Angiotensin II Type AT2 Receptor mRNA Expression and Renal Vasodilatation Are Increased in Renal Failure

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Abstract—Kidney failure is associated with changes in renal vascular responses to angiotensin (Ang) II. We characterized expression of Ang II receptors and the renal vasoconstrictor and vasodilator responses to Ang II in kidneys from sham-operated and kidney failure rats. In the isolated perfused kidney of sham-operated rats, Ang II (1, 2, 4, and 8 ng) increased perfusion pressure by 27±6, 41±10, 54±11, and 74±12 mm Hg, respectively. These responses were amplified by 62±10% (P<0.05) in kidney failure rats. Losartan (1 μmol/L), an angiotensin type 1 (AT1) receptor blocker, abolished renal vasoconstriction induced by Ang II, unmasking a renal vasodilatation that was greater in kidney failure rats. CGP-42112 (1 μmol/L) or PD 123,319 (1 μmol/L), angiotensin type 2 (AT2) receptor ligands, blunted Ang II–induced renal vasodilatation. In the renal tissue of kidney failure rats, there was a marked increase in expression of AT1 and AT2 mRNA receptor. Ang II–induced vasodilatation was blunted by eicosatetraynoic acid (1 μmol/L), the all-purpose inhibitor of arachidonic acid metabolism; clotrimazole (1 μmol/L), an inhibitor of epoxygenase-dependent arachidonic acid metabolism; or No-nitro-L-arginine methyl ester (L-NAME; 1 μmol/L), an inhibitor of NO synthesis. On stimulation with Ang II, 20-HETE was the predominant product released into the renal effluent of sham-operated rats, whereas epoxy-eicosatrienoic acids were the predominant products released into the effluent of kidney failure rats. These data suggest that during development of kidney failure, there is induction of the AT2 receptors, which may account for increased Ang II–dependent vasodilatation through the predominant release of epoxyeicosatrienoic acids.

Key Words: receptors, angiotensin • cytochrome P450 • kidney failure • angiotensin II • arachidonic acid

The kidney is an important target organ for angiotensin (Ang) II, where it plays a critical role in the regulation of kidney function. Two major isoforms of the Ang II receptor have been described: type 1 (AT1) and type 2 (AT2).1 Most of the known effects of Ang II are attributable to the AT1 receptor,2 and little is known concerning the function of the AT2 receptor. The AT2 receptor is widely expressed in fetal tissues and decreases during development.3 Renal AT2 receptor was demonstrated to be involved in regulation of pressure natriuresis4 and in preglomerular afferent arteriolar dilatation.5 Also, a role was proposed for AT2 receptor in vascular development.6

Cytochrome P450–derived arachidonic acid (CYP-AA) metabolites released in response to Ang II.7 The biological activities of CYP-AA are potentially of great importance in renal function in view of the capacity of these metabolites to regulate various renal mechanisms.8 Activation of the AT2 receptor increases production of epoxygenase-derived metabolites in the rabbit afferent arteriole.9 Furthermore, in the absence of AT2 receptor, microsomal AA ω-hydroxylase activity was greatly reduced, suggesting that the capacity to produce 20-HETE in response to Ang II is strongly linked to the AT2 receptor.9 Kidney failure is characterized by increased renal vascular tone and cellular hypertrophy. As AT2 receptors were implicated in vascular development,6 we propose that the cellular hypertrophy of kidney failure may be due to upregulation of AT2 receptor. We further propose that activation of AT2 receptors releases dilator CYP-AA metabolites that provide a counterregulatory mechanism to diminish the increased vascular tone in kidney failure. Thus, we explored the role of AT2 receptors in kidney failure by evaluating (1) Ang II–induced renal vascular responses during AT1 or AT2 receptor blockade and (2) expression of AT2 mRNA receptor. We also identified the mediator of Ang II–induced vasodilatation by evaluating Ang II responses in the presence of inhibitors of AA metabolism and by measuring the release of CYP-AA metabolites on Ang II stimulation.

Methods

Experiments were performed on male Wistar rats weighing 250 to 300 g. Induction of kidney failure was performed as described.15 Development of kidney failure was evaluated by measuring blood pressure and urinary excretion of protein, sodium, and water as previously reported.10

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Isolated Perfused Kidneys of Rats
Two days after surgery, kidneys from sham-operated or kidney failure rats were dissected and mounted in Langendorff preparation system.11 A decrease in perfusion pressure indicated vasodilatation; whereas increase in perfusion pressure indicated vasoconstriction. Data were expressed as absolute changes (Δ) in perfusion pressure from basal value.

Ang II–Induced Renal Vascular Responses
After 30 minutes stabilization, Ang II at doses of 1, 2, 4, 8, 16, and 32 ng was randomly injected as boluses into the perfusate line of the isolated kidneys. Each dose was administered when perfusion pressure had returned to the basal (preinjection) value. Responses to Ang II were then repeated in the presence of losartan (1 μmol/L, Merck Sharp & Dohme), an AT1 receptor antagonist. To characterize the oxygenase(s) involved in losartan-independent vasodilator response to Ang II, kidneys were perfused with losartan (1 μmol/L) in presence of one of the following inhibitors, which was added to the perfusion solution: CGP-42112A (1 μmol/L, Merck Sharp & Dohme), an AT2 receptor partial agonist; PD 123,319 (1 μmol/L, Merck Sharp & Dohme), an AT2 receptor antagonist; eicosatetraynoic acid (ETYA) (1 μmol/L, Cayman), the inhibitor of all pathways of AA metabolism; indomethacin (2.7 μmol/L, Sigma), a cyclooxygenase inhibitor; clotrimazole (1 μmol/L, Sigma), an epoxygenase-dependent inhibitor of CYP-AA metabolism; or Nω-nitro-L-arginine methyl ester (L-NAME) (1 μmol/L, Sigma), an inhibitor of NO synthesis. Thirty minutes of inhibitor-tissue contact was allowed before responses to Ang II (16 ng) were reevaluated. Responses to U46619 (Cayman), the thromboxane A2-mimetic, and acetylcholine (1 μmol/L, Sigma) were used as controls to demonstrate inhibitors selectivity.

Renal Release of CYP-AA Metabolites
Kidneys from sham-operated or kidney failure rats were perfused as previously described. Following a stabilization period of 30 minutes, losartan (1 μmol/L) was added to the perfusion solution; after a 30-minute drug-tissue contact time, the renal perfusate was collected for 5 minutes (~50 mL). Subsequently, a bolus injection of Ang II (32 ng) was made, and renal perfusate was again collected for 5 minutes. CYP-AA metabolites were extracted and purified by reverse-phase high-performance liquid chromatography.7 Derivatization and mass spectrometric analysis (MSA) were performed by adding 5 μL pentafluorobenzyl bromide (Sigma) and 5 μL N, N-diisopropylethylamine (Sigma) to a sample dissolved in acetonitrile (100 μL), 20-HETE, and epoxy-eicosatrienoic acid (EET) measured by MAS.7

RT-PCR Analysis of AT1 and AT2 Receptors
Two days after surgery, kidneys from sham-operated or kidney failure rats were removed, and 2 μg of total RNA were converted to
cDNA. Polymerase chain reaction (PCR) was performed in a Perkin-Elmer Gene Amp 2400 PCR system for 35 cycles at 94°C for 1 minute, 64°C for 1 minute, and 72°C for 1 minute, followed by a 10-minute extension at 72°C. Primers specific for AT, AT, and GAPDH were used for the reverse transcription (RT)-PCR, and the final PCR products were 506, 336, and 715 bp in size, respectively. PCR products were size-fractionated by agarose gel electrophoresis. DNA bands were analyzed with a densitometer system (Kodak EDAS 120 system). Values of each band are in arbitrary units.

Statistics Analysis
All results are expressed as mean±SE. Multiple comparisons were by 1-way ANOVA. Differences were analyzed using Student’s t test or Dunnet’s test. Differences were considered significant when P<0.05.

Results
In sham-operated rats, mean arterial blood pressure, urinary protein, urinary Na⁺ excretion, and urine volume were 100±3 mm Hg, 21±4 mg/d, 1.5±0.2 mEq/d, and 10±2 mL/d. These values were markedly increased in kidney failure rats to 140±4 mm Hg (n=5, P<0.05), 60±9 mg/d (n=5, P<0.05), 2.5±0.2 mEq/d (n=5, P<0.05), and 28±2 mL/d (n=5, P<0.05), respectively.

Ang II–Induced Renal Vascular Responses
Ang II (1, 2, 4, and 8 ng) increased renal perfusion pressure by 27±6, 41±10, 54±11, and 74±12 mm Hg, respectively, in isolated kidneys from sham-operated rats and by 37±8, 64±9, 99±12, and 128±17 mm Hg, respectively in isolated kidneys from kidney failure rats. Losartan (1 µmol/L) abolished the increases in perfusion renal pressure in response to Ang II in both groups of rats (Figure 1), unmasking a vasodilator effect of Ang II that was evident at doses of Ang II >4 ng. The renal vasodilatation was greater in the kidneys from kidney failure rats (Figure 1). PD 123,319, the AT₂ receptor antagonist, blunted the vasodilator response to Ang II as did CGP-42112A, the AT₂ receptor partial agonist. ETYA or clotrimazole inhibited Ang II–induced renal vasodilatation to a similar degree in kidneys from sham-operated and kidney failure rats (Figure 2). Indomethacin did not affect Ang II–induced renal vasodilatation (Figure 2). On the other hand, L-NAME decreased Ang II–induced renal vasodilatation (Figure 2). In the absence of inhibitors, U46619 (1 µmol/L) increased perfusion pressure by 58±18 and 62±20 mm Hg in sham-operated and kidney failure rats, respectively. And in the presence of the inhibitors used, increased perfusion pressure by 60±6 and 62±11 mm Hg respectively. In the absence of inhibitors, acetylcholine (1 µmol/L) decreased perfusion pressure by 25±3 and 27±4 mm Hg in sham-operated and kidney failure rats, respectively, and in the presence of the inhibitors used, acetylcholine decreased perfusion pressure by 28±3 and 25±4 mm Hg, respectively.

Expression of AT₁ and AT₂ Ang II Receptors
Expression of AT₁ mRNA receptor was similar in sham-operated and kidney failure rats. AT₁/GAPDH mRNA ratio was 1.7±1.4 and 2±1.6 (arbitrary units) (n=3, P<0.05) in renal tissue from sham-operated and kidney failure rats, respectively. The expression of AT₂ mRNA receptor in renal tissue from sham-operated rats was very low, whereas there was a marked and distinct expression of AT₂ mRNA in the renal tissue from kidney failure rats. Thus, the ratio of AT₂/GAPDH mRNA was 0.25±0.01 and 1.3±0.2 (arbitrary units) (n=3, P<0.05) in the renal tissue from sham-operated and kidney failure rats, respectively.

Ang II–Induced Renal Release CYP-AA Metabolite
Compared with sham-operated rats, basal release of EET in renal effluent from kidney failure rats, whereas that of 20-HETE decreased by 60±5% in renal effluent of kidney failure rats. Figure 3 shows that Ang II elicited a 1.6-fold increase in the release of EETs (P<0.01) in renal effluent of kidney failure rats, but there was no change in the release of EETs in sham-operated rats. After challenge with Ang II (32 ng), there was a 1.8-fold increase in 20-HETE release in sham operated rats (P<0.01) and a 2.2-fold increase in the release of 20-HETE in kidney failure rats (P<0.01) (Figure 3).

Discussion
Our data revealed that Ang II–induced vasoconstriction is mediated by AT₁ receptor, inasmuch as AT₁ receptor block-
ade abolished the vasoconstriction. Ang II–induced renal vasoconstriction was greater in kidney failure. An increase in Ang II–induced effect may represent a critical phase in the development of hypertension, and this is similar to the observations made during the development of genetic or angiotensin-dependent hypertension in which increased response to Ang II was demonstrated in different vessels.\textsuperscript{11–13} Enhancement in Ang II–induced vascular response was reported to be due to alterations in Ang II receptor population.\textsuperscript{13} NO synthesis,\textsuperscript{11} or cyclooxygenase activity.\textsuperscript{14} An enhanced vascular response to Ang II in hypertension is consistent with a prohypertensive mechanism. However, during the initial phases of renal damage and hypertension, antihypertensive mechanisms are activated to mitigate against the damaging effects of the ongoing pathology. Such may be the case in this study, in which Ang II produced a renal vasodilatation that was unmasked after abolishing the AT\textsubscript{1}-mediated vasoconstriction. Ang II–induced vasodilatation was attenuated by the antagonist of AT\textsubscript{2} receptor. These results are consistent with the hypothesis that Ang II effects are balanced by the activation of both types of receptors. Thus, in the cardiovascular system, Ang II–induced pressor response is coupled to the AT\textsubscript{1} receptor, whereas the vasodepressor response is coupled to the AT\textsubscript{2} receptor.\textsuperscript{15} This observation is in agreement with studies that demonstrated the involvement of AT\textsubscript{2} receptors with vasodilatation.\textsuperscript{15}

Having demonstrated AT\textsubscript{1} and AT\textsubscript{2} receptors mediating qualitatively distinct responses in the kidney, we next explored whether alterations in Ang II receptor status may account for the greater vascular responses to Ang II in kidney failure. In agreement with other studies, our data demonstrated a predominance of AT\textsubscript{1} mRNA receptors that were equally expressed in sham-operated and kidney failure rats. However, expression of AT\textsubscript{2} mRNA receptor was very low in the sham-operated rats but markedly elevated in renal tissue from kidney failure rats.

The mechanism for the increased AT\textsubscript{2} receptor mRNA expression in renal tissue from kidney failure rats is not known. However, based on the upregulation of Ang II receptor expression following stimulation of the renin-angiotensin system\textsuperscript{16} and increased levels of Ang II in renal hypertension,\textsuperscript{17} it is possible that increased expression of AT\textsubscript{2} mRNA may be due to increased Ang II levels.

We explored the participation of AA metabolites in Ang II–induced vasodilatation. Blockade of all the pathways of AA metabolism with ETYA blunted the renal vasodilatation elicited by Ang II, implicating an AA metabolite in Ang II effect. This observation, coupled with the lack of effect of indomethacin, the cyclooxygenase inhibitor, and the diminution of the Ang II effect by clotrimazole, suggested that a CYP- AA metabolite may be responsible for Ang II–induced renal vasodilatation.\textsuperscript{8} Indeed, 20-HETE produces endothelium- and cyclooxygenase-dependent vasoconstriction,\textsuperscript{18} and recent reports implicated one or more EETs as the endothelium-derived hyperpolarizing factor.\textsuperscript{19} We explored further the role of CYP- AA metabolism in this response by measuring the levels of HETEs and EETs in the effluent of isolated perfused kidney challenged with Ang II. There is a clear change in the profile of CYP- AA metabolites synthesized by renal tissue of kidney failure rats: production of EET increased whereas 20-HETE levels decreased in the renal effluent from kidney failure rats. To demonstrate the relevance of the production of 20-HETE or EETs in response to Ang II, we calculated the ratio of the constrictor (20-HETE) to that of the vasodilators (EETs) and obtained a ratio of 11±2 in sham-operated rats, suggesting a predominance of constrictor 20-HETE, whereas this ratio was 1±1 in kidney failure rats, suggesting a diminution of the constrictor 20-HETE, which accounts for the greater vasodilatation in kidney failure. Thus, we can suggest that in the presence of AT\textsubscript{1} blockade, Ang II stimulates the release of both vasoconstrictor (20-HETE) and vasodilators (EETs) CYP- AA metabolites in both sham-operated and kidney failure rats, suggesting that vascular response to Ang II depends on a balance between vasoconstrictor/vasodilator effects. However, in kidney failure, there is an AT\textsubscript{2}-dependent diminution in the constrictor CYP- AA component.

In conclusion, we have presented evidence that in kidney failure produced by renal ablation, AT\textsubscript{1} receptor stimulation mediates Ang II–induced vasoconstriction, whereas AT\textsubscript{2} receptor activation mediates a counterregulatory vasodilatation. We further demonstrate that AT\textsubscript{2} receptor-dependent vasodilatation is coupled to generation of CYP- AA metabolites and that there is upregulation of AT\textsubscript{2} receptor mRNA expression in kidney failure, which accounts for enhanced vasodilatation.

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