Interference of angiotensin II and enalapril with hepatic blood flow regulation

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Twenty male pigs (median weight 40.0 kg, IQR 38.0–41.25 kg) were randomized to receive AT-II (n = 8) from 5 to 61 ng/kg per min, enalapril (n = 8) from 3 to 24 μg/kg per h, or saline (n = 4). HABR was assessed by occluding portal vein and expressed as (I) ratio between changes in Qha and Qpv. AT-II infusion increased mean arterial blood pressure from 74 (66–77) mmHg to 116 (109–130) mmHg (median, IQR; P < 0.0001) and decreased cardiac output, Qpv, and renal artery flow (−24%, −28% and −45%, respectively). The fraction of cardiac output of Qha, carotid, and femoral flows increased. With enalapril, blood pressure decreased, whereas cardiac output was maintained with flow redistribution favoring hepatic and renal arteries. In AT-II group, dQha/dQpv increased from 0.06 (0.03, 0.17) to 0.24 (0.13, 0.31) (P = 0.002), but Cha during acute portal vein occlusion decreased from 4.3 (1.6, 6.6) to 2.9 (1.2, 3.7) ml/mmHg (P = 0.003). Both variables remained unchanged in the enalapril group and in controls. AT-II infusion reduces portal flow in parallel with cardiac output and induces a dose-dependent redistribution of flow, favoring brain, hepatic artery, and peripheral tissues at the expense of renal perfusion. During HABR, AT-II decreases Cha but increases Qha compensation, likely as result of increased hepatic arterial perfusion pressure. Enalapril had no effect on HABR.

hepatic artery buffer response; angiotensin II; angiotensin-converting enzyme inhibitor; enalapril

THE RENIN-ANGIOTENSIN SYSTEM (RAS) with its main effector peptide angiotensin II plays a major role in cardiovascular homeostasis (8, 27). Angiotensin II acts through its binding to specific cellular receptors, angiotensin receptors type 1 (AT1) and type 2 (AT2). AT1 receptors mediate vasoconstriction, aldosterone synthesis, as well as aldosterone and vasopressin secretion. Effects mediated by the AT2 receptor include apoptosis, inhibition of cell growth, inflammatory cell recruitment, and activation of the kinin/nitric oxide system (8, 27).

Reduction in portal vein flow is a well-recognized effect of angiotensin II (7, 30, 38). RAS and sympathetic nervous system activation and nonosmotic release of antidiuretic hormone as consequences of peripheral arterial vasodilation are involved in the pathogenesis of decompensated cirrhosis (2, 40). Sinusoidal structural changes and increased vascular tone (due to reduced bioavailability of nitric oxide and increased production of vasoconstrictors as angiotensin, endothelin, cysteiny1 leukotrienes, and thromboxane) are initial events in pathophysiology of portal hypertension (3, 23). Additionally, a local hepatic RAS seems to potentiate the process (5, 25). This seems to be countered by a recently described alternative RAS system arm (11), with actions mediated by another angiotensin-converting enzyme (ACE2), activating angiotensin-1–7, which could exert protective effects by reducing hepatic fibrosis (36).

Theoretically, enalapril as ACE inhibitor could block the impact of endogenous angiotensin II. Short-term enalapril infusion can quickly reduce hepatic venous pressure gradient in patients with hepatitis B liver cirrhosis (9), and there is accumulating evidence that RAS blockade could prevent the progression of liver fibrosis and portal hypertension (15). However, in advanced disease, systemic hypotension induced by ACE inhibitors can activate other vasoconstrictive pathways, maintain increased intrahepatic resistances (16), and induce or worsen kidney dysfunction (14).

The hepatic artery buffer response (HABR) is a physiological protective mechanism by which hepatic artery flow increases to defend global liver perfusion in response to portal vein flow reduction. The effect of angiotensin II and enalapril on the HABR has not been explored so far. Before designing future studies using angiotensin II as a therapeutic option in critical conditions in which liver flow could be affected, that issue should be addressed.

The aim of this experimental study was to evaluate the effects of increasing doses of angiotensin II and ACE inhibition on regional blood flow distribution and HABR. We hypothesized that angiotensin II reduces portal blood flow but preserves HABR. In contrast, enalapril may improve regional perfusion but impair HABR by decreasing the capacity of the hepatic artery to further dilate when portal flow decreases.

MATERIALS AND METHODS

The study was performed in accordance with the National Institutes of Health guidelines for the care and use of experimental animals, and with the approval of the Animal Care Committee of the Canton of Bern, Switzerland.

Twenty male pigs (median weight 40.0 kg, IQR 38.0–41.25 kg) were studied after block randomization to three different groups: angiotensin II (n = 8), enalapril (n = 8), and control (placebo, n = 4). After a few days of acclimatization in the veterinary hospital, and 12-h fasting with free access to water, the pigs were premedicated with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg). After orotracheal intubation and an ear vein cannulation, anesthesia was induced with intravenous midazolam (0.5 mg/kg, plus atropine 0.02 mg/kg). Anesthesia was maintained with continuous intravenous infusions of propofol (4–8 mg/kg per h) and fentanyl (30 μg/kg per h) (reduced to 5 μg/kg per h at the end of the surgery). The stomach was
kept empty by a large-bore orogastric tube. Ringer’s acetate (12 ml/kg) as a bolus was infused before surgery, and a continuous infusion of 10 ml/Kg per h was maintained throughout the study. Core temperature was targeted to 38.0 to 39.5°C, using room temperature control and a fan.

Animals were ventilated with a volume-controlled mode (Servo I; Maquet, Rastatt, Germany), adjusted to a positive-end expiratory pressure of 5 cmH2O, a FiO2 to maintain PaO2 levels between 100 and 150 mmHg, and a minute ventilation adjusted to PaCO2 levels between 35 and 45 mmHg (with a tidal volume of 8 ml/kg and a respiratory rate adjusted from 20–28 breaths/min).

Surgical Preparation

Before surgery, all animals received 1.5 g of intravenous cefuroxime. Under sterile conditions, right carotid artery, right external and internal jugular veins, left external jugular vein, and left femoral vein were cannulated under direct visualization. Doppler flow probes (Transonic, Ithaca, NY) were placed around carotid and left femoral arteries. Right external jugular vein access was used for fluoroscopy-guided coronary sinus catheterization (Cordis; Johnson & Johnson, Jacksonville, FL), right internal jugular vein for fluoroscopy-guided right hepatic vein catheterization (pulmonary artery catheter; Edwards Lifesciences, Munich, Germany), and left external jugular vein for pulmonary artery catheterization (Edwards Lifesciences).

A median laparotomy was performed to allow hepatic artery (indirectly, through gastroduodenal artery), right renal vein, and portal vein (through splenic or mesenteric veins) catheterizations and flow probe placement around hepatic artery, portal vein, and right renal artery. Occluders were placed around celiac trunk and portal vein. A urinary catheter was inserted into the bladder under direct visualization.

Study Protocol

After surgery and 1 h of stabilization, randomization was performed, and baseline data and blood samples were obtained. In the first animal, HABR was absent (see Calculations and Statistical Analysis). We speculated that the animal was hypovolemic. Therefore, the protocol was adapted in subsequent animals, and fluid challenges performed after a first pilot (26, 28, 29, 31, 43). The dose range for angiotensin II was defined using the maximum and minimum systemic dose found in literature, including animal studies, with adjustments performed after a first pilot (26, 28, 29, 31, 43). The dose range for enalapril was defined based on human doses, adjusted per body weight of the animal. Different time intervals (half-hourly increases in doses of angiotensin II, and hourly increases for enalapril) were chosen for the two drugs according to their pharmacokinetic profiles.

Control animals received hourly increasing amounts of saline, based on the average of expected volumes for drug infusion in enalapril and angiotensin II groups.

HABR Measurements

Variables for the assessment of the HABR were calculated as follows: 1) relative blood flow compensation, \( \frac{dQ_{ha}/dQ_{pv}}{\Delta Q_{ha}/\Delta Q_{pv}} \) (change in Qha/change in Qpv in response to portal vein occlusion); 2) hepatic arterial conductance, during portal vein occlusion (Cha, portal) = \( \frac{Q_{ha}}{\Delta Q_{ha}/\Delta Q_{pv}} \) divided by difference between hepatic arterial pressure before and after celiac trunk occlusion, and without portal vein occlusion (Cha) = \( \frac{Q_{ha}}{\Delta Q_{ha}} \) before portal vein occlusion divided by the difference between actual hepatic arterial pressure and the pressure during celiac trunk occlusion with released portal vein flow (Cha).

After the last measurements, sedation was deepened, and tissue was sampled for another study. The animals were then euthanized by an overdose of potassium chloride.

Monitoring

Hemodynamic and respiratory data were collected using two sets of modular patient monitors (S/5 Compact Critical Care monitor; Datex-Ohmeda, Helsinki, Finland), a continual cardiac output/SvO2 monitor (Edwards Lifesciences, Irvine, CA), and a multichannel flowmeter (TS 420 flowmeter; Transonic Systems). Urine output and fluid balance were recorded.

Data were recorded at a sampling frequency of 100/s using a data acquisition and signal analysis software (Soleasy/Labview; National Instruments, Austin, TX) and in an electronic patient data management system (Clinisoft; GE Healthcare, Helsinki, Finland), in which 2-min medians were stored.

Blood Sampling and Analysis

Blood gas analysis (GEM Premier 3000 analyzer; GEM, Bohemia, NY), hemoglobin (Hb), and oxygen saturation (SaO2) measurements (OSM3; Radiometer, Copenhagen, Denmark; precalibrated for pig blood) were performed at baseline and thereafter at hourly intervals from all vascular sites (carotid artery, pulmonary artery, coronary sinus, portal vein, hepatic vein, and right renal vein). ACE activity was measured hourly (Thermo Infinity ACE Liquid Stable Reagent; Thermo Scientific, Sunnyvale, CA) and angiotensin II concentrations (Enzyme Immunoassay kit; Enzo Life Science, Lausen, Switzerland). In addition, creatinine at baseline and end of experiment and hourly diuresis were measured. Urinary creatinine for calculation of creatinine clearance (4 h) was measured in the end of the experiment.

Determination of Plasma Angiotensin II Concentration

The angiotensin II Enzyme Immunoassay kit (Enzo Life Science) was used for the quantitative determination of angiotensin II in the plasma samples. The kit contains assay buffer (Tris buffer containing proteins and preservatives), angiotensin II standard, 96-well microtiter plate coated with goat anti-rabbit polyclonal antibody, biotinylated angiotensin II conjugate, streptavidin conjugated to horseradish peroxidase (streptavidin-HRP), wash buffer (Tris-buffered saline containing detergents), 3,3',5',tetramethylbenzidine (TMB) substrate, stop solution (1 N solution of hydrochloric acid), and plate sealers. The assay was initiated by adding 50 µl of standards (final concentrations in the wells: 3.9, 15.6, 62.5, 250, 1,000, and 10,000 pg/ml) and plasma samples to the bottom of the appropriate wells of the microtiter plate coated with a goat-anti-rabbit IgG antibody. Into the blank wells, 50 µl of wash buffer was added. To determine nonspecific binding, 75 µl of the assay buffer was added into the nonspecific binding wells. Afterward, 25 µl of rabbit polyclonal antibody to angiotensin II was added into each well except the blank and nonspecific binding wells. The plate was then sealed and incubated for 1 h at room temperature on a plate shaker (500 revolution/min). Afterward, 25 µl of angiotensin II conjugated to biotin was added (1 h incubation at room temperature on a plate shaker, 500 revolution/min). During this incubation, the specific
Determination of Plasma ACE Activity

As ACE activity is inhibited in blood containing the chelating agent EDTA, blood samples were collected in separate tubes containing heparin instead of EDTA. ACE activity was measured in heparinized plasma samples using Infinity ACE Liquid Stable Reagent enzymatic kit (Thermo Scientific) according to the manufacturer’s instructions.

Table 1. Hemodynamic and oxygen variables

| Variables                  | Group  | BL       | T1       | T2       | T3       | T4       | P<  
|---------------------------|--------|----------|----------|----------|----------|----------|-----
| MAP, mmHg                 | ENL    | 75.4     | 65.3     | 68.2     | 60.2     | 59.7     | 0.006 |
|                           | ANGII  | 74.0     | 89.6     | 99.9     | 100.8    | 112.6    | <0.001 |
|                           | PLAC   | 59.7     | 58.8     | 56.8     | 64.5     | 63.1     | 0.454 |
| Heart rate, beats/min     | ENL    | 96.0     | 97.0     | 103.0    | 111.0    | 96.0     | 0.771 |
|                           | ANGII  | 97.5     | 79.5     | 75.5     | 77.0     | 71.0     | 0.638 |
|                           | PLAC   | 77.5     | 81.0     | 95.5     | 100.0    | 70.0     | 0.001 |
| Cardiac output, ml/kg per min | ENL  | 115.2    | 104.5    | 108.2    | 100.2    | 94.5     | 0.013 |
|                           | ANGII  | 121.6    | 117.0    | 112.7    | 100.4    | 93.7     | 0.076 |
|                           | PLAC   | 103.1    | 103.0    | 91.3     | 98.1     | 99.7     | 0.525 |
| Stroke volume, ml/kg      | ENL    | 1.27     | 1.06     | 1.00     | 1.01     | 1.07     | 0.039 |
|                           | ANGII  | 1.43     | 1.17     | 1.21     | 1.15     | 1.24     | 0.141 |
|                           | PLAC   | 1.32     | 1.19     | 0.93     | 0.90     | 1.00     | 0.015 |
| MPAP, mmHg                | ENL    | 15.3     | 15.7     | 16.2     | 16.2     | 14.4     | 0.179 |
|                           | ANGII  | 15.2     | 15.9     | 16.1     | 17.5     | 16.9     | 0.006 |
|                           | PLAC   | 12.5     | 14.3     | 15.0     | 13.7     | 13.7     | 0.048 |
| CVP, mmHg                 | ENL    | 4.0      | 3.6      | 3.4      | 3.2      | 3.4      | 0.567 |
|                           | ANGII  | 3.4      | 3.2      | 4.4      | 4.6      | 5.0      | 0.042 |
|                           | PLAC   | 2.1      | 3.1      | 3.0      | 2.7      | 2.9      | 0.558 |
| SvO2, %                   | ENL    | 53.5     | 49.3     | 49.6     | 51.1     | 50.4     | 0.150 |
|                           | ANGII  | 55.7     | 50.3     | 47.7     | 49.5     | 51.6     | 0.020 |
|                           | PLAC   | 49.7     | 47.3     | 45.7     | 52.5     | 52.0     | 0.910 |

Values are medians (IQR). ENL, Enalapril group (n = 7); ANGII, angiotensin-II group (n = 8); PLAC, placebo group (n = 4); MAP, mean arterial pressure; MPAP, mean pulmonary artery pressure; CVP, central venous pressure; BL: baseline (no drug infusion); T1/T2/T3/T4: times after increased doses of the study drugs. *Friedman’s test (within-group effect). †Wilcoxon signed-rank test vs. baseline (P < 0.05). Boldface denotes statistically significant P values (P < 0.05).

Table 2. Regional absolute flows

| Variables                  | Group  | Baseline | T1       | T2       | T3       | T4       | P<  
|---------------------------|--------|----------|----------|----------|----------|----------|-----
| Carotid flow, ml/kg per min | ENL    | 6.08     | 5.31     | 5.34     | 4.92     | 5.50     | 0.663 |
|                           | ANGII  | 7.19     | 6.57     | 8.12     | 8.50     | 8.87     | 0.126 |
|                           | PLAC   | 5.48     | 5.06     | 6.29     | 6.34     | 5.95     | 0.185 |
| Femoral flow, ml/kg per min | ENL    | 2.19     | 2.53     | 2.58     | 2.37     | 2.47     | 0.978 |
|                           | ANGII  | 2.87     | 3.14     | 2.82     | 2.99     | 2.95     | 0.951 |
|                           | PLAC   | 1.54     | 1.56     | 1.86     | 2.15     | 2.27     | 0.199 |
| Hepatic artery flow, ml/kg per min | ENL  | 1.69     | 2.21     | 2.56     | 1.92     | 2.11     | 0.087 |
|                           | ANGII  | 1.80     | 1.44     | 1.44     | 2.48     | 2.59     | 0.005 |
|                           | PLAC   | 1.51     | 1.48     | 2.36     | 2.04     | 2.21     | 0.938 |
| Portal vein flow, ml/kg per min | ENL  | 22.21    | 17.87    | 17.90    | 20.04    | 20.00    | 0.622 |
|                           | ANGII  | 21.73    | 17.39    | 18.47    | 16.76    | 15.72    | 0.001 |
|                           | PLAC   | 21.96    | 22.01    | 21.22    | 23.33    | 24.17    | 0.592 |
| Renal artery flow, ml/kg per min | ENL  | 4.69     | 5.46     | 5.07     | 4.49     | 4.60     | 0.704 |
|                           | ANGII  | 5.74     | 3.40     | 3.28     | 2.99     | 2.94     | 0.001 |
|                           | PLAC   | 8.10     | 7.91     | 7.90     | 8.64     | 7.10     | 0.331 |
| Unmeasured flows, ml/kg per min | ENL  | 59.93    | 53.52    | 52.91    | 48.10    | 45.32    | 0.203 |
|                           | ANGII  | 66.90    | 44.37    | 44.66    | 48.16    | 44.57    | 0.006 |
|                           | PLAC   | 50.53    | 43.77    | 37.14    | 38.72    | 43.23    | 0.116 |

Values are medians (IQR). Enalapril group (n = 7); angiotensin-II group (n = 8); placebo group (n = 4). *Friedman’s test (within-group effect). †Wilcoxon signed-rank test vs. baseline (P < 0.05). Boldface denotes statistically significant P values (P < 0.05).
The Infinite ACE reagent of the kit was comprised of 55 mmol/L Tris buffer pH 8.2, the ACE substrate \(N-[3-(2-furyl)-acryloyl]-L-phenylalanyl]glycylglycine (FAPGG), and additives. The assay is based on the continued kinetic method first described by Holmquist et al. (22). First, 30 μl of plasma samples were mixed with 300 μl of Infinite ACE reagent, and ACE activity was monitored using a kinetic assay type on a VERSAmax microplate reader (Molecular Devices) at 340 nm and 37°C. In the assay, FAPGG is hydrolyzed by plasma ACE to FAP and glycylglycine (FAPGG → FAP + glycylglycine). FAP has a low absorbance at 340 nm, and the hydrolysis of FAPGG by ACE results in a decrease in absorbance at 340 nm. Glycylglycine does not absorb light at this condition. For adequate quality control, normal and elevated Thermo Scientific assayed control materials (TR85101) were run as unknown samples. Calibration was performed using Thermo Scientific ACE calibrator (TR85201), and results were calculated with the calibration factor as follows: ACE activity (U/l) = [(ΔAbsorbance/min of unknown sample)/(ΔAbsorbance/min of calibrator)] × calibrator value.

**Calculations and Statistical Analysis**

The sum of unmeasured regional flows was calculated as cardiac output – \([2 \times \text{(carotid + renal + femoral blood flow)} + \text{hepatic artery + portal vein blood flow}].\) Because true zero flow conditions in portal vein and hepatic artery could not be achieved consistently during the HABR assessments, zero flow pressures were calculated by linear extrapolation of the flows measured with open and occluded vessel. At the point of zero flow, the corresponding pressure was calculated on the extrapolated blood pressure line. For statistical analysis, the SPSS software package (version 20.0) was used. Data are presented as median (interquartile range) and as full range in the four controls. Changes over time were assessed by Friedman’s test. In the case of a significant Friedman’s test but a biphasic response, a post hoc Wilcoxon signed-rank test was performed to compare baseline with the most aberrant interim value. Kruskal-Wallis test was performed to test between-group differences of kidney function. Twenty animals were initially foreseen, but 22 were studied. In two animals,
the protocol could not be completed because of malignant tachyarrhythmia (during vascular catheterization and during the baseline HABR maneuvers, respectively). All data from these two animals were excluded from the analysis. Hemodynamic values of the first animal studied (randomized to enalapril group) were also excluded because of absent hepatic artery buffer response, most likely as a result of hypovolemia (see Study Protocol). The impact of blood flow distribution was evaluated based on changes in fractional blood flows. Statistical significance was considered at $P < 0.05$.

RESULTS

Hemodynamics

**Global hemodynamics.** In angiotensin II group, blood pressure increased by 70% with maximum doses ($P < 0.0001$), whereas it decreased in enalapril group by 18% ($P = 0.002$) (Table 1). Cardiac output but not stroke volume decreased in angiotensin II group ($-24%$; $P = 0.004$), whereas cardiac output remained unchanged in enalapril group (Table 1). Control animals remained hemodynamically stable.

**Regional hemodynamics.** With increasing angiotensin II doses, renal artery and portal vein blood flow decreased ($-45%$, $P = 0.001$; $-28%$, $P < 0.0001$, respectively). Hepatic artery blood flow decreased early ($-24%$, $P = 0.005$) but subsequently fully recovered (Table 2). The other regional flows and all regional blood flows in enalapril and control groups remained unchanged.

The regional blood flow distribution, expressed as a fraction of cardiac output was modified with angiotensin II. Carotid,
femoral, and hepatic artery fractional flows increased and remained elevated, whereas the increase in portal vein fractional flow was transient. In contrast, renal fractional blood flow decreased \((P = 0.002)\). In the enalapril group, both hepatic \((+37\%)\) and renal \((+31\%)\) artery fractional blood flows increased (Table 3).

**HABR**

With increasing angiotensin II doses, acute portal vein occlusion induced an increasing hepatic arterial blood flow compensation \((dQ_{ha}/dQ_{pv}; P = 0.002, \text{Fig. 1})\). The increased \(dQ_{ha}/dQ_{pv}\) was the result of an (early) increasing \(dQ_{ha}\) \((P =\) \(\text{Fig. 4. Conductance in hepatic artery with and without portal vein occlusion. PV: portal vein.} P\) value: Friedman (time-effect). Enalapril \((n = 7)\); Angiotensin II \((n = 8)\); Placebo \((n = 4)\).
Table 4. Kidney function

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Baseline</th>
<th>END</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis, ml/kg</td>
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<td>1.42 (1.20, 3.09)</td>
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<tr>
<td>Creatinine, mg/dl</td>
<td>ENL</td>
<td>1.07 (0.90, 1.31)</td>
<td>1.10 (0.87, 1.41)</td>
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<td>0.310</td>
</tr>
<tr>
<td>CRE, mg/dl</td>
<td>ANGII</td>
<td>1.02 (0.90, 1.17)</td>
<td>1.04 (0.98, 1.16)</td>
<td>0.207</td>
<td></td>
</tr>
<tr>
<td>PLAC</td>
<td>1.00 (0.90, 1.19)</td>
<td>1.04 (0.96, 1.14)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means (IQR). Angiotensin II group (n = 8), Enalapril group (n = 7), placebo group (n = 4). Baseline: before start of the randomized study drug, END: after maximum dose of the randomized study drug. *P = group effect at END (Kruskal-Wallis test), †P = time effect (Wilcoxon signed ranks test).

0.044 and a (later) decreasing dQpv (P < 0.001; Fig. 2). Enalapril had no effect. During celiac trunk occlusion and maintained portal vein occlusion, increasing hepatic artery pressure values were measured in angiotensin animals with progressively higher doses (Fig. 3). Hepatic arterial conductance during portal vein occlusion decreased in angiotensin group (P = 0.001) and tended to increase in enalapril group (P = 0.060; Fig. 4).

Kidney Function

Four hours of angiotensin and enalapril infusion had no effect on diuresis, creatinine, and creatinine clearance (Table 4).

Angiotensin II Levels and ACE Activity

Increasing angiotensin II infusion rates increased angiotensin II levels, reaching a plateau at the two highest doses (Fig. 5). Angiotensin II plasma concentrations were similar in enalapril and control animals, even in the presence of high doses of enalapril. ACE activity was reduced close to zero in enalapril group, and there was a slight and early reduction in angiotensin II animals (Fig. 6).

DISCUSSION

The present study has two main findings. 1) Both angiotensin II and enalapril induced blood flow redistribution; angiotensin II favored the hepatic artery, the brain, and the peripheral tissues at the expense of renal blood flow, whereas enalapril increased fractional hepatic artery and renal blood flows. 2) Angiotensin II decreased hepatic arterial conductance during portal vein occlusion, but, despite this, progressive reduction of portal flow was better compensated by HABR.

Angiotensin II reduces blood flow to the kidney (29) and, if infused directly into the portal vein, also to the liver (38). The clinical relevance of these alterations and potential adaptive mechanisms involved in intact organisms are largely unexplored. In vitro experiments in isolated arteries suggest that the hepatic artery is one of the least responsive arteries to the angiotensin II vasoconstrictive effect, and superior mesenteric artery is the most responsive one (41). Our data demonstrate that portal vein blood flow, which gets tributaries from both the superior mesenteric artery and celiac trunk, does not decrease in excess to cardiac output during increasing doses of angiotensin. During single-pass perfusion of rat livers, both intraarterial and intraportal infusion of angiotensin II decreased blood flow in the respective vessels (37). The portal vein expresses AT receptors in pigs (32) and in humans and other mammals (35). In addition, angiotensin II may also induce contraction of activated stellate cells and thereby increase portal vein resistance (4). Our intact experimental model demonstrates that the hepatic artery resistance increases less in response to angiotensin II than the portal vein and systemic vascular resistance do. Within the angiotensin II dose range we

Fig. 5. Angiotensin II plasmatic levels. P value = Friedman test (time-effect). Enalapril group (n = 8); Angiotensin II group (n = 8); Placebo group (n = 4).

Fig. 6. Angiotensin converting-enzyme (ACE) plasmatic activity. P value = Friedman test (time-effect). Enalapril group (n = 8); Angiotensin-II group (n = 8); Placebo group (n = 4).
used, hepatic artery conductance measured at unrestricted portal flow decreased only transiently and fully recovered at higher doses. We assume that the increase in hepatic arterial vascular resistance induced by angiotensin II at higher doses was counteracted by a progressive HABR-mediated vasodilatation as a consequence of simultaneously decreasing portal vein blood flow.

The lack of an increase in absolute hepatic artery blood flow with enalapril despite decreased zero hepatic artery blood flow pressure and increasing hepatic arterial conductance may have been related to unchanged cardiac output and critically reduced perfusion pressure in our model. However, ACE inhibition in healthy volunteers, in patients after cardiac surgery, and in patients with congestive heart failure did not change the absolute splanchnic and hepatic blood flows (13, 34, 42).

The clinical relevance of the HABR is a partial compensation of decreasing portal vein oxygen delivery by increasing hepatic arterial oxygen supply (24). Systemic infusion of angiotensin II is likely to modify the HABR because selective infusion to portal vein and hepatic artery reduces the respective blood flows (37). We found that angiotensin II infusion decreased hepatic arterial conductance but increased hepatic artery blood flow compensation when portal vein blood flow was acutely decreased. Because absolute hepatic artery blood flow compensation increased only during the lowest angiotensin dose, and remained at the same levels at higher doses, increasing (relative) blood flow compensation (dQha/dQpv) at higher doses was the result of maintained artery flow increase with a numerically decreasing portal flow reduction (dQpv). Because HABR is adenosine mediated (12), the maximal response should be dependent on the rate of adenosine production or release rather than on the magnitude of portal blood flow reduction. Therefore, dQha/dQpv does not necessarily change in parallel with hepatic arterial conductance. From the clinical point of view, dQha/dQpv is probably more relevant because it reflects the actual compensation of liver blood flow. The decrease in hepatic artery conductance (dQha/dQha) during increasing angiotensin dose infusion was mainly related to increased perfusion pressure because hepatic blood flow compensation remained similar at all angiotensin doses.

With enalapril, hepatic arterial conductance tended to increase during assessment of HABR. Mathematically, this was the result of decreasing hepatic arterial perfusion pressure with maintained hepatic (and portal) flow changes during the HABR maneuver. Because (weak) hepatic arterial autoregulation has been reported (12), it is difficult to determine whether or not enalapril exhibited a small effect on the HABR.

Our results obtained in healthy pigs may therefore have limited clinical relevance in disease states such as liver cirrhosis, where several vasoactive substances such as nitric oxide, cyclooxygenase-derivatives, and carbon monoxide are activated (reviewed in Ref. 6). In these circumstances, splanchic vessels are dilated, and their reaction to vasoconstrictors is diminished. On the other hand, there is evidence that the intrahepatic RAS is considerably upregulated in the diseased liver (33) (reviewed in Ref. 19). In the perfused cirrhotic rat liver, the vasoconstriction response to angiotensin II is increased (20). Furthermore, there are data indicating that, in rats, liver angiotensin-(1–7), which is converted by ACE2 from angiotensin II (19), may antagonize the increase in portal pressure mediated by angiotensin II (21). Because the HABR is diminished in liver cirrhosis (1, 18), the net effect of angiotensin II on liver blood flow in this condition may considerably differ from what we found in healthy animals.

Our study has limitations. In this intact animal model, it was not possible to achieve systematically zero flow conditions because of collateral circulation. Zero flow pressure was therefore calculated assuming a linear relationship between pressure and flow in the lowest range. This may have introduced small errors in our estimate of conductance. Furthermore, despite the use of doses analogous to human maximum doses, angiotensin II blood concentrations were not significantly reduced during enalapril treatment. Potential effects of physiological angiotensin II concentrations on the HABR could therefore not be evaluated. Finally, anesthesia with propofol and fentanyl may have altered hemodynamics, and we do not know whether similar results would have been observed in conscious animals. Nevertheless, in piglets, propofol and fentanyl seem to have only minimal effects on cardiac function (17, 39), and systemic and regional hemodynamics remain stable during 72 h of propofol/fentanyl (10).

Angiotensin II infusion is associated with decreasing cardiac output and blood flow redistribution at the expense of portal and renal perfusion. However, the fraction of cardiac output supplying the portal vein is unaltered, and fractional hepatic artery flow increased. Hepatic arterial conductance is decreased, but hepatic arterial flow compensation for a given portal flow reduction increased. If anything, enalapril supported the HABR. Effects of pressure manipulation with other drugs on HABR should be evaluated in future studies.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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