Regulation of renal 12(S)-hydroxyeicosatetraenoic acid in diabetes by angiotensin AT1 and AT2 receptors

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Abdel-Rahman EM, Abadir PM, Siragy HM. Regulation of renal 12(S)-hydroxyeicosatetraenoic acid in diabetes by angiotensin AT1 and AT2 receptors. Am J Physiol Regul Integr Comp Physiol 295: R1473–R1478, 2008. First published September 17, 2008; doi:10.1152/ajpregu.90699.2008.—Diabetes is associated with increased production of 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE]. The mechanisms involved in this process remain unclear. We hypothesized that hyperglycemia and angiotensin II (ANG II) regulate renal 12(S)-HETE production via a balance between angiotensin AT1 and AT2 receptors activities. Using a microdialysis technique, renal interstitial fluid (RIF) levels of ANG II and 12(S)-HETE were monitored in normal control and streptozotocin-induced diabetic rats at baseline and then weekly thereafter for 12 wk. In a second group of normal and diabetic rats, 3 wk after development of diabetes, we monitored RIF 12(S)-HETE levels in response to acute AT1 receptor blockade with valsartan or AT2 receptor blockade with PD123319 individually or combined. Two weeks after induction of diabetes there was a 404% increase in ANG II (P < 0.05), a 149% increase in 12S-HETE (P < 0.05), and a 649% increase in urinary albumin excretion (P < 0.05). These levels remained elevated throughout the study. PD123319 given alone had no effect on 12(S)-HETE. Valsartan decreased 12(S)-HETE by 61.6% (P < 0.0001), a response that was abrogated when PD123319 was given with valsartan. These data demonstrate that hyperglycemia increases renal ANG II and 12(S)-HETE levels. The increase in 12(S)-HETE is mediated via AT1 receptor. The attenuation of the effects of AT1 receptor blockade by PD123319 suggests that AT2 receptor contributes to the downregulation of renal 12(S)-HETE production.

diabetes mellitus; angiotensin II; kidneys; urinary albumin excretion

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The U.S. RENAL DATA SYSTEM reports a continuous increase in the incidence of end-stage renal disease (ESRD) in diabetic patients over the past two decades (10). The pathophysiology of diabetic nephropathy is not well understood. It is well established that increased activity of the renin angiotensin system (RAS) contributes to the development of this disease (7, 19, 31). Angiotensin II (ANG II) is the most effector hormone of the RAS and most of its effects are mediated by its AT1 and AT2 subtype receptors. Previous studies demonstrated the correlation between elevated blood glucose and RAS activity. Hyperglycemia stimulates the expression of angiotensinogen gene (45) through synthesis of diacylglycerol and the protein kinase C signal transduction pathway, increases kidney renin mRNA and ACE activities (47), enhances the expression of AT1 receptors (48), and increases renal ANG II production (37). The involvement of the AT1 receptor in the development of diabetic nephropathy is well established based on trials demonstrating reduction in albuminuria and slowing the progression to ESRD by blocking this receptor activity of (7, 19, 31). Despite this knowledge, the role of the AT2 receptors in diabetic kidney disease is not known.

12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] is the lipid product of 12-lipoxygenase (12-LO) and is produced in the kidney, vascular smooth muscle, and adrenal cells (18, 22). Previous studies demonstrated involvement of 12-LO and its metabolite 12(S)-HETE in vascular disease, hypertension, and diabetes (8, 17, 30); yet, there are very limited studies describing its role in the development of diabetic nephropathy (2, 15). Rat mesangial cells exposed to high glucose levels demonstrated increase in 12-LO mRNA and protein expression, suggesting a role for 12-LO pathway in the pathogenesis of diabetic nephropathy (15). The mechanisms through which 12(S)-HETE may contribute to the pathogenesis of diabetic nephropathy are not well established. Previous studies suggested that 12(S)-HETE plays a role in angiogenesis (28), atherosclerosis (24), inflammation (4), modulating ANG II-induced aldosterone secretion (27), and activation of PKC (21). Enhancing 12-LO pathway and 12(S)-HETE activities may augment the arteriolar vasoconstrictor effect of ANG II (16, 23) and facilitate its stimulatory actions on cellular calcium entry into cultured vascular smooth muscle cells (25), while inhibition of 12-LO activities attenuates the vasoconstrictor action of ANG II (52).

In this study, we hypothesized that in the presence of diabetic nephropathy, enhanced renal ANG II levels increases 12(S)-HETE production via the AT1 receptor, a process that is attenuated by the AT2 receptor.

MATERIALS AND METHODS

In vivo microdialysis technique. For the determination of renal interstitial fluid (RIF) ANG II and 12(S)-HETE, we constructed a microdialysis probe as previously described (38, 44). Substances with a molecular mass >40,000 Da cannot cross the dialysis membrane. This molecular mass cutoff allows free passage of ANG II and 12(S)-HETE. In vitro, best recoveries were observed with a perfusion rate of 3 μl/min and were 47% for ANG II and 52% for 12(S)-HETE (39, 40, 44). Negligible amounts of these substances stick to polyethylene tubes (39).

Blood pressure measurements. Systolic blood pressure was measured in conscious animals by the tail cuff method as previously...
described (38, 40) using an automated sphygmomanometer (model 679; IITC/Life Sciences Instruments, Colorado Springs, CO). Blood pressures were recorded at 10-min intervals for 30 min in each experimental day, and values were averaged for each day.

Animal preparation. All protocols were approved by the University of Virginia Animal Care Committee. Male Sprague-Dawley rats (Harlan Sprague Dawley, Harlan Teklad, Madison, WI) weighing 350–400 g were studied. For in vivo determinations of RIF 12(S)-HETE and ANG II, the rats were placed under general anesthesia with ketamine (80 mg/kg im) and xylazine (8 mg/kg im), and the left kidneys were exposed by a left lateral abdominal incision. A microdialysis probe was placed in the cortex of each rat left kidney. All RIF measurements were made 7 days after probes were implanted. For collection of RIF, the inflow tube of the dialysis probe was connected to a gas-tight syringe filled with lactated Ringers solution and perfused at 3 μl/min. The effluent was collected from the outflow tube of the dialysis probe for 60-min sample periods in plastic nonheparinized tubes and stored at −80°C until assayed for ANG II and 12(S)-HETE. Diabetes was induced in 16 animals by a single intravenous injection of streptozotocin (STZ; 30 mg/kg body wt; Sigma, St. Louis, MO) 7 days after insertion of the microdialysis tubes. Blood glucose from tail vein was monitored 2 days after STZ injection and then once weekly using glucose measuring strips (Accu-Check: Boehringer Mannheim). Body weight and 24-h urine collections for measurement of urinary albumin excretion (UAE) were obtained at the beginning of the study and then once weekly. Nondiabetic animals (n = 16) were used as normal control.

Renal 12(S)-HETE and ANG II levels in normal and diabetic rats. RIF ANG II and 12(S)-HETE levels were monitored in diabetic (n = 8) and normal control (n = 8) animals while on normal sodium intake. RIF collections were made at the beginning of the study and then weekly thereafter up to 12 wk.

Renal ANG II and 12(S)-HETE in response to insulin administartion. To test whether elevated blood glucose plays a role in regulation of renal production of ANG II and 12(S)-HETE, normal (n = 8) and diabetic (n = 8) rats were treated with intravenous regular insulin 0.4 U·kg−1·h−1 for 5 h (Eli Lilly, Indianapolis, Indiana) at day 21 after onset of diabetes. Blood glucose levels were monitored hourly during the study. Insulin treatment was stopped if blood glucose decreased to 70 mg/dl. RIF ANG II and 12(S)-HETE were monitored before and at the end of insulin infusion. Blood glucose levels were maintained between 60 and 100 mg/dl.

Effects of AT1 and AT2 receptors blockade on RIF ANG II and 12(S)-HETE. To investigate the relationship between ANG II receptor subtypes and 12(S)-HETE, the angiotensin AT1 receptor blocker, valsartan 10 mg/kg iv (Novartis Pharmaceuticals, East Hanover, NJ), or the AT2 receptor blocker PD123319 50 μg·kg−1·min−1 iv (Parke-Davis, Ann Arbor, MI) were administered individually or combined for 8 h. This treatment was given to normal (n = 8) and diabetic (n = 8) animals (n = 8) at day 21 of the study.

Specimen collection, storage, and assays. Specimens of RIF ANG II and 12(S)-HETE were collected and stored at −80°C until they were assayed. The RIF ANG II samples were collected in tubes containing a mixed inhibitor solution containing 5 mmol/l EDTA, 10 mmol/l pepstatin, 20 mmol/l enalaprilate, and 1.25 mmol/l and 1.10-phenanthroline and kept on ice, which is known to prevent ANG II formation or degradation, and evaporated to dryness under N2 (Savant Instruments, Holbrook, NY). Measurement of RIF ANG II was done as previously described (44) using enzyme immunoassay kit (SIP-BIO, France). This assay sensitivity is 0.5 pg/ml. RIF 12(S)-HETE was measured by ELISA (Assay Design, Ann Arbor, MI). The assay sensitivity for 12(S)-HETE is 146.3 pg/ml. UAE was measured (14) by ELISA using Neprat (Exocell, Philadelphia, PA). The assay sensitivity for urinary albumin is 0.3 μg/ml.

Statistical analysis of data. Comparison among different treatment and control groups were examined by one-way ANOVA. Comparisons between, before, and after treatment groups were examined using t-test using SPSS program (SPSS, Chicago, IL). Data are expressed as means ± SE. Differences of P < 0.05 were considered significant.
RIF 12(S)-HETE levels in the normal control and diabetic groups. In the control normoglycemic rats, the RIF 12(S)-HETE was 1349.7 ± 178.9 pg/ml at baseline (not significantly different from the baseline of the diabetic group) and did not significantly change throughout the study (Fig. 2B). RIF 12(S)-HETE increased in diabetic rats (Fig. 2B) by the second week of the study from 1774.5 ± 61.5 pg/ml to 2636.7 ± 143.5 pg/ml (P < 0.05) and continued to be elevated throughout the duration of the study peaking at day 63 to 6039.1 ± 531.7 pg/ml (P < 0.001).

Correlation between blood glucose, ANG II, 12(S)-HETE, and albuminuria. In the diabetic rats, there was a positive correlation between blood glucose level and both RIF 12(S)-HETE (r = 0.81, P < 0.001) and RIF ANG II (r = 0.79, P < 0.001) levels. Similarly, urinary UAE correlated positively with both RIF 12(S)-HETE (r = 0.73, P < 0.0001) and ANG II (r = 0.85, P < 0.0001) levels. A positive correlation was also noted between RIF ANG II and RIF 12(S)-HETE (r = 0.83, P < 0.0001).

RIF ANG II and 12(S)-HETE response to insulin in diabetic rats. In normal control rats, blood glucose decreased during insulin administration from 78.2 ± 0.6 mg/dl to 66.8 ± 0.4 mg/dl (P < 0.05). There were no significant changes in RIF ANG II or 12(S)-HETE levels in response to insulin treatment in control animals (Fig. 3, A and B). In diabetic rats, insulin treatment decreased the blood glucose level from 358.3 ± 19.4 mg/dl to a normal value of 88.4 ± 2.3 mg/dl (P < 0.01). RIF ANG II decreased from 20.3 ± 0.4 pg/ml at baseline to 3.8 ± 0.6 pg/ml (P < 0.001) by the end of insulin treatment (Fig. 3A). Similarly, when blood glucose was reduced in the diabetic rats, there was significant reduction in 12(S)-HETE level (Fig. 3B) from 2240 ± 149.8 pg/ml to 1051.3 ± 157.9 pg/ml (P < 0.001).

Changes in RIF ANG II and 12(S)-HETE levels in response to valsartan or PD123319, individually and combined. RIF ANG II levels in the nondiabetic control group increased slightly following individual treatment with valsartan or PD123319 or their combination and were significantly lower than its levels in the diabetic group (Fig. 4A).

In diabetic rats, RIF ANG II levels were elevated at baseline compared with normal control animals and increased further (P < 0.001) during treatment with valsartan or PD123319, individually and combined (Fig. 4A). Valsartan alone increased RIF ANG II levels from 18.5 ± 1.6 pg/ml to 30.1 ± 2.1 pg/ml (P < 0.001). Similarly, PD123319 alone or combined with valsartan and PD123319 treatment increased RIF ANG II from 20.9 ± 2.6 pg/ml and 18.7 ± 0.9 pg/ml to 30.1 ± 2.1 pg/ml (P < 0.001) and 39.8 ± 1.5 pg/ml (P < 0.0001), respectively (Fig. 4A).

RIF 12(S)-HETE level changes in response to valsartan or PD123319, individually and combined. In normoglycemic control rats, 12(S)-HETE levels did not show significant changes with different treatments (Fig. 4B). Twenty-one days after onset of diabetes, RIF 12(S)-HETE was 2833.6 ± 277.5 pg/ml and decreased in response to valsartan to 1087.1 ± 269.3 pg/ml (P < 0.0001). There were no significant changes in 12(S)-HETE levels when PD123319 was given alone. However, the decrease in 12(S)-HETE level with valsartan alone (Fig. 4B) was partially abrogated when PD123319 was given together with valsartan (P < 0.05).

Fig. 2. Renal interstitial levels of angiotensin II (A) and 12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] in conscious normoglycemic control (■) and streptozotocin-induced diabetic (●) rats (n = 8 each group). +P < 0.05, *P < 0.001 vs. control.

Fig. 3. Renal interstitial levels of angiotensin II (A) and 12(S)-HETE (B) in conscious normoglycemic control (white bars) and streptozotocin-induced diabetic (black bars) rats (n = 8 each group) in response to insulin treatment (n = 8 each group). *P < 0.001 vs. baseline of diabetic rats.
This study shows that 12(S)-HETE is present locally in the RIF. These levels are increased in STZ-induced diabetic rats and correlate positively with blood glucose level. The results suggest that the increase in renal production of 12(S)-HETE is related to the elevated blood glucose. This finding was confirmed by reduction in the level of renal 12(S)-HETE following insulin treatment and normalization of blood glucose.

Current knowledge of the mechanisms regulating renal production of 12(S)-HETE in diabetes is not well established. Furthermore, the status of in vivo 12(S)-HETE levels in diabetes is controversial with some studies showing increases (2, 15, 20), while others showed no change or decrease in levels (34, 46).

In our study, we confirmed the presence of diabetic nephropathy by the presence of microalbuminuria as early as 2 wk after induction of diabetes. The increase in albuminuria correlated positively with both renal ANG II and 12(S)-HETE levels, suggesting a role for both ANG II and 12(S)-HETE in the pathogenesis of diabetic nephropathy. The reduction in renal 12(S)-HETE levels during valsartan treatment confirms the involvement of ANG II in mediating renal production. We further confirmed that ANG II increases 12(S)-HETE levels in diabetes via AT1 receptor stimulation. Several mechanisms were suggested to explain the contribution of AT1 receptor to the pathogenesis of diabetic nephropathy (9, 32, 35, 53). Stimulation of renal 12(S)-HETE production by the AT1 receptor could be another mechanism that contributes to the deleterious effects of AT1 receptor in development of diabetic nephropathy. Our study supports the notion that there is a cross talk between the AT1 receptor and 12(S)-HETE. A recent report demonstrated increased AT1 receptor expression by 12(S)-HETE (51). Furthermore, 12(S)-HETE may contribute to development of diabetic nephropathy through enhancing the renal vasoconstrictor effects of ANG II (52), TGF-β production (17), matrix formation (33), and COX-2 production (50).

In contrast to AT1 receptor, the potential role of AT2 receptor in diabetic nephropathy or its influence on 12(S)-HETE is not known. Abrogation of the effects of the AT1 receptor blockade on 12(S)-HETE by PD123319 supports the involvement of the AT2 receptor in regulation of this factor.
Our study suggests that AT2 receptor inhibits renal 12(S)-HETE production in diabetic rats and is in agreement with previous reports suggesting a protective role of AT2 in the kidney (43). Several mechanisms could contribute to the inhibitory effect of the AT2 receptor on 12(S)-HETE production. AT2 receptor expression was reported to be reduced in diabetes (5, 49), which is also associated with reduction in renal NO production (3). Reduction in renal NO enhances production of renal renin and ANG II (42, 44) and increases 12-LO activities (20). Our study also shows that ANG II level is increased further when combined with blockade of the AT1 and AT2 receptors. These results are consistent with recent reports of AT2 receptor inhibition of renin and ANG II production (42, 44). Taken together, the increase in ANG II levels and AT1 receptor activity, combined with the reduction of AT2 expression and NO activity could explain the marked increase in 12(S)-HETE levels in diabetic nephropathy.

We conclude that hyperglycemia increases renal ANG II, which in turn increases renal 12(S)-HETE levels via AT1 receptor. To our knowledge, this is the first study demonstrating that AT2 receptor plays a role in modulating renal 12(S)-HETE in diabetes. Future studies are needed to define the exact mechanisms by which AT2 receptor modulates renal 12(S)-HETE and to further clarify how they contribute to the pathogenesis of diabetic nephropathy.

**Perspectives and Significance**

This study demonstrates that hyperglycemia increases renal ANG II, which in turn increases renal 12(S)-HETE levels via AT1 receptor. To our knowledge, this is the first study demonstrating that AT2 receptor plays a role in modulating renal 12(S)-HETE in diabetes. Future studies are needed to define the exact mechanisms by which the AT2 receptor modulates renal 12(S)-HETE and to further clarify how they contribute to the pathogenesis of diabetic nephropathy.

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