The Roles of Angiotensin II Receptors in the Portosystemic Collaterals of Portal Hypertensive and Cirrhotic Rats

Hui-Chun Huang^{a, c} Ching-Chih Chang^{b, c} Sun-Sang Wang^{a, c} Fa-Yauh Lee^{a, c}
Tzu-Hua Teng^a Jing-Yi Lee^{a, e} Han-Chieh Lin^{a, c} Chiao-Lin Chuang^{b, c}
Shou-Dong Lee^{a, c}

Divisions of ^aGastroenterology and ^bGeneral Medicine, Department of Medicine, Taipei Veterans General Hospital, ^cFaculty of Medicine, and ^dDepartment and Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

Key Words

Angiotensin II • Angiotensin receptor • Cirrhosis • Endothelial nitric oxide synthase • Perfusion • Collateralization

Abstract

Background/Aims: In liver cirrhosis/portal hypertension, collaterals as varices may bleed and are influenced by vaso-responsiveness. An angiotensin blockade ameliorates portal hypertension but the influence on collaterals is unknown.

Methods: Portal hypertension and cirrhosis were induced by portal vein (PVL) and common bile duct ligation (BDL). Hemodynamics, real-time PCR of angiotensin II receptors (AT₁R, AT₂R) in the left adrenal vein (LAV, sham) and spleno-renal shunt derived from LAV (PVL, BDL) were performed. With an in situ collateral perfusion model, angiotensin II vaso-responsiveness with different preincubations was evaluated: (1) vehicle; (2) AT₁R blocker losartan; (3) losartan plus nonselective nitric oxide synthase (NOS) inhibitor (N^ω-nitro-L-arginine); (4) AT₂R blocker PD123319; (5) PD123319 plus N^ω-nitro-L-arginine; (6) N^ω-nitro-L-arginine, and (7) losartan plus inducible NOS inhibitor aminoguanidine. **Results:** LAV

AT₁R and AT₂R expression decreased in PVL and BDL rats. Losartan attenuated angiotensin II-elicited vasoconstriction but PD123319 had no effect. N^ω-nitro-L-arginine but not aminoguanidine reversed the losartan effect. **Conclusions:** Angiotensin receptors are downregulated in the collateral vessel of portal hypertensive and cirrhotic rats. The AT₁R blockade attenuates the angiotensin II vasoconstrictive effect, suggesting AT₁R mediates collateral vasoconstriction and the influence of AT₂R is negligible. The lack of aminoguanidine influence indicates that endothelial NOS participates in the losartan effect.

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Introduction

Renin-angiotensin system is pivotal in circulatory function and body fluid homeostasis [1] in which angiotensin II plays a key role [2]. The vascular effects of an-

Hui-Chun Huang and Ching-Chih Chang contributed equally to this work.

giotensin II are mediated by two subtypes of receptors, type 1 (AT₁R) and 2 (AT₂R) [3]. Previous studies have indicated that angiotensin II mainly induced vasoconstriction via AT₁R whereas AT₂R mediated vasodilatation [4, 5]. Siragy et al. [6] and Siragy and Carey [7] discovered that AT₂R stimulation engendered a vasodilator cascade composed of bradykinin, nitric oxide (NO) and guanosine cyclic 3',5'-monophosphate. However, the interactions between angiotensin II and NO is rather complex. It has been reported that angiotensin II stimulates NO synthesis via AT₂R in endothelial cells [8]. AT₁R activation also elicited endothelial NO synthesis in rat carotid arteries [9], although the extent of AT₁R-mediated NO release is not sufficient to overcome the overriding contractile response exerted by the same receptor [10].

A recent study has indicated that angiotensin II may elevate portal pressure via the enhancement of the adrenergic vasoconstrictory effect and a direct contractile influence on stellate cells [11]. On the other hand, a worse splanchnic vascular contractile response to angiotensin II in portal hypertensive rats has been found [12], suggesting its diverse actions in different vascular beds. In liver cirrhosis and portal hypertension, the portosystemic collaterals develop in response to the increased intrahepatic resistance, trying to divert stagnant portal venous blood flow to systemic circulation. Among them, gastroesophageal varices have been notorious for massive hemorrhage with high morbidity and mortality. The portosystemic collateral vascular response to vasoconstrictors is, actually, pivotal in the control of gastroesophageal variceal hemorrhage. Furthermore, since portal pressure is influenced by portal inflow, intrahepatic resistance and portosystemic collateral vascular resistance, the collaterals also participate in the control of portal pressure. Nevertheless, the influence of angiotensin II in this distinct vascular bed has not been evaluated. We herein surveyed the presence and expression levels of angiotensin II receptors in the most prominent intra-abdominal portosystemic collateral vessel, splenorenal shunt that is derived from the left adrenal vein in normal rats [13]. Furthermore, the collateral vascular responses to angiotensin II influenced by different angiotensin II receptor antagonists in portal hypertensive and cirrhotic rats were evaluated.

Animals and Methods

Animal Model

Male Sprague-Dawley rats (300–350 g) were maintained at 24°C with a 12-hour light-dark cycle and free access to food and water. Surgery and hemodynamic studies were performed under

ketamine hydrochloride anesthesia (100 mg/kg; i.m.). Portal hypertension was induced by partial portal vein ligation (PVL) [14] and liver cirrhosis by common bile duct ligation (BDL) [15–17], respectively. Corresponding sham groups received sham operations without ligations and were treated in the same way. To avoid coagulation defects, BDL rats received weekly vitamin K injections (50 µg/kg; i.m.). The study was approved by the Taipei Veterans General Hospital Animal Committee. The principles of laboratory animal care [Guide for the Care and Use of Laboratory Animals, DHEW publication No. (NIH) 85-23, rev. 985, Office of Science and Health Reports, DRR/NIH, Bethesda, Md., USA.] were followed.

In situ Perfusion Preparation

As previously described [18, 19], in brief, both jugular veins were cannulated with 16-gauge Teflon cannulas as outlets of perfusate. The inlet was an 18-gauge Teflon cannula inserted in the superior mesenteric vein. To exclude the liver from perfusion, the portal vein was tied. The animal was transferred into a chamber (37 ± 0.5°C). Perfusion was started via the mesenteric cannula by a roller pump (model 505S; Watson-Marlow Ltd., Falmouth, UK) with Krebs solution equilibrated with 95% (v/v) O₂ and 5% (v/v) CO₂ by a silastic membrane lung [20]. Pneumothorax was created by opening slits through the diaphragm to increase pulmonary arterial resistance and prevent the perfusate from entering the left heart. Experiments were performed 25 min after starting perfusion at a constant rate of 20 ml/min for PVL rats and 12 ml/min for BDL rats. As the flow rate was kept constant, the measured perfusion pressure reflected collateral vascular resistance. Only one concentration-response curve was performed in each preparation and the contracting capability was challenged with a 125-mM potassium chloride solution after testing the experimental agents.

Measurement of Systemic and Portal Hemodynamics

The right femoral arteries and mesenteric vein were cannulated with PE-50 catheters connected to Spectramed DTX transducers (Spectramed Inc., Oxnard, Calif., USA). The external zero reference was at the level of the midportion of the rat. Continuous recordings of mean arterial pressure, heart rate and portal pressure were performed on a multichannel recorder (model RS 3400, Gould Inc., Cupertino, Calif., USA) [21, 22].

Total Ribonucleic Acid Isolation

Total ribonucleic acid (RNA) was extracted from the splenorenal shunt, the most prominent intra-abdominal portosystemic collateral vessel in the cirrhotic and portal hypertensive rat, with the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). Only undegraded RNA free of genomic DNA contamination was used. The extracted RNA was quantified by UV absorbance at 260 nm, quickly frozen on ice and stored at –80°C for real-time PCR analysis.

Real-Time Quantitative RT-PCR

One microgram of total RNA was reverse-transcribed to cDNA with Superscript II reverse transcriptase and poly dT priming (Life Technologies, Rockville, Md., USA). Quantitative RT-PCR was carried out on a LightCycler (LightCycler 480, Roche Diagnostics, Mannheim, Germany) and a standard LightCycler amplification cycle protocol was established for each gene.

The primers are: β -actin: 5'-TTGTAACCAACTGGGACGAT-ATGG-3' (sense), 5'-GATCTTGATCTTCATGGTGCTAGG-3' (antisense); inducible NO synthase (iNOS): 5'-AGACGCAC-AGGCAGAGGT-3' (sense), 5'-AGGCACACGCAATGATGG-3' (antisense); endothelial NOS (eNOS): 5'-CTGCTGCCCCGAG-ATATCTTC-3' (sense), 5'-CAGGTACTGCAGTCCCTCCT-3' (antisense); AT₁R: 5'-ACTCTTTTCCTACCGCCCTTC-3' (sense); 5'-TTAGCCCAAATGGTCCTCTG-3' (antisense); AT₂R: 5'-CA-ATCTGGCTGTGGCTGACTT-3' (sense), 5'-TGCACATCACA-GGTCCAAAGA-3' (antisense) [23, 24]. A total volume of 18 μ l of LightCycler master mix was pipetted into LightCycler glass capillaries and 2 μ l of the cDNA product (diluted tenfold) was added as a PCR template. The capillaries were placed in the LightCycler carousel and centrifuged in a specific LightCycler centrifuge. The first segment of the amplification cycle consisted of a denaturation program of 95°C for 10 min. The second segment consisted of denaturation (95°C for 15 s), primer annealing (58°C for 5 s), elongation (72°C for 10 s) and a quantification program repeated for 40 cycles. The third segment consisted of a melting curve program (95°C for 0 s, 57°C for 15 s and a linear temperature transition at 0.05°C/s from 57 to 95°C with continuous fluorescence acquisition). The final segment consisted of a cooling program to 40°C. An internal housekeeping gene, β -actin, was used to normalize the differences in RNA isolation, RNA degradation and the efficiencies of the RT. The abundance of mRNA was determined by real-time RT-PCR normalized to the abundance of β -actin mRNA. LightCycler analysis software (Roche Diagnostics) allowed the quantitative analysis.

Study Design

Systemic and portal hemodynamics were evaluated on the 8th day after PVL and the 43rd day after BDL. Following this two studies were performed: first, real-time PCR analysis of AT₁R and AT₂R of splenorenal shunt in PVL, BDL and their corresponding sham groups; second, with an in situ collateral perfusion model and different preincubations (30 min before the beginning throughout the whole course of the concentration-response relationship study), the collateral vascular responses to angiotensin II (3×10^{-10} , 10^{-9} , 3×10^{-9} , 10^{-8} , 3×10^{-8} , 10^{-7} M) in PVL and BDL rats were evaluated. This was done in 7 steps: (1) Krebs solution (vehicle control); (2) AT₁R blocker, losartan (3×10^{-5} M); (3) losartan plus a nonselective NOS inhibitor (N^G -nitro-L-arginine, NNA, 10^{-4} M); (4) AT₂R blocker, PD123319 (10^{-6} M); (5) PD123319 plus NNA; (6) NNA, and (7) losartan plus aminoguanidine, a selective iNOS inhibitor (10^{-4} M).

Drugs

Angiotensin II, losartan, PD123319, NNA, aminoguanidine and reagents for Krebs solution were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All solutions were freshly prepared on the days of experiment.

Statistical Analysis

Results are expressed as mean $\pm$ standard error of the mean (mean $\pm$ SEM). The changes in perfusion pressure (mm Hg) over the baseline were calculated for each concentration in each preparation. The concentration-response curves were fitted by non-linear regression using the computer software Prism (Graph Pad Software Inc., San Diego, Calif., USA). Differences between the dose-response curves were analyzed by two-way ANOVA for re-

Table 1. Body weight and baseline hemodynamics in PVL rats

	n	BW g	MAP mm Hg	HR beats/min	PP mm Hg
Krebs	16	330 $\pm$ 5	107 $\pm$ 4	378 $\pm$ 15	15.5 $\pm$ 0.7
Lo	7	335 $\pm$ 16	107 $\pm$ 8	355 $\pm$ 24	17.5 $\pm$ 1.4
Lo + NNA	6	329 $\pm$ 7.2	91 $\pm$ 8	356 $\pm$ 31	14.1 $\pm$ 1.1
Lo + AG	7	293 $\pm$ 12	108 $\pm$ 7	280 $\pm$ 239	20.5 $\pm$ 1.2
NNA	6	303 $\pm$ 16	108 $\pm$ 4	427 $\pm$ 10	15.6 $\pm$ 1.2
PD	7	313 $\pm$ 6	100 $\pm$ 5	334 $\pm$ 22	15.5 $\pm$ 1.8
PD + NNA	8	322 $\pm$ 7	100 $\pm$ 4	341 $\pm$ 18	16.2 $\pm$ 0.8

BW = Body weight; MAP = mean arterial pressure; HR = heart rate; PP = portal pressure; Krebs = Krebs solution (vehicle control); Lo = losartan; AG = aminoguanidine; PD = PD123319. All $p > 0.05$.

Table 2. Body weight and baseline hemodynamics in BDL rats

	n	BW g	MAP mm Hg	HR beats/min	PP mm Hg
Krebs	9	311 $\pm$ 16	93 $\pm$ 6	283 $\pm$ 23	17.2 $\pm$ 0.8
Lo	7	302 $\pm$ 17	96 $\pm$ 7	283 $\pm$ 22	18.1 $\pm$ 2.8
Lo + NNA	6	298 $\pm$ 16	96 $\pm$ 10	280 $\pm$ 16	17.5 $\pm$ 1.6
Lo + AG	6	293 $\pm$ 12	108 $\pm$ 7	280 $\pm$ 18	20.5 $\pm$ 1.2
NNA	8	284 $\pm$ 14	99 $\pm$ 6	280 $\pm$ 17	21.3 $\pm$ 1.9
PD	8	292 $\pm$ 22	106 $\pm$ 8	302 $\pm$ 25	17.1 $\pm$ 1.4
PD + NNA	8	308 $\pm$ 15	97 $\pm$ 6	271 $\pm$ 15	17.5 $\pm$ 1.6

All $p > 0.05$.

peated measures using SPSS 15.0.1 for Windows (SPSS Inc., Chicago, Ill., USA). For comparison of mRNA expression, the unpaired Student t test was used. A two-tailed $p < 0.05$ was considered significant.

Results

Baseline Hemodynamics

Tables 1 and 2 show the body weights and baseline hemodynamics of PVL and BDL rats before the perfusion studies. Body weight, heart rate, mean arterial pressure and portal pressure were similar among these groups ($p > 0.05$).

Splenorenal Shunt Angiotensin Receptors mRNA Expression

Compared with the sham rats, AT₂R mRNA expressions were significantly decreased in PVL rats (AT₂R/ β -

Fig. 1. AT₁R and AT₂R mRNA expressions in splenorenal shunts of PVL (**a, b**), BDL (**c, d**) and corresponding sham-operated rats. Compared with the corresponding sham groups, AT₂R mRNA expressions were significantly decreased in PVL rats and AT₁R and AT₂R decreased in BDL rats.

Fig. 2. Concentration-response curves to angiotensin (Ang) II in portosystemic collateral vascular beds of PVL (**a**) or BDL (**b**) rats when preincubated with Krebs solution (vehicle control), losartan (Lo), or PD123319 (PD), expressed as absolute increase over the baseline value. **a** In PVL rats, compared with the vehicle group, losartan significantly decreased the perfusion pressure changes to angiotensin II. **b** In BDL rats, compared with the vehicle group, losartan significantly reduced the perfusion pressure changes to angiotensin II.

actin: 0.347 ± 0.089 vs. 0.118 ± 0.039 , $p = 0.035$, fig. 1b). Although AT₁ mRNA expression was lower in the PVL group, it did not reach a statistical significance (AT₁R/ β -actin: 0.508 vs. 0.409 , $p = 0.421$, fig. 1a). Both AT₁R and AT₂R mRNA expressions were significantly decreased in BDL rats (AT₁R/ β -actin: 0.724 ± 0.114 vs. 0.417 ± 0.056 , $p = 0.017$, fig. 1c; AT₂R/ β -actin: 0.270 ± 0.070 vs. 0.097 ± 0.022 , $p = 0.036$, fig. 1d). There was no significant difference between AT₁R expression of PVL and BDL rats and between AT₂R expression of PVL and BDL rats ($p > 0.05$).

Concentration-Response Relationships to Angiotensin II with AT₁R or AT₂R Blockade in PVL or BDL Rats

Figure 2 shows the concentration-response curves of portosystemic collaterals to angiotensin II with different preincubations in PVL rats: Krebs solution (control), losartan, or PD123319. Compared with the control group, in PVL rats losartan significantly decreased the collateral vascular bed perfusion pressure changes to angiotensin II ($p < 0.001$). The perfusion pressure changes elicited by losartan was also lower than PD123319 ($p = 0.001$). PD123319 did not significantly influence the perfusion pressure changes to angiotensin II (fig. 2a).

Fig. 3. Concentration-response curves to angiotensin (Ang) II in portosystemic collateral vascular beds of PVL rats with the following preincubations: Krebs solution (vehicle control), losartan (Lo), losartan plus NNA, or NNA (**a**) and Krebs solution (vehicle control), PD123319 (PD), PD123319 plus NNA, or NNA (**b**), expressed as absolute increase over the baseline value. **a** Losartan plus NNA significantly reversed the attenuation of pressure response to an-

giotensin II by losartan alone. In addition, NNA exerted the highest perfusion pressure changes in all groups, which was attenuated by losartan. **b** PD123319 plus NNA significantly enhanced the pressure response to angiotensin II by PD123319 alone. NNA exerted the highest perfusion pressure changes in all groups which were not influenced by PD123319.

Fig. 4. Concentration-response curves to angiotensin (Ang) II in portosystemic collateral vascular beds of BDL rats with the following preincubations: Krebs solution (vehicle control), losartan (Lo), losartan plus NNA, or NNA (**a**) and Krebs solution (vehicle control), PD123319 (PD), PD123319 plus NNA, or NNA (**b**), expressed as absolute increase over the baseline value. **a** Losartan plus NNA elicited higher perfusion pressure changes than those by losartan

alone, but did not reach statistical significance (all $p > 0.05$). NNA exerted the highest perfusion pressure changes in all groups, which was attenuated by losartan. **b** PD123319 plus NNA significantly enhanced the pressure response to angiotensin II by PD123319 alone. NNA exerted the highest perfusion pressure changes in all groups which were not influenced by PD123319 ($p > 0.05$).

In BDL rats, compared with the control group, losartan significantly reduced the perfusion pressure changes to angiotensin II ($p < 0.001$). Losartan elicited lower perfusion pressure changes than PD123319 ($p < 0.001$). PD123319 did not significantly influence the perfusion pressure changes to angiotensin II (fig. 2b).

Concentration-Response Relationships to Angiotensin II with AT₁ and/or NNA/Aminoguanidine Blockade in PVL Rats

Figure 3a depicts the concentration-response curves to angiotensin II in PVL rats with different preincubations: Krebs solution (control), losartan, losartan plus NNA or

Fig. 5. Concentration-response curves to angiotensin (Ang) II in portosystemic collateral vascular beds of PVL (a) and BDL (b) rats, preincubated with Krebs solution (vehicle control), losartan (Lo), or losartan plus aminoguanidine (AG), expressed as absolute increase over the baseline value. The perfusion pressure changes to angiotensin II influenced by losartan were not modified by aminoguanidine, an iNOS inhibitor, both in PVL (a) and BDL (b) rats.

NNA alone. Losartan plus NNA significantly reversed the attenuation of pressure response to angiotensin II by losartan alone ($p = 0.034$). NNA exerted the highest perfusion pressure changes in all groups whereas it was attenuated by losartan co-preincubation ($p < 0.001$). The perfusion pressure changes to angiotensin II influenced by losartan was not modified by the addition of aminoguanidine, an iNOS inhibitor (fig. 5a).

Concentration-Response Relationships to Angiotensin II with AT_2 and/or NNA Blockade in PVL Rats

Figure 3b shows the concentration-response curves to angiotensin II in PVL rats with different preincubations: Krebs solution (control), PD123319, PD123319 plus NNA or NNA alone. PD123319 plus NNA significantly enhanced the pressure response to angiotensin II by PD123319 alone ($p < 0.001$). NNA exerted the highest perfusion pressure changes in all groups which were not influenced by PD123319 co-preincubation.

Concentration-Response Relationships to Angiotensin II with AT_1 and/or NNA/Aminoguanidine Blockade in BDL Rats

Figure 4a depicts the concentration-response curves to angiotensin II in BDL rats with different preincubations: Krebs solution (control), losartan, losartan plus NNA or NNA alone. Losartan plus NNA elicited higher perfusion pressure changes than those by losartan alone, but did not reach statistical significance ($p > 0.05$). NNA exerted the highest perfusion pressure changes in all groups whereas it was attenuated by losartan co-preincubation ($p < 0.001$). The perfusion pressure changes to angiotensin II influenced by losartan was not modified by the addition of aminoguanidine, an iNOS inhibitor (fig. 5b).

Concentration-Response Relationships to Angiotensin II with AT_2 and/or NNA Blockade in BDL Rats

Figure 4b demonstrates the concentration-response curves to angiotensin II in BDL rats with different preincubations: Krebs solution (control), PD123319, PD123319 plus NNA or NNA alone. PD123319 plus NNA significantly enhanced the pressure response to angiotensin II by PD123319 alone ($p = 0.011$). NNA exerted the highest perfusion pressure changes in all groups which were not influenced by PD123319 co-preincubation.

Discussion

The current study has five major findings: in portosystemic collaterals of PVL and BDL rats, (1) AT_1 R and AT_2 R are present and more downregulated than those of the corresponding control groups; (2) the AT_1 R blockade with losartan alleviates angiotensin II-induced vasoconstrictive effects; (3) PD123319, an AT_2 R blocker, does not significantly influence the collateral vascular responsiveness to angiotensin II; (4) in the presence of NOS inhibition with NNA, losartan attenuates NNA-enhanced collateral vasoconstrictive response to angiotensin II, and (5) the selective iNOS inhibitor aminoguanidine did not influence the collateral vascular responsiveness modified by losartan. Since the vascular NO synthesis is mainly mediated via iNOS and eNOS, a crucial role of eNOS in the losartan effect is suggested. However, the potential influence of another constitutive NOS, the neuronal NOS, should be taken into consideration.

In the portosystemic collateral vascular bed, the downregulation of AT_2 R in PVL rats and AT_1 R and AT_2 R

in BDL rats is noted. An increase in angiotensin II level as the result of the activation of the renin-angiotensin system is evidenced in cirrhosis and correlates with portal hypertension [25, 26]. However, splanchnic hyposensitivity to angiotensin II is common in cirrhotic and portal hypertensive states. It occurs despite elevated plasma levels of angiotensin II [27, 28]. Downregulation of AT receptors, either qualitative or quantitative, may play a role in the vascular hyposensitivity. Hennenberg et al. [29] have proposed that in humans and rats with cirrhosis, hepatic arterial and aortic hypocontractility to angiotensin II is due to enhanced β -arrestin-2 binding to receptors, resulting in AT₁R desensitization without affecting AT₁R expression. Nevertheless, the nature of the conductance vessels may not be representative of the collateral resistance vessels. Our current data suggest that the downregulation of angiotensin receptors may pave the way for hyporesponsiveness to angiotensin II in portosystemic collaterals.

The current finding that the AT₁R blockade decreased the portosystemic collateral vasoconstrictive response to angiotensin II in PVL and BDL rats indicates that AT₁R mediates the angiotensin II-induced vasoconstriction in this vascular bed. This is consistent with the previous report that angiotensin II-induced vasoconstriction was mediated through AT₁R, which could be reversed by losartan [30]. Regarding the relevant studies on portal systems, it has been found that losartan, an AT₁R antagonist, elicited a significant reduction in the hepatic venous pressure gradient [31]. The portal hypotensive effect of losartan was attributed to the reduction in intrahepatic vascular resistance. Arroyo et al. [32] also reported that saralasin significantly decreased portal pressure in cirrhotic patients. In that study, those who showed a substantial reduction in portal pressure demonstrated a marked reduction of the hepatic vascular resistance. Interestingly, another study showed that losartan at 3 mg/kg intravenously reduced portal pressure via an increase in splanchnic vascular resistance and decrease in portal inflow. However, at higher doses, losartan reduced portal pressure by a decrease in peripheral vascular resistance [33]. Since portal pressure is determined by the net effects of splanchnic vascular resistance, intrahepatic resistance and portosystemic collateral vascular resistance, the attenuation of the angiotensin II pressor effect by losartan on collaterals may contribute, at least partly, to its portal hypotensive action.

The AT₂R blockade with PD123319 did not significantly influence the collateral vasoconstrictive response

to angiotensin II. In the portal system, Fernandes et al. [34] showed that angiotensin II contracted mesenteric venules and portal veins by activating both AT₁R and AT₂R. Pelet et al. [35] also demonstrated that angiotensin II-induced contraction in rat portal vein was mediated through AT₁R and AT₂R activation, in which AT₁R was responsible for about 80% of the maximal contractile response. In other vascular beds, AT₂R stimulation induced vasoconstriction in untreated mesenteric resistance arteries from spontaneously hypertensive rats [36]. Furthermore, in young hypertensive rats, an angiotensin II-induced contraction was decreased by PD123319 [37]. In rats with hemorrhagic shock, selective stimulation of AT₂R in the presence of the AT₁R blockade increased cerebrovascular resistance elicited by angiotensin II [38]. Moreover, in the perfused hydronephrotic kidney, PD 123319 reduces angiotensin II-induced contraction of intralobular renal arteries [39]. Such a vasoconstrictive action in the renal circulation may be mediated by cytochrome P450 metabolites such as 20-HETE [40, 41]. Although contradictory results showed that stimulation of AT₂R induces relaxation in several vascular territories [42, 43], the vascular action of AT₂R seems to be diverse and influenced by pathologic conditions, hemodynamic derangements and multiple substances.

In the current study, the attenuation of angiotensin II-induced vasoconstriction by losartan was reversed by NNA, but not aminoguanidine, an inducible NOS inhibitor, suggesting the participation of eNOS in the mechanism. Furthermore, NNA plus losartan attenuated the enhanced angiotensin II vasoconstrictive effect exerted by NNA alone, especially in PVL rats. This is in agreement with a previous study which demonstrated that the reduction of angiotensin II-mediated vasoconstriction elicited by losartan can be reversed by NO inhibition [30]. The authors postulated that in the presence of AT₁R antagonists, angiotensin II binds to unblocked AT₂R and then stimulates NO synthesis [30]. A similar finding showed that the AT₁R blockade in patients with coronary artery disease led to endothelial NO release which was mediated by AT₂R stimulation [44]. This is supported by the finding that AT₁R activation increased endothelial NO synthesis in rat isolated carotid arteries [9].

In summary, AT₁R and AT₂R are present and downregulated in the portosystemic collaterals of portal hypertensive and cirrhotic rats. The AT₁R blockade attenuates the pressor effects of angiotensin II in the collaterals, suggesting that AT₁R mediates the vasoconstrictive effect. Endothelial NOS activation plays a role in the AT₁R-

induced response. The findings indicate the vascular hyposensitivity to angiotensin II related to receptor down-regulation and the lack of influence of AT₂R in this vascular bed in portal hypertension and cirrhosis.

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