HHS Public Access

Author manuscript

Endocrinology. Author manuscript; available in PMC 2023 October 26.

Published in final edited form as:

Endocrinology. 2003 June ; 144(6): 2229–2233. doi:10.1210/en.2003-0010.

The Angiotensin II Type 1 Receptor Mediates Renal Interstitial Content of Tumor Necrosis Factor-α in Diabetic Rats

HELMY M. SIRAGY, M.D.,

ALAA AWAD,

PETER ABADIR,

RANDY WEBB

The Department of Medicine (H.M.S., A.A., P.A.), University of Virginia School of Medicine, Charlottesville, Virginia 22904; and Novartis Pharmaceuticals Corporation (R.W.), East Hanover, New Jersey 07936

Abstract

A unique microdialysis technique was used to demonstrate that increased levels of angiotensin II (Ang II) and consequent stimulation of the Ang II type 1 (AT_1) receptor increase the renal content of TNF-α in diabetes. Recovery of Ang II and TNF-α in renal interstitial fluid (RIF) was measured in conscious rats before and weekly for 12 wk after induction of diabetes with streptozocin and in response to oral valsartan (10 mg/kg·d). Recovery of Ang II in RIF was significantly higher in diabetic rats than in nondiabetic rats. In diabetic rats, RIF recovery of TNF-α increased by approximately 67% over baseline, whereas it was unchanged in nondiabetic rats. AT_1 receptor blockade with valsartan prevented the increase in TNF-α in the diabetic group. This study shows that diabetes is associated with an increase in the vasoconstrictive hormone Ang II and the inflammatory cytokine TNF-α, both of which play a role in accelerating renal function decline in diabetic nephropathy. The study also confirms that valsartan reduces intrarenal level of TNF-α by acting on Ang II at the AT_1 receptor level. This finding of a potential antiinflammatory effect for valsartan is new and in addition to its known antihypertensive effects.

The prevalence of diabetes and diabetic nephropathy has increased rapidly in the United States over the past decade, a trend that is expected to continue (1). The number of patients with end-stage renal disease nearly doubled during the decade; in most patients, renal failure was a result of diabetic nephropathy due to type 2 diabetes (2). In patients with diabetic nephropathy, results of clinical trials of angiotensin-converting enzyme (ACE) inhibitors in type 1 diabetes and of angiotensin II (Ang II) type 1 (AT_1) receptor blockers (ARBs) in type 2 diabetes suggest that antihypertensive agents that target the reninangiotensin system (RAS) can slow the progression of renal disease independent of their effects on blood pressure (2–4).

The pathogenesis of diabetic nephropathy is complex. High extracellular glucose concentrations, a determinant of progressive nephropathy, activate intracellular signaling

Address all correspondence and requests for reprints to: Helmy M. Siragy, M.D., Box 801409, Department of Medicine, University of Virginia Health Sciences Center, 450 Ray C. Hunt Drive, Charlottesville, Virginia 22908. hms7a@virginia.edu.

cascades and alter the expression of cytokines and growth factors (4). Among the cytokines with an implicated role in diabetic nephropathy is TNF-α, a monocyte-macrophage derived cytokine that activates transcription factors such as nuclear factor *k*B, which induce expression of genes involved in inflammation and cell growth (5–7).

An activated RAS, via its principal effector Ang II, promotes proteinuria and accelerates the decline in renal function in diabetes through both hemodynamic (increased glomerular capillary pressure) and nonhemodynamic (stimulation of cellular hypertrophy and accumulation of extracellular matrix) effects (4). The deleterious effects of the RAS are thought to be due in part to induction of cytokines and growth factors by Ang II and interaction of Ang II with the AT_1 receptor, which is widely distributed in the kidney (4, 8). In addition to the systemic RAS, the intrarenal RAS is activated in diabetic nephropathy.

The present study was conducted to determine whether hyperglycemia associated with diabetes increases renal production of TNF-α due to increased production of Ang II and stimulation of the AT_1 receptor. Renal interstitial fluid (RIF) recovery of Ang II and TNF-α was measured in rats before and after induction of diabetes with streptozocin (STZ). One group received valsartan, a specific and potent ARB, to determine the effect of AT_1 receptor blockade on RIF recovery of TNF-α. An *in vivo* renal microdialysis technique that has been used previously to measure renal levels of bradykinin, nitric oxide, cGMP, prostaglandin E_2, and Ang II was used in the present study (9–16). Measurements were made in conscious animals over a continuous time course, with each animal serving as its own control in addition to having a time control. Advantages of measuring RIF recovery over blood or urine concentrations include the following: potential unwanted hemodynamic changes that may occur with repeated blood sampling in small animals are avoided; interstitial levels of hormones/autocoids reflect local changes in the organ that may not be reflected in measurements of circulating substances; concentrations in the interstitium, which is closer to target receptors, may differ from the concentration in the circulation; substances can be formed or degraded in the urine and, thus, do not reflect concentrations within the target organ; and the molecular weight cutoff of the microdialysis membrane helps exclude undesirable substances such as degrading enzymes and carrier proteins (10, 14).

Materials and Methods

Renal microdialysis technique

The renal microdialysis technique used in this study was developed by our laboratory and has been described previously (9–16). In brief, each end of a single hollow-fiber dialysis tube (5.0 mm long, 0.1-mm inner diameter) with a 40,000 molecular mass cutoff (Hospal, Meyzieu, France) was inserted into the manually dilated ends of two 300-mm-long (inflow and outflow) hollow polyethylene tubes with 0.12-mm inner diameter and 0.65-mm outer diameter (BAS Co., Indianapolis, IN).

Animal preparation

The experiments, which were approved by the University of Virginia Animal Research Committee and conducted in accordance with institutional guidelines, were performed

in 4-wk-old conscious male Sprague-Dawley rats (Harlan Teklad, Madison, WI) with a microdialysis catheter inserted into the outer renal cortex. Rats underwent general anesthesia with im ketamine (80 mg/kg) and xylazine (8 mg/kg), and the right and left kidneys of each rat were exposed via a midline abdominal incision (14). A 31-gauge needle was inserted into the renal capsule to a depth of approximately 1 mm from the outer cortex and passed a distance of 5 mm before it exited the capsule. The needle tip was inserted into one end of the dialysis probe and pulled back through the renal capsule to place the dialysis fiber within the renal cortex. The dialysis probe inflow and outflow tubes were tunneled sc through a bevel-tipped stainless steel tube and emerged near the interscapular region. The exterior ends of the tubes were sutured to the skin at the exit site and covered with a stainless steel spring to protect them from rat-inflicted damage.

The rats were housed under controlled conditions (temperature, 21 ± 1 C; humidity, 60 ± 10%; lighting, 8 h/20 h) and were allowed 7 d to recover and become acclimatized to the laboratory sampling as well as to avoid any influence of anesthesia or surgery on the experiment. All animals consumed a normal sodium diet (0.28% NaCl, Bioserve, Frenchtown, NJ). Experiments were started promptly at 0800 h daily to avoid diurnal variation in experimental parameters. For collection of RIF, the inflow tube was connected to a gas-tight syringe filled with lactated Ringer's solution, which was perfused at a rate of 3 μl/min. The effluent was collected from the outflow tube for 60-min sample periods on the same day every week for the 12 wk of the study.

Assessment of AT_1 receptor blockade on TNF-α

Rats were placed in metabolic cages and randomly assigned to one of four study groups of eight rats each: a normal (untreated) control group that received a regular sodium diet; a normal control group treated with valsartan (Novartis Pharmaceuticals, East Hanover, NJ) 10 mg/kg·d orally; an STZ-induced diabetes group; and an STZ-induced diabetes group treated with valsartan 10 mg/kg·d. After an overnight fast, baseline measurements were taken of basal RIF Ang II and TNF-α; then two groups of rats were injected iv with STZ 35 mg/kg (Sigma, St. Louis, MO) to induce diabetes. One group with STZ-induced diabetes received valsartan. RIF samples were collected from both kidneys, and the samples from both kidneys were pooled for measurements, which were made on the same day each week during the study, with wk 1 as baseline (before induction of diabetes with STZ). Animals were monitored for body weight; systolic blood pressure; and levels of blood glucose, 24-h urinary albumin, and TNF-α during the 12-wk study.

Assessment of RIF Ang II and TNF-α in response to insulin treatment

Rats were placed in metabolic cages and were randomly assigned to a normal (untreated) control group (n = 8) and an STZ-induced diabetes group (n = 8). Diabetes was induced with STZ as above. After an overnight fast, both groups were treated with iv administration of 0.4 U/kg·h regular insulin in 0.9% NaCl (Eli Lilly & Co., Indianapolis, IN) for 5 h. Blood and RIF glucose levels were monitored hourly during the study. Insulin infusion was discontinued if blood glucose decreased to less than 60 mg/dl. RIF glucose, Ang II, and TNF-α were monitored before and at the end of insulin infusion. Blood glucose levels were

maintained between 60 and 100 mg/dl. To avoid blood glucose levels below 60 mg/dl in normal animals, insulin was mixed with 10% dextrose in water (20 μl/min).

Analytical methods

Blood glucose concentrations were monitored daily using the Accu-Check glucometer (Mannheim Boehringer, Indianapolis, IN), which uses approximately 4 μl of whole blood. Hemodynamic measurements, including heart rate, systolic and diastolic blood pressure, and mean arterial pressure, were measured using a Natsumi BP Pulse Monitor (Peninsula Laboratories, Inc. Belmont, CA). Urinary and RIF recovery levels of TNF-α were measured by a specific ELISA (PharMingen, San Diego, CA), which has no cross-reactivity with interleukins or interferon-γ. RIF recovery of Ang II was measured using a specific ELISA with a sensitivity of 0.5 pg/ml, as described previously (17). Urinary albumin was measured by a specific ELISA (Exocell Inc., Philadelphia, PA).

Statistical analysis

Treatment and control groups were compared using ANOVA, including a repeated measure term, using the general linear models procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Data are expressed as mean ± se α level. *P* values were calculated from the least squares measure of a linear model. A difference of *P* less than 0.05 was considered statistically significant.

Results

Fasting blood glucose concentrations in the diabetes-induced groups indicated that the animals were in a diabetic state (346 ± 11 mg/dl for STZ-treated animals that did not receive valsartan and 338 ± 13 mg/dl for STZ-animals that did receive valsartan; $P < 0.001$ compared with control animals). Body weight increased as expected for normal rats. In contrast, body weight changes were modest but not significant in diabetic rats. There were no changes in blood pressure throughout the study in any group. Systolic blood pressures were 113.2 ± 1.5 mm Hg at baseline, 114.9 ± 1.1 mm Hg at wk 6, and 112.9 ± 0.7 at wk 12 in the diabetic rats. Valsartan treatment did not cause any significant changes in blood pressure in the diabetic group (ranging from 112.8 ± 2.1 mm Hg at baseline to 113.4 ± 2.1 mm Hg at wk 12) or in the normal animals (113.1 ± 1.5 mm Hg at baseline and 114 ± 2.5 mm Hg at wk 12).

At baseline before insulin infusion, blood and RIF glucose concentrations, respectively, were 70 ± 5 mg/dl and 42 ± 3 mg/dl in normal animals, and 325 ± 15 mg/dl and 253 ± 12 mg/dl in diabetic animals. At the end of 5 h of insulin administration, blood and RIF glucose levels, respectively, were 68 ± 7 mg/dl (*P* = not significant) and 33 ± 2 mg/dl ($P < 0.001$) in normal animals and 93 ± 11 mg/dl and 51 ± 4 mg/dl in diabetic animals ($P < 0.001$, compared with levels before insulin treatment). TNF-α recovery in RIF did not change significantly in response to insulin treatment in normal control animals; it was 2.15 ± 0.01 pg/min at baseline and 2.20 ± 0.01 pg/min at the end of insulin treatment. In the diabetic group, TNF-α recovery in RIF significantly decreased in response to insulin treatment, from 10.21 ± 0.50 pg/min to 2.10 ± 0.02 pg/min ($P < 0.0001$).

Urinary TNF-α and albumin were measured in the untreated diabetes group and the valsartan-treated diabetes group. In the diabetes group, urinary TNF-α and albumin rose from 16.1 ± 6.5 pg/ml and 3.96 ± 0.93 μg/24 h at baseline, respectively, to 294.18 ± 36.94 pg/ml and 341 ± 53.4 μg/24 h at wk 6, and 337.72 ± 34.91 pg/ml and 934 ± 88.7 μg/24 h at wk 12 ($P < 0.0001$ compared with baseline). In the valsartan-treated diabetes group, baseline urine TNF-α and albumin were 17.11 ± 4.23 pg/ml and 3.29 ± 0.199 μg/24 h, and although levels increased over the course of the study, they were statistically significantly lower than those in the diabetes group that did not receive valsartan, rising to 42.34 ± 6.21 pg/ml and 57.1 ± 3.99 μg/24 h at wk 6, and 31.88 ± 5.76 pg/ml and 63.5 ± 9.12 μg/24 h at wk 12 ($P < 0.0001$ compared with diabetes group).

RIF Ang II in normal and diabetic rats

RIF recovery of Ang II in normal (untreated control) rats remained nearly constant throughout the study (0.30 ± 0.001 pg/min at baseline, and 0.31 ± 0.002 pg/min at wk 12; Fig. 1). Valsartan treatment increased RIF Ang II recovery rate in normal rats from 0.30 ± 0.002 pg/min at baseline to 0.36 ± 0.001 pg/min at wk 12. In contrast, RIF recovery of Ang II in diabetic rats was significantly higher after induction of diabetes (0.40 ± 0.01 pg/min at wk 3; $P < 0.001$) and continued to increase significantly ($P < 0.0001$) through wk 11, remaining at that level (0.73 ± 0.01 pg/min) at wk 12. In STZ-induced diabetic rats treated with valsartan, RIF Ang II recovery increased even higher, reaching 0.62 ± 0.04 pg/min by wk 3 ($P < 0.001$) and continuing to increase through wk 8, remaining at 0.92 ± 0.01 pg/min at wk 12 ($P < 0.0001$).

Ang II recovery in RIF did not change in response to insulin administration in normal animals; it was 0.31 ± 0.004 pg/min at baseline and 0.31 ± 0.002 pg/min at the end of insulin administration. In contrast, Ang II recovery in RIF decreased from 0.62 ± 0.004 pg/min to 0.33 ± 0.01 pg/min ($P < 0.001$) in diabetic animals in response to insulin treatment.

TNF-α in normal and diabetic rats and response to valsartan

RIF recovery of TNF-α in normal (untreated control) rats remained constant throughout the study [2.20 ± 0.02 pg/min at wk 1 (baseline) and 2.19 ± 0.01 pg/min at wk 12]. In diabetic rats, RIF recovery of TNF-α was significantly higher ($P < 0.0001$) throughout the study compared with recoveries in the control and valsartan-treated groups (Fig. 2). At baseline (wk 1), RIF recovery of TNF-α was similar among the four groups: control group, 2.20 ± 0.02 pg/min; valsartan-treated control group, 2.21 ± 0.01 pg/min; diabetes group, 2.16 ± 0.01 pg/min; valsartan-treated diabetes group, 2.18 ± 0.01 pg/min. At wk 12, RIF recovery of TNF-α was 11.8 ± 0.8 pg/min ($P < 0.0001$) in the diabetes group (about 80% increase from baseline at wk 1) and 5.6 ± 0.01 pg/min ($P < 0.0001$) in the valsartan-treated diabetes group. There were no significant changes in RIF TNF-α recovery in the valsartan-treated control group throughout the study. Thus, administration of 10 mg/kg·d oral valsartan to diabetic rats significantly reduced the diabetes-associated increase in TNF-α throughout the 12-wk experimental period (Fig. 2).

Discussion

We report here the first chronic *in vivo* measurements of renal tissue recovery of Ang II and TNF-α, and present for the first time data collected over an extended time course in a unique conscious rat model of diabetes. Our model allows direct sampling of RIF and provides accurate measurement of Ang II in the target renal tissue, because the intrarenal concentration of Ang II is about 1000-fold higher than the circulating (plasma) level (11). Local changes in hormone and TNF-α levels may not be detectable in the circulation, and these factors may be degraded in the urine (14, 15). In addition, our use of conscious animals avoids anestheticinduced elevations of Ang II levels (11) as well as the interfering effects of anesthesia on hemodynamics and other parameters.

Although high levels of extracellular glucose stimulated angiotensinogen expression in studies of isolated rat proximal tubular cells in culture (4), it had been assumed that Ang II is not significantly increased in diabetes, based on studies of animal models in which levels of Ang II were measured in blood or in kidney tissue isolated from anesthetized animals (4, 18). However, we have clearly shown in our model of STZ-induced diabetes in intact, conscious animals that renal levels of Ang II are significantly increased at the target tissue level, where Ang II is believed to act primarily through the AT_1 receptor. Valsartan, an Ang II receptor antagonist (ARB), selectively blocks the AT_1 receptor (19), unlike ACE inhibitors that act nonspecifically to block an early enzymatic process in Ang II synthesis. Valsartan increases the tissue Ang II level through direct blockade of the AT_1 short negative feedback loop, which, in turn, stimulates the increase in renin secretion. During blockade of the AT_1 receptor, the excess Ang II may be renoprotective, because it is available to interact with the AT_2 receptor (20). The renoprotective effects of the AT_2 receptor stem from its ability to increase generation of bradykinin, nitric oxide, and cGMP, as well as its antiproliferative and antifibrotic properties (8, 9, 14, 15).

Diabetes is associated with increased levels of vasoconstrictors such as Ang II (19), which can up-regulate the expression of inflammatory cytokines, including TNF-α, as well as growth factors, adhesion molecules, and transcription factors (7). TNF-α may play a role in the development of diabetic nephropathy. Increased levels of the cytokines TNF-α and IL-1 have been detected in isolated glomerular basement membranes from rats with STZ-induced diabetes (5). In patients with coronary artery disease and increased levels of inflammatory molecules including TNF-α, treatment with an ARB reduced these markers of inflammation in the circulation (21). Antihypertensive therapy with both ACE inhibitors (in type 1 diabetes) and ARBs (in type 2 diabetes) has also had a beneficial effect in treating diabetic renal disease (19). In patients with diabetic nephropathy, ACE inhibitors and ARBs have been shown in clinical trials to be renoprotective independent of their ability to lower blood pressure (22–25). In diabetic patients with advanced renal failure, levels of both TNF-α and proteinuria were correlated. Treatment with pentoxifylline, an immune modulator with anti-TNF-α activity, reduced proteinuria, possibly via its anticytokine activity (26).

The source of TNF-α in RIF is not well established. Although TNF-α can be produced by inflammatory cells, it can also be produced by the glomeruli, tubules, and blood vessels (27–29). The increase in RIF TNF-α could reflect an increase in production or a decrease

in degradation or clearance. In this study, we could not specifically distinguish between these possibilities. However, in our studies, there was a concomitant increase in urinary TNF-α that suggests an increase in its production. The exact mechanism through which Ang II can stimulate TNF-α production is not known. The increase in renal production of Ang II stimulates gene expression and, therefore, renal production of TNF-α, as well as other proinflammatory mediators, including IL-1 and IL-6, possibly via activation of the transcription factor nuclear factor-*k*B (27), release of prostaglandin E_2 (29), and reduction of intracellular cAMP (30). This is consistent with our data showing reduction in RIF TNF-α during AT_1 receptor blockade. Insulin treatment over a 5-h time course lowered blood glucose levels to less than 100 mg/dl. This was associated with reduced recovery of Ang II and TNF-α in RIF. These results support the hypothesis that the moderate hyperglycemia produced in our rat model using a relatively small dose of STZ stimulates renal production of Ang II, which in turn stimulates the AT_1 receptor to increase TNF-α production. In this study, due to the small volume of RIF that could be collected, we were not able to measure the levels of inflammatory cytokines other than TNF-α; this is an area of future interest.

We show in this study that TNF-α is increased in the diabetic kidney as a whole or at the level of the renal cortex, because the microdialysis probes for collection of RIF were placed in the renal cortices of the animals we studied. The administration of the ARB valsartan inhibited the diabetes-associated recovery of TNF-α. This increase in TNF-α must be mediated via the AT_1 receptor, because valsartan decreased TNF-α levels. In two previous studies in diabetic rats, elevated levels of TNF-α mRNA were not decreased after treatment with an ACE inhibitor or ARB. However, these studies only looked at mRNA in isolated glomeruli and did not quantify actual TNF-α production (31, 32). In Ang II-dependent hypertension, renal levels of TNF-α are higher than those of normotensive controls (33). This is consistent with other studies reporting an increase in TNF-α via the AT_1 receptor (21).

The findings of the present study indicate that inflammatory cytokine TNF-α may mediate the progression of diabetic kidney disease, at least partially, through stimulation of the AT_1 receptor. This study also clearly demonstrates that hyperglycemia is a major stimulus for initiation of the reninangiotensin cascade. Therapeutic modalities such as angiotensin receptor blockade may arrest this process. As we learned, administration of the ARB valsartan reduced the increase in TNF-α, thereby protecting the kidney from inflammation. Other inflammatory factors, such as acute phase reactants, may also be implicated in the pathophysiology of diabetic nephropathy. A shift of focus from growth factors to the role of cytokines in diabetic nephropathy offers a new direction for research, perhaps into areas such as the link between change in TNF-α levels and renal function and histology. Attempts to block that change, directly or indirectly, may even extend beyond incipient investigations into the use of ARBs as a therapeutic modality to the possibility of direct TNF antagonism to slow the progression of renal disease.

We conclude that in addition to its antihypertensive actions, valsartan seems to protect the kidney from inflammation. This antiinflammatory effect is not a result of lowering of blood pressure but is a result of direct action on cytokine TNF-α at the level of the kidney.

Acknowledgments

This work was supported by Grants HL-47669 and HL-57503 (to H.M.S.) from the National Institutes of Health (NIH) and a research grant from Novartis Pharmaceuticals Corporation. H.M.S. was the re cipient of Research Career Development Award K04-HL-03006 from NIH.

Abbreviations:

ACE	Angiotensin-converting enzyme
Ang II	angiotensin II
ARB	AT_1 receptor blocker
AT_1	Ang II type 1
RAS	reninangiotensin system
RIF	renal interstitial fluid
STZ	streptozocin

References

1. Mokdad AH, Ford ES, Bowman BA, Nelson DE, Engelgau MM, Vinicor F, Marks JS 2000 Diabetes trends in the US: 1990–1998. Diabetes Care 23:1278–1283 [PubMed: 10977060]
2. Hostetter TH 2001 Prevention of end-stage renal disease due to type 2 diabetes. N Engl J Med 345:910–912 [PubMed: 11565525]
3. Opie LH 2001 Renoprotection by angiotensin-receptor blockers and ACE inhibitors in hypertension. Lancet 358:1829–1831 [PubMed: 11741617]
4. Burns KD 2000 Angiotensin II and its receptors in the diabetic kidney. Am J Kidney Dis 36:449–467 [PubMed: 10977776]
5. Hasegawa G, Nakano K, Sawada M, Uno K, Shibayama Y, Ienaga K, Kondo M 1991 Possible role of tumor necrosis factor and interleukin-1 in the development of diabetic nephropathy. Kidney Int 40:1007–1012 [PubMed: 1762301]
6. Leong KG, Karsan A 2000 Signaling pathways mediated by tumor necrosis factor α. Histol Histopathol 15:1303–1325 [PubMed: 11005254]
7. Klahr S, Morrissey JJ 2000 The role of vasoactive compounds, growth factors and cytokines in the progression renal disease. Kidney Int 57(Suppl 75):S77–S14
8. Siragy HM 2000 AT_1 and AT_2 receptors in the kidney: role in disease and treatment. Am J Kidney Dis 36(Suppl 1):S4–S9
9. Siragy HM, de Gasparo M, Carey RM 2000 Angiotensin type 2 receptor mediates valsartan-induced hypotension in conscious rats. Hypertension 35: 1047–1077
10. Siragy HM, Jaffa AA, Margolius HS, Carey HM 1996 Renin-angiotensin system modulates renal bradykinin production. Am J Physiol 271:155–163
11. Siragy HM, Howell NL, Ragsdale NV, Carey RM 1995 Renal interstitial fluid angiotensin: modulation by anesthesia, epinephrine, sodium depletion, and renin inhibition. Hypertension 25:1021–1024 [PubMed: 7737709]
12. Siragy HM, Ibrahim MM, Jaffa AA, Mayfield R, Margolius HS 1994 Rat renal interstitial bradykinin, prostaglandin E2, and cyclic guanosine 3', 5'-mono-phosphate. Effects of altered sodium intake. Hypertension 23:1068–1070 [PubMed: 8206596]
13. Siragy HM, Jaffa AA, Margolius HS 1993 Stimulation of interstitial bradykinin by sodium depletion. Am J Hypertens 6:863–866 [PubMed: 8267942]

14. Siragy HM, Carey RM 1997 The subtype-2 (AT_2) angiotensin receptor mediates renal production of nitric oxide in conscious rats. J Clin Invest 100:264–269 [PubMed: 9218502]
15. Siragy HM, Carey RM 1996 The subtype-2 (AT_2) angiotensin receptor regulates renal cyclic guanosine 3', 5'-monophosphate and AT_1 receptor-mediated prostaglandin E_2 production in conscious rats. J Clin Invest 97:1978–1982 [PubMed: 8621783]
16. Carey RM, Jin X-H, Wang Z-Q, Siragy HM 2000 Nitric oxide: a physiological mediator of the type 2 (AT_2) angiotensin receptor. Acta Physiol Scand 168: 65–71 [PubMed: 10691781]
17. Siragy HM, Carey RM 1999 Protective role of the angiotensin AT_2 receptor in a renal wrap hypertension model. Hypertension 103:167–174
18. Zimpelmann J, Kumar D, Levine DZ, Wehbi G, Imig JD, Navar LG, Burns KD 2000 Early diabetes mellitus stimulates proximal tubule renin mRNA expression in the rat. Kidney Int 58:2320–2330 [PubMed: 11115066]
19. Ruilope LM 2001 Valsartan and the kidney: review of preclinical and clinical data. Adv Ther 18:57–65 [PubMed: 11446269]
20. Goodfriend TL, Elliott ME, Catt KJ 1996 Angiotensin receptors and their antagonists. N Engl J Med 334:1649–1654 [PubMed: 8628362]
21. Navalkar S, Parthasarathy S, Santanam N, Khan BV 2001 Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. J Am Coll Cardiol 37:440–444 [PubMed: 11216960]
22. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD, for the Collaborative Study Group 1993 The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. N Engl J Med 329:1456–1462 [PubMed: 8413456]
23. Lewis EJ, Hunsicker LG, Clarke WR, Berl T, Pohl MA, Lewis JB, Ritz E, Atkins RC, Rohde R, Raz I, for the Collaborative Study Group 2001 Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 345:851–860 [PubMed: 11565517]
24. Brenner BM, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving H-H, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S, for the RENAAL Study Investigators 2001 Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 345:861–869 [PubMed: 11565518]
25. Parving H-H, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P, for the Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria Study Group 2001 The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. N Engl J Med 345:870–878 [PubMed: 11565519]
26. Navarro JF, Mora C, Rivero A, Gallego E, Chahin J, Macia M, Mendez M, Garcia J 1999 Urinary protein excretion and serum tumor necrosis factor in diabetic patients with advanced renal failure: effects of pentoxifylline administration. Am J Kidney Dis 33:458–463 [PubMed: 10070909]
27. Ruiz-Ortega M, Ruperez M, Lorenzo O, Esteban V, Blanco J, Mezzano S, Egido J 2002 Angiotensin II regulates the synthesis of proinflammatory cytokines and chemokines in the kidney. Kidney Int Suppl 82:S12–S22
28. Wang D, Pedraza PL, Abdullah HI, McGiff JC, Ferreri NR 2002 Calciumsensing receptor-mediated TNF production in medullary thick ascending limb cells. Am J Physiol Renal Physiol 283:F963–F970 [PubMed: 12372772]
29. Ferreri NR, Escalante BA, Zhao Y, An S-J, McGiff JC 1998 Angiotensin II induces TNF production by the thick ascending limb: functional implications. Am J Physiol 274:F148–F155 [PubMed: 9458834]
30. Yokoyama T, Sekiguchi K, Tanaka T, Tomaru K, Arai M, Suzuki T, Nagai R 1999 Angiotensin II and mechanical stretch induce production of tumor necrosis factor in cardiac fibroblasts. Am J Physiol 276:H1968–H1976 [PubMed: 10362677]
31. Fukui M, Nakamura T, Ebihara I, Makita Y, Osada S, Tomino Y, Koide H 1994 Effects of enalapril on endothelin-1 and growth factor gene expression in diabetic rat glomeruli. J Lab Clin Med 123:763–768 [PubMed: 8195682]

32. Kato S, Luyckx CA, Ots M, Lee K-W, Ziai F, Troy JL, Brenner BM, Mackenzie HS 1999 Renin-angiotensin blockage lowers MCP-1 expression in diabetic rats. Kidney Int 56:1037–1048 [PubMed: 10469372]

33. Ferreri NR, Zhao Y, Takizawa H, McGiff JC 1997 Tumor necrosis factor-α-angiotensin interactions and regulation of blood pressure. J Hypertens 15: 1481–1484 [PubMed: 9431855]

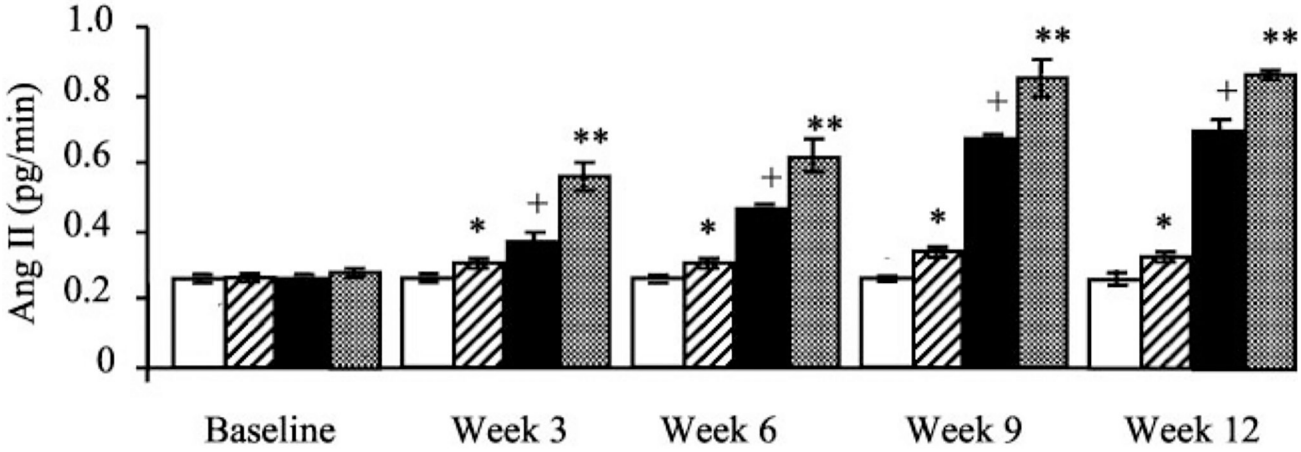

FIG. 1.

Ang II recovery in RIF in normal rats (*white bars*), normal rats treated with valsartan (*cross-hatch bars*), diabetic rats (*black bars*), and diabetic rats treated with valsartan (*gray bars*). Values are mean ± SE (n = 8). +, $P < 0.0001$ *vs.* normal; *, $P < 0.01$ and **, $P < 0.0001$ *vs.* normal and diabetes without treatment. Baseline values were obtained before induction of diabetes with STZ.

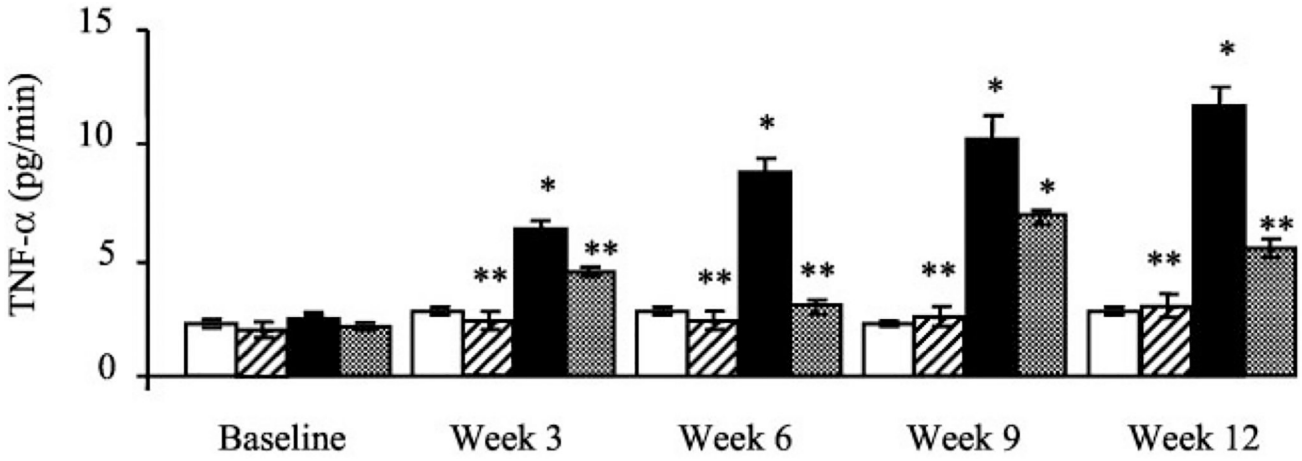

FIG. 2.

TNF-α recovery in RIF in normal rats (*white bars*), normal rats treated with valsartan (*cross-hatch bars*), diabetic rats (*black bars*), and diabetic rats treated with valsartan (*gray bars*). Values are mean ± SE (n = 8). *, $P < 0.0001$ *vs.* normal; **, $P < 0.01$ *vs.* diabetes without treatment. Baseline values were obtained before induction of diabetes with STZ.