Regulation of renal 12(S)-hydroxyeicosatetraenoic acid in diabetes by angiotensin AT$_1$ and AT$_2$ receptors

Emaad M. Abdel-Rahman, Peter M. Abadir, and Helmy M. Siragy

Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia

Submitted 18 August 2008; accepted in final form 15 September 2008

Abdel-Rahman EM, Abadir PM, Siragy HM. Regulation of renal 12(S)-hydroxyeicosatetraenoic acid in diabetes by angiotensin AT$_1$ and AT$_2$ 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 AT$_1$ and AT$_2$ 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 AT$_1$ receptor blockade with valsartan or AT$_2$ 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 AT$_1$ receptor. The attenuation of the effects of AT$_1$ receptor blockade by PD123319 suggests that AT$_2$ receptor contributes to the downregulation of renal 12(S)-HETE production.

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 effecter hormone of the RAS and most of its effects are mediated by its AT$_1$ and AT$_2$ 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 AT$_1$ receptors (48), and increases renal ANG II production (37). The involvement of the AT$_1$ 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 AT$_2$ receptors in diabetic kidney disease is not known.

12(S)-hydroxyeicosatetraenoic acid [12(S)-HETE] is the lipid product of 12-lypoxigenase (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), modulation of ANG II-induced aldosterone secretion (27), and activation of PKC (21). Enhancing 12-LO pathway and 12(S)-HETE activities 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 AT$_1$ receptor, a process that is attenuated by the AT$_2$ 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

Address for reprint requests and other correspondence: H. M. Siragy, P.O. Box 801409, Univ. of Virginia Health System, Charlottesville, VA 22908-1409 (e-mail: hms7a@virginia.edu).
R1474

ANGIOTENSIN RECEPTORS REGULATE 12(S)-HETE IN DIABETES

found (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 in −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 administration. 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 (Speedy Vac; Savant Instruments, Holbrook, NY). Measurement of RIF ANG II was done as previously described (44) using enzyme immunoassay kit (SPI-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 Nephrat (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.

RESULTS

Blood glucose, blood pressure, body weight, and UAE. Blood glucose ranged between 78.5 ± 3.1 to 80 ± 3.3 mg/dl in normal control rats. Diabetes was induced by STZ treatment as demonstrated by the rise of baseline mean blood glucose level from 81.7 ± 1.6 mg/dl to 356.1 ± 41.3 mg/dl, 2 days after STZ injection (P < 0.0001). The elevated blood glucose level was maintained throughout the 12-wk study period ranging from 332.9 ± 28.3 mg/dl to 452.7 ± 16.8 mg/dl. There was no significant difference in systolic blood pressure between the diabetic and the control groups throughout the study, ranging from 113.2 ± 1.5 mmHg and 111.8 ± 1.3 mmHg at baseline to 112.2 ± 0.7 mmHg and 110.1 ± 0.7 mmHg by week 12, respectively. There were no significant changes in systolic blood pressure with individual treatment with valsartan or PD123319 or their combination. At baseline the average body weight of the normal control group was 398 ± 4.8 g and progressively increased to 423 ± 10.2 g at week 12. In the diabetic animals, the average body weight at baseline was 395 ± 11.3 g and increased to 404 ± 12.4 and 398.2 ± 3.1 g at weeks 6 and 12, respectively. There was no significant difference in body weights between normal and diabetic rats.

UAE in normal control rats was low, ranging from 3.68 ± 1.12 to 3.90 ± 0.94 μg/24 h throughout the study period. In contrast, UAE in diabetic rats (Fig. 1) increased from 3.96 ± 0.93 μg/24 h at baseline to 25.7 ± 2.3 μg/24 h (P < 0.05) by the end of the second week and continued to increase progressively thereafter up to week 12, reaching 93.6 ± 8.87 μg/24 h (P < 0.0001).

RIF ANG II levels in the normal control and diabetic groups. There were no significant changes in RIF ANG II in normal control rats throughout the study ranging from 3.6 ± 0.5 pg/ml to 3.4 ± 0.8 pg/ml (Fig. 2A). In contrast, in the diabetic group there was a progressive significant increase in RIF ANG II levels (Fig. 2A) from 3.3 ± 0.9 pg/ml at baseline to 13.4 ± 3.1 pg/ml by the second week, and levels continued to be elevated throughout the duration of the study peaking at day 63 to 24.8 ± 3.1 pg/ml.

![Fig. 1. 24-h urinary albumin excretion rate in conscious normoglycemic control (■) and streptozotocin-induced diabetic (●) rats (n = 8 each group). WK, week. +P < 0.05, *P < 0.0001 vs. control.](http://ajpregu.physiology.org/ by tenzo.33.6 on April 1, 2017)
ANGIOTENSIN RECEPTORS REGULATE 12(S)-HETE IN DIABETES

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.](http://ajpregu.physiology.org/)

![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 (●) and albuminuria.](http://ajpregu.physiology.org/)
This study shows that 12(S)-HETE is present locally in the RIF. These levels are increased in STZ-induced diabetic rat model by the second week, and continue throughout the 12-wk duration of the study. This rise in RIF 12(S)-HETE in diabetic rats was not mirrored by a similar rise in the control rats and correlated positively with blood glucose level. These results suggest that the increase in renal production of 12(S)-HETE level 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). Renal mesangial cells cultured in high-glucose medium demonstrated an increase in 12(S)-HETE mRNA and protein (15). ANG II also was reported to increase 12(S)-HETE production by renal microvasculature in normoglycemic state (52).

Similar to 12(S)-HETE, our study shows increased renal ANG II levels in diabetic rats throughout the 12-wk duration of the study. This finding is consistent with our previous finding (36) of increased renal ANG II production in diabetic rats. The mechanisms responsible for the increased renal ANG II in diabetes are still being elucidated. An increase in renal cortical renin mRNA (1), enhanced macula densa signaling pathway for renin production (6), or increased activity of plasma and tissue angiotensin converting enzyme (13) are among the factors that may lead to the observed increase in renal ANG II production in diabetes.

In the present study, we focused on monitoring changes in the kidney ANG II and 12(S)-HETE levels by renal microdialysis technique. This method has advantages over monitoring these substances in blood or urine. ANG II level in renal tissue is about 1,000-fold higher than its systemic (41) concentration, suggesting the importance of ANG II in the local regulation of renal physiology and pathophysiology. Other studies also confirmed that renal concentrations of ANG I and ANG II are substantially higher than the corresponding plasma concentrations (29). Similarly, the measurement of circulating 12(S)-HETE is not totally reliable, since it can be formed in large amounts ex vivo in collected blood samples (12).

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.

DISCUSSION

This study shows that 12(S)-HETE is present locally in the RIF. These levels are increased in STZ-induced diabetic rat model by the second week, and continue throughout the 12-wk duration of the study. This rise in RIF 12(S)-HETE in diabetic rats was not mirrored by a similar rise in the control rats and correlated positively with blood glucose level. These results suggest that the increase in renal production of 12(S)-HETE level 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). Renal mesangial cells cultured in high-glucose medium demonstrated an increase in 12(S)-HETE mRNA and protein (15). ANG II also was reported to increase 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 AT$_2$ receptor inhibits renal 12(S)-HETE production in diabetic rats and is in agreement with previous reports suggesting a protective role of AT$_2$ in the kidney (43). Several mechanisms could contribute to the inhibitory effect of the AT$_2$ receptor on 12(S)-HETE production. AT$_2$ 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 AT$_1$ and AT$_2$ receptors. These results are consistent with recent reports of AT$_2$ receptor inhibition of renin and ANG II production (42, 44). Taken together, the increase in ANG II levels and AT$_1$ receptor activity, combined with the reduction of AT$_2$ 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 AT$_1$ receptor. To our knowledge, this is the first study demonstrating that AT$_2$ receptor plays a role in modulating renal 12(S)-HETE in diabetes. Future studies are needed to define the exact mechanisms by which AT$_2$ 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 AT$_1$ receptor. To our knowledge, this is the first study demonstrating that AT$_2$ receptor plays a role in modulating renal 12(S)-HETE in diabetes. Future studies are needed to define the exact mechanisms by which the AT$_2$ receptor modulates renal 12(S)-HETE and to further clarify how they contribute to the pathogenesis of diabetic nephropathy.

**REFERENCES**


