the study session started. Beginning at 7 AM an oral water load of 100 ml tap water was given every 30 min until 2:30 PM. From 8 AM, the patient was placed in the seated position (which is the most neutral position with regard to sodium excretion) (2), and plastic cannulas for blood sampling and administration of tracers were inserted. The patients received a bladder catheter. Priming doses of 99mTc-diethyleneetriamine-pentaacetaetate (99mTc-DTPA) and 131I-hippuran were given at 8:45 AM, followed by a continuous intravenous infusion of the tracers. After a 90-min steady-state period and a 60-min basal period, sildenafil or placebo tablets were administered. From 10:30 AM, urine and blood were collected during eight consecutive 30-min periods for determination of 99mTc-DTPA, 131I-hippuran, sodium, potassium, and osmolality. From 10:30 AM, blood was drawn every 60 min for determination of plasma renin activity (PRA), ANG II, aldosterone, ANG, osmolality, hemoglobin, and hematocrit. Basal values in plasma of bilirubin, albumin, creatinine, sodium, potassium and prothrombin-proconverting test activity (PPPA), activated partial thromboplastin time (APTT), platelets, and alanine aminotransferase (ALT) were determined. Mean arterial blood pressure (MAP) and heart rate (HR) were measured every 6 min by standard cuff on an automatic recorder (Colin BP 10011S, Colin Electronics).

**Determination of kidney parameters.** Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured as renal clearance of 99mTc-DTPA and 131I-hippuran using the constant-infusion clearance technique. A loading dose of 99mTc-DTPA (0.5 mBq/kg body wt) and 131I-hippuran (0.025 mBq/kg body wt) was followed by infusion of 99mTc-DTPA (0.05 mBq/kg body wt·h⁻¹) and 131I-hippuran (0.02 mBq/kg body wt·h⁻¹). GFR and ERPF were standardized to a body surface area of 1.73 m². MAP was calculated as [diastolic BP + (systolic BP – diastolic BP)/3]. Free water clearance (C120) was determined by subtracting osmolar clearance (Cosm) from the urinary flow rate (V), where Cosm = Uosm × V/Posm. Posm is osmolality of plasma, and Uosm is the osmolality of urine. Plasma and urine electrolyte concentrations were determined by flame photometry (IL 243 flame photometer, Instrumentation Laboratory, Lexington, MA). Osmolality was measured within 24 h by freezing-point depression (Osmomat, 030D, Gnotec).

**Plasma concentrations of hormones.** ANP and ANG II in plasma were quantified by radioimmunoassay after acidification with 4% acetic acid and extraction in C18 Sep-Pak cartridges (Waters, Milford, MA) (11). ANP was determined with antibody Ab 8798 (Peninsula) and the tracer IM 187 (Amersham Pharmacua Biotech) (9). The detection limit was 2.0 pg/ml. The intra-assay coefficient of variation was 4%, and recovery was 60–70%. ANG II immunoactivity was determined with antibody Ab 5030682 (Panum); the tracer was from Gastroup Hospital, Copenhagen, Denmark. The detection limit was 1.4 ng/l. The intra-assay coefficient of variation was 4%, and recovery was 96%. Plasma renin concentration was determined as the generation of ANG I from endogenous angiotensinogen and measured by immunoassay of generated ANG I as described (32). PRA (in mIU/l) was calibrated to renin standards from the National Institute for Biological Standards and Control (Potters Bar, Herts, UK). Plasma
aldosterone concentration was measured with a commercial kit (Coat-A-Count Aldosterone, DPC, Los Angeles, CA). The detection limit was 11 pg/ml. Plasma cGMP concentration was measured by use of a commercial kit (cGMP EIA Kit, Cayman Chemical, Ann Arbor, MI) after precipitation with ice-cold ethanol, evaporation under a stream of nitrogen, and resuspension in phosphate buffer. The intra-assay coefficient of variation was 5%, the interassay coefficient of variation was 12%, and recovery was 100%. The concentration of cGMP in urine was measured by use of a commercial kit (Biotrak, cGMP enzyme-linked immunosassay, Amersham Pharmacia). The intra-assay coefficient of variation was 6%, the interassay coefficient of variation was 2.5%, and recovery was 86%.

Data presentation and statistical analysis. All values listed in tables are medians and range. Baseline values from the 2 study days were compared with a paired Student’s t-test or with a Wilcoxon matched-pairs-signed-rank test. Since baseline values did not significantly different, absolute changes between the 2 study days were subjected to one-way ANOVA for repeated measures. Bonferroni’s multiple comparison test was applied to evaluate differences between mean values. In case parameters did not have a Gaussian distribution, Friedman two-way ANOVA was used for evaluation of change over time, and Dunn’s test was performed when overall P < 0.05. A P value <0.05 was considered statistically significant.

**RESULTS**

Expression of PDE5 in human kidneys. We performed quantitative PCR with internal plasmid standards and a RNase protection assay to elucidate whether PDE5 was expressed in human kidney. PDE5 was expressed at significant levels in human kidney, and there was no significant difference in PDE5 mRNA level between the cortex and inner medulla (n = 6 and n = 4 separate samples, respectively, Fig. 1A). In the same RNA samples, renin was detected in the cortex at >60-fold higher levels compared with in the medulla (Fig. 1A). A specific human PDE5 antisense probe was hybridized with total RNA isolated from the inner medulla of two separate human nephrectomy specimens. The assay was linear when various amounts of total RNA were hybridized with the probe and documented significant expression of PDE5 (Fig. 1B). In microdissected rat nephron segments, PDE5 transcripts were detected predominantly in inner medullary collecting ducts (IMCD) and also in inner medullary thin limbs of Henle’s loop and in the convoluted and straight parts of the proximal tubules (Fig. 1C). As a positive control for equal loading and equal reverse transcription, β-actin was amplified and detected in all dissected segments (Fig. 1C, bottom). Histological sections of

![Fig. 1](http://ajprenal.physiology.org/)

**Fig. 1.** A: expression of phosphodiesterase type 5 (PDE5) mRNA in human kidney cortex (n = 6) and inner medulla (IM; n = 4; left). Human nephrectomy specimens were separated in the cortex and inner medulla, and total RNA was analyzed by real-time RT-PCR using internal hPDE5-plasmid standards. Columns represent mean starting quantity of cDNA. Right: results from a similar experiment in which human kidney renin mRNA was quantitated in the same RNA samples as PDE5. B: autoradiograph displaying the result of RNase protection assay for PDE5 mRNA in human renal IM. Total RNA from IM of 2 nephrectomy specimens. The assay was linear when various amounts of total RNA were hybridized with the probe and documented significant expression of PDE5 (Fig. 1B). In microdissected rat nephron segments, PDE5 transcripts were detected predominantly in inner medullary collecting ducts (IMCD) and also in inner medullary thin limbs of Henle’s loop and in the convoluted and straight parts of the proximal tubules (Fig. 1C). As a positive control for equal loading and equal reverse transcription, β-actin was amplified and detected in all dissected segments (Fig. 1C, bottom). Histological sections of human kidney cortex and IMCD were stained for PDE5 immunoreactivity with antibodies raised in rabbit. Bar = 50 μm.
human kidneys were immunolabeled with a specific PDE5 antibody. Sections displayed distinct labeling associated with the perinuclear cytoplasm of inner medullary collecting duct cells and thin limb cells of Henle’s Loop (Fig. 1D), in agreement with PDE5 mRNA expression along the rat nephron. PDE5 immunoreactive protein was also observed in vascular smooth muscle cells of arteries and arterioles in human kidney, and labeling was found in the perinuclear area (Fig. 1D, bottom). In the absence of primary antibody no labeling was observed after reaction with chromogen substrate (DAB).

**Basal functional and biochemical parameters of included patients.** Clinical data and medication of the cirrhotic patients that completed the study are shown in Table 1 and basal biochemical profiles on the 2 different study days in Table 2. Baseline values of systemic and renal hemodynamic parameters and measured hormones and electrolytes are shown in Table 3 for both study days. Patients did not differ with respect to baseline values on the day of sildenafil treatment compared with the day of placebo treatment (Tables 2 and 3). Absolute values for MAP and HR and relative changes with time are shown for all patients in Fig. 2, A and B. MAP and HR were similar on sildenafil and placebo treatment days (Table 3). Absolute values for MAP and HR and relative changes with time are shown for all patients in Fig. 2, A and B. MAP was significantly decreased after 120 min and remained significantly lower at the last point of measurement, 180 min (Fig. 2A). In contrast, HR was significantly increased only 60 min after sildenafil treatment and then stabilized at a level not different from control (Fig. 2B). MAP and HR did not change with time after placebo treatment. Renal perfusion (ERPF) and GFR were not significantly different in the individual patients on the 2 experimental days. Administration of sildenafil did not affect GFR and ERPF (Table 4). Accordingly, the filtration fraction was unaffected. In some of the patients, GFR was decreased. There were elevated bilirubin and elevated levels of renin-angiotensin-aldosterone and ANP in plasma compared with normal reference values (Tables 2 and 3).

**Effect of sildenafil on renal and systemic hemodynamics in cirrhotic patients.** The effectiveness of sildenafil was validated by determination of cGMP in plasma and urine. In response to sildenafil, plasma cGMP concentration was significantly increased 120 and 180 min after administration, whereas cGMP levels did not change significantly after placebo (Fig. 2C). In contrast to plasma, urinary cGMP excretion (UcGMP/V, Table 4) did not change significantly in response to sildenafil. Baseline MAP and HR were similar on sildenafil and placebo treatment days (Table 3). Absolute values for MAP and HR and relative changes with time are shown for all patients in Fig. 2, A and B. MAP was significantly decreased after 120 min and remained significantly lower at the last point of measurement, 180 min (Fig. 2A). In contrast, HR was significantly increased only 60 min after sildenafil treatment and then stabilized at a level not different from control (Fig. 2B). MAP and HR did not change with time after placebo treatment. Renal perfusion (ERPF) and GFR were not significantly different in the individual patients on the 2 experimental days. Administration of sildenafil did not affect GFR and ERPF (Table 4). Accordingly, the filtration fraction was unaffected. In some of the patients, GFR was decreased. There were elevated bilirubin and elevated levels of renin-angiotensin-aldosterone and ANP in plasma compared with normal reference values (Tables 2 and 3).
lower than normal whereas other patients had above normal levels of GFR. In all cases, GFR was closely correlated with ERPF ($r = 0.82$, $P < 0.0001$, placebo day, $r = 0.70$, $P < 0.0001$, sildenafil day).

**Effect of sildenafil on plasma concentration of hormones.**

The change with time of plasma renin activity (PRA), plasma ANG II, and aldosterone concentrations in each individual on the 2 experimental days is shown in Fig. 3, A–C. Average PRA increased significantly 60 min after sildenafil compared with placebo treatment day and stayed elevated through the period of observation (Fig. 3A). In response to sildenafil treatment, average plasma ANG II concentration increased significantly only after 60 min (Fig. 3B). As expected, a close correlation was found between PRA and plasma ANG II levels on both placebo and sildenafil treatment days ($r = 0.93$, $P < 0.0001$ and $r = 0.94$, $P < 0.0001$, respectively, not shown), and this correlation was also found between plasma ANG II and aldosterone levels after placebo treatment ($r = 0.67$, $P < 0.0001$) and after sildenafil treatment ($r = 0.64$, $P < 0.0001$, not shown). Similar to ANG II, plasma aldosterone concentration was significantly higher only after 60 min in response to sildenafil treatment (Fig. 3C). The basal, elevated, levels of plasma ANP did not change in response to intervention in the study (Table 4).

**Effect of sildenafil on urinary excretion of sodium, potassium, and water in cirrhotic patients.** All patients were in positive sodium balance (Table 3). Three patients excreted nominally sodium-free urine, one patient on the day of placebo treatment, and two patients on the day of sildenafil treatment. On the day of placebo treatment, average baseline sodium excretion ($U_{Na}V$) was 107 (0–1,975) μmol/h, which was not statistically different from the value on the day of sildenafil treatment 209 (0–2,345) μmol/h. Figure 4 displays absolute values of urinary sodium excretion for all patients on the 2 study days. Despite the various levels of sodium excretion prior to medication, the patients responded with similar directional changes to sildenafil. $U_{Na}V$ decreased significantly compared with placebo. Excretion of potassium was not different on two study days and did not change throughout the study (Table 4). Baseline urine flow rate and free water clearance (UV and $C_{1200}$) were similar and did not change throughout the study (Table 4).

**DISCUSSION**

In the present study, we tested the hypothesis that inhibition of cGMP-specific PDE5 in the kidneys promoted natriuresis in cirrhotic patients with marked sodium retention and ascites.
We used the crossover design to exploit the benefits of “within-subject” comparison and the smaller group size required. Sodium intake was standardized and diuretic treatment was stopped prior to each experiment. The data showed that PDE5 mRNA was expressed at comparable levels in normal human kidney cortex and medulla. Immunoreactive PDE5 protein was associated with medullary collecting ducts and arterial vascular smooth muscle in human kidney sections similar to localization of PDE5 mRNA in microdissected rat nephron segments (21). This indicated local transcription and translation of the enzyme. Systemic inhibition of PDE5 in decompensated cirrhotic patients by oral administration of sildenafil at (50 mg) increased plasma cGMP concentration, reduced mean arterial pressure, decreased renal sodium excretion, and led to an activation of the RAAS that was seen before the decrease in mean arterial pressure.

Baseline values of hemodynamic parameters and plasma hormone levels were similar on the 2 days of intervention (Tables 2–4). The patients stopped diuretic treatments, and despite ingestion of a diet with a fixed low-sodium content (100 mmol) for 5 days, the patients were in apparent positive sodium balance with a median value of excreted sodium of 17 mmol/day (range 0.1–52 mmol/day). This is expected based on the elevated levels of renin-angiotensin-aldosterone seen at baseline in these patients and is comparable to results from a study by Warner et al. (43), where decompensated patients had a mean urinary sodium output of 37 × 13 mmol/day after 5 days on a fixed 100-mmol sodium diet. There was a marked variability in baseline sodium excretion, GFR, and effective renal plasma flow (Tables 3 and 4). Two patients exhibited a markedly higher sodium excretion on both study days (Fig. 4).

Despite the baseline differences among patients, the relative hormonal, antinatriuretic, and hypotensive responses to sildenafil were remarkably similar within each patient. This indicates that the response to sildenafil was relatively independent of the level of kidney function. The study design with consecutive measurements in each patient on 2 independent study days and subsequent within-subject comparison allowed detection of small differences in functional parameters. The present observation of an antinatriuretic effect of sildenafil is not consistent with the observed natriuretic effect in cirrhotic rats after administration of the PDE5 antagonist zaprinast (4). The IC50 of zaprinast and sildenafil are 2.0 μM and 3.5 nM, respectively, indicating a superior PDE5 affinity of sildenafil (24). In decompensated cirrhotic rats, zaprinast increased sodium excretion and GFR in doses not affecting blood pressure (15–30 μg·kg−1·min−1) and normalized renal tissue cGMP concentration (4). In the present cirrhotic patients, plasma cGMP concentration was increased in response to sildenafil, as seen in healthy subjects (8), whereas no change in urinary cGMP was observed. In healthy supine subjects, sildenafil (40–80 mg intravenously) has been reported to cause a transient 7- to 9-mmHg decrease in systolic and diastolic blood pressures (14), whereas oral sildenafil (50–100 mg) did not affect blood pressure (8, 31). Based on these results and results from cirrhotic patients where 50 mg were well tolerated (27), we chose the lowest dose (50 mg orally) and still observed a mild, delayed decrease in MAP. This is in line with results from hypertensive subjects (20) and suggests that we did achieve systemic inhibition of PDE5. We cannot exclude that the failure of sildenafil to promote natriuresis and urinary cGMP excretion is a result of incomplete inhibition of PDE5 in the kidneys, but a more likely explanation for the sodium-retaining effect is the early activation of the renin-angiotensin system. A regular dose-response study in cirrhotic patients might provide information as to whether effects of sildenafil on kidney function are obscured by blood pressure changes or higher doses are required to obtain PDE5 inhibition in kidney

Table 4. Effect of 50 mg sildenafil on plasma ANP and renal functional parameters on the 2 study days

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
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<tbody>
<tr>
<td><strong>Plasma ANP, pg/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo day</td>
<td>70 (34–132)</td>
<td>69 (38–122)</td>
<td>78 (36–142)</td>
<td>76 (37–136)</td>
</tr>
<tr>
<td>Sildenafil day</td>
<td>68 (42–110)</td>
<td>69 (41–110)</td>
<td>65 (38–116)</td>
<td>70 (35–133)</td>
</tr>
<tr>
<td><strong>Uosm, mosmol/kgH2O</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo day</td>
<td>4.5 (1.0–12.1)</td>
<td>6.4 (2.3–18.5)</td>
<td>6.4 (1.3–17.7)</td>
<td>5.2 (1.8–14.2)</td>
</tr>
<tr>
<td>Sildenafil day</td>
<td>5.6 (3.5–8.7)</td>
<td>5.4 (2.8–11.5)</td>
<td>5.9 (3.8–13.3)</td>
<td>7.1 (2.9–15.8)</td>
</tr>
<tr>
<td><strong>Diuresis, ml/h</strong></td>
<td>59 (23–257)</td>
<td>136 (33–245)</td>
<td>141 (59–300)</td>
<td>170 (78–419)</td>
</tr>
<tr>
<td>Sildenafil day</td>
<td>133 (30–323)</td>
<td>98 (31–202)</td>
<td>77 (30–237)</td>
<td>130 (33–250)</td>
</tr>
<tr>
<td><strong>UcGMPV, pmol/100 min</strong></td>
<td>403 (262–760)</td>
<td>196 (133–914)</td>
<td>199 (138–560)</td>
<td>169 (138–560)</td>
</tr>
<tr>
<td>Sildenafil day</td>
<td>312 (97–849)</td>
<td>438 (70–735)</td>
<td>394 (64–720)</td>
<td>279 (53–580)</td>
</tr>
<tr>
<td><strong>CH2O, ml/min</strong></td>
<td>59 (32–164)</td>
<td>77 (37–115)</td>
<td>87 (39–148)</td>
<td>104 (41–152)</td>
</tr>
<tr>
<td>Placebo day</td>
<td>1,560 (805–5,678)</td>
<td>1,826 (1,210–5,500)</td>
<td>1,470 (846–5,436)</td>
<td>2,054 (1,049–4,392)</td>
</tr>
<tr>
<td>Sildenafil day</td>
<td>1,540 (937–4,292)</td>
<td>1,170 (826–4,580)</td>
<td>1,199 (409–3,360)</td>
<td>1,540 (579–3,102)</td>
</tr>
<tr>
<td><strong>GFR, ml/min⁻¹·1.73 m²⁻¹</strong></td>
<td>116 (37–136)</td>
<td>112 (24–119)</td>
<td>112 (41–138)</td>
<td>106 (24–133)</td>
</tr>
<tr>
<td>Placebo day (n = 6)</td>
<td>123 (66–164)</td>
<td>114 (54–152)</td>
<td>87 (57–127)</td>
<td>111 (41–152)</td>
</tr>
<tr>
<td>Sildenafil day (n = 6)</td>
<td>692 (251–925)</td>
<td>598 (201–1,077)</td>
<td>607 (110–1,448)</td>
<td>524 (87–1,188)</td>
</tr>
<tr>
<td><strong>ERPF, ml/min⁻¹·1.73 m²⁻¹</strong></td>
<td>628 (388–1,120)</td>
<td>651 (416–1,095)</td>
<td>656 (136–1,180)</td>
<td>621 (106–1,287)</td>
</tr>
</tbody>
</table>

Values are expressed as median and (range). Uosm, UcGMPV, and UcV: urinary cGMP execution, osmolarity, and potassium excretion, respectively; GFR, glomerular filtration rate; ERPF, effective renal plasma flow. No significant changes were detected between the 2 study days.
tissue. However, this does not seem warranted based on published data on the bioavailability, clearance, and mild side effects of 50 mg sildenafil in patients with liver impairment (27). Thus if PDE5 was incompletely inhibited in the kidneys with the present dose, it is not likely that renal PDE5 inhibition can be pharmacologically achieved at all in this sensitive patient group because of significant cardiovascular side effects.

We observed a transient increase in HR, as seen sometimes (8), but not always (14, 31), in healthy subjects. This was likely due to an increased activity of the sympathetic nervous system because plasma norepinephrine levels increased by 30% after sildenafil in healthy control subjects (31). The early stimulation of HR by sildenafil could be caused by a direct effect on the central nervous system or by peripheral effects on venous return. It was less likely due to a drop in arterial blood pressure, which was first significantly lowered after 2 h. The increase in heart rate at 60 min was accompanied by increases in plasma renin, ANG II, and aldosterone concentrations before any

Fig. 3. Effect of sildenafil on plasma renin activity (PRA), plasma ANG II concentration, and aldosterone concentration (n = 8). Shaded areas in left panels A–C represent normal hormone concentration ranges. A: changes in absolute PRA in each patient at the 2 different intervention days (left) and relative changes in PRA in response to sildenafil and placebo compared with the premedication level during intervention (right). Values are means ± SE. **P < 0.01. *P < 0.05. B: changes in plasma ANG II concentration in each patient at the 2 different intervention days (left) and relative changes in ANG II in plasma in response to sildenafil and placebo compared with the premedication level (right). Values are means ± SE. *P < 0.05. C: changes in absolute plasma aldosterone concentration in each patient at the 2 different intervention days (left) and relative changes in aldosterone in plasma in response to sildenafil and placebo compared with the premedication level (right). Values are means ± SE. #P < 0.05.

Fig. 4. Effect of sildenafil on sodium excretion (UNaV) in decompensated cirrhotic patients. Left: absolute values of UNaV in each patient during administration of sildenafil and placebo. Right: relative change in UNaV in decompensated cirrhotic patients after sildenafil and placebo compared with baseline value. Values are means ± SE. *P < 0.05 and **P < 0.01 between the 2 study days.
detectable decrease in blood pressure. The rapid activation of the RAAS could be a direct stimulatory effect on renin-secreting juxtaglomerular cells or it could be secondary to increased sympathetic activity.

The pharmacokinetics of sildenafil are affected by hepatic impairment (27, 39). In a subset of cirrhotic patients, plasma concentrations of both sildenafil and its active metabolite were increased by 50%, resulting in a systemic exposure (area under the curve) ~85% higher than in weight- and age-matched healthy controls (27). Although the hypoensive effect of sildenafil was reported to be independent of dose (20), it is likely that a higher systemic exposure in patients with liver cirrhosis contributed to the hypoensive effects observed in 3 of 9 patients. It is well known that the modest effect of sildenafil on systemic hemodynamics may be augmented by nitric oxide donors, which promote cGMP formation (20). Endogenously elevated plasma nitric oxide levels occur in patients with cirrhosis (13) and may have contributed to an enhanced sensitivity of blood pressure to sildenafil.

ANP regulates sodium excretion, especially in states of sodium excess (18, 41). However, several studies have shown the influence of ANP on sodium excretion to be inferior to the influence of RAAS and the sympathetetic nervous system. Overexpression of ANP in transgenic mice resulted in slight hypotension and normal sodium balance at the expense of increased plasma renin activity and aldosterone (5, 21, 23). Moreover, sodium-restricted humans did not suppress RAAS in response to ANP infusion (6). These findings are in agreement with our results and support the view that the natriuretic effect of elevated ANP is not sufficient to overcome the effect of an activated RAAS in decompensated cirrhosis. This is in accord with other studies in which cirrhotic patients who were unable to increase sodium excretion by different volume-expanding maneuvers had more advanced liver disease, more avid sodium retention, higher RAAS activity, and a more hypoensive response to ANP compared with a group of patients responding with increased natriuresis (10, 34). Thus these studies stress that a successful natriuretic effect of ANP in the cirrhotic patient depends on suppression of antinatriuretic systems. Other possible mechanisms of ANP resistance could be diminished delivery of sodium to the ANP-sensitive part of the nephron (1, 26); increased renal neutral endopeptidase activity, limiting access of ANP to receptor sites (30); and alteration in renal ANP receptor density or function with a higher ratio of clearance receptors to biologically active receptors (11). We did not determine intrarenal levels of cGMP after sildenafil, and therefore a relative insensitivity of cellular pathways distal to cGMP could potentially also have contributed.

We conclude that administration of a PDE5-selective inhibitor (sildenafil 50 mg) to decompensated cirrhotic patients did not promote natriuresis. On the contrary, treatment with sildenafil caused rapid activation of the RAAS and sodium retention which was associated with a decrease in arterial blood pressure.

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