Renin Response to 12-Hydroxyeicosatetraenoic Acid Is Increased in Diabetic Rats

Indra Antonipillai, Elke Jost-Vu, Rama Natarajan, Jerry Nadler, and Richard Horton

Eicosanoids (prostaglandins) can alter renin secretion and angiotensin (ANG) II action. We have studied the effects of both prostacyclin and a lipoxygenase (LO) product, 12-hydroxyeicosatetraenoic acid (12-HETE), on renin in normal and streptozotocin-induced diabetic rats. 12-HETE is not only a potent inhibitor of basal renin secretion but also a key mediator of ANG II–induced renin inhibition. We have also examined the effects of ANG II on 12-HETE formation in normal and diabetic animals. Both plasma (3.9 ± 0.9 vs. 0.8 ± 0.1 ng ANG I · ml^{-1} · h^{-1}, $P < 0.01$) and tissue (38 ± 6 vs. 21 ± 2 ng ANG I · mg tissue^{-1} · h^{-1}, $P < 0.05$) renin activity levels were markedly reduced in diabetic animals. Iloprost (10^{-6} mol/l), a stable analog of prostacyclin, had similar stimulatory effects on renin secretion in both normal and diabetic tissues, but the response was enhanced by LO inhibition in diabetic tissue. 12-HETE (10^{-7} mol/l) had an exaggerated effect on renin inhibition in diabetic tissue (78 ± 2% normal vs. 65 ± 4% diabetic, $P < 0.05$). Similarly, ANG II (10^{-8} mol/l) inhibition of renin was significantly enhanced in diabetic rats ($P < 0.001$). However, ANG II did not produce an exaggerated increase in 12-HETE in diabetic renal tissue. Insulin reversed the inhibitory effects of ANG II on renin in normal rats, but it blunted the effect of ANG II in diabetic rats. These studies suggest that, while the capacity of renal cortical tissue to synthesize 12-HETE in response to ANG II is not altered, 12-HETE and ANG II actions are exaggerated in diabetes, and this may contribute to reduced renin production. *Diabetes* 44:321–325, 1995

Vasodilator prostaglandins (PGs) in the cyclooxygenase (CO) pathway play a role in renal blood flow and renin secretion (1,2). Prostacyclin or a metabolite produced in either the vessel wall or juxtaglomerular (JG) cells is a key secretagogue of renin secretion, since blockade of the system alters renin release both in vivo and in vitro (2,3). Arachidonic acid (AA) is also the substrate for another group of enzymes, the lipoxygenases (LOs), which convert AA into 12- and 15-hydroperoxyeicosatetraenoic acids (HPETEs) and their respective hydroxyeicosatetraenoic acids (HETEs) (4). 12-HETE is a major arachidonate LO product in both isolated human and rat glomeruli, and it is produced by vascular smooth muscle and adrenal cells (5–7). We have shown that both CO and LO products of AA play a dual regulatory role in renin secretion with prostacyclin as a renin secretagogue and products of the LO pathway, such as 12-HPETE and 12-HETE, as inhibitors of renin release (8). Angiotensin (ANG) II increases 12-HETE production, and the action of ANG II on renin and aldosterone is blocked by a number of LO inhibitors (7,9).

Several studies suggest that the diabetic state alters the renin–ANG II and PG systems (10). Diabetic vessels generate increased 15-HETE (11). Endothelial and smooth muscle cells cultured under hyperglycemic conditions produce increased amounts of HETEs (12). HETEs are potentially vasoactive and can inhibit both renin and prostacyclin synthesis (8,11). Plasma renin levels are lower in chronic diabetic patients, and a generalized disorder in renin processing with increased prorenin levels is present in diabetic patients with hyporeninemic hypoaldosteronism (10).

Recently, we reported that in rats with streptozotocin (STZ)-induced type I diabetes, ANG II has an exaggerated inhibitory effect on renal renin secretion (13). In the current study, we have explored whether there is an abnormal interaction of renin with these eicosanoids. Herein, we have examined whether decreased levels of renin in STZ-induced type I diabetes are a result of increased 12-HETE formation or action by ANG II or of a reduced responsiveness to prostacyclin.

RESEARCH DESIGN AND METHODS

Studies were conducted in male Sprague-Dawley rats weighing 180–220 g. Diabetes was induced by a single injection of 45 mg/kg STZ, freshly dissolved in citrate buffer (pH 4.5), into the tail vein of rats, as described previously (13). Age- and/or weight-matched control rats were given a similar injection of citrate buffer alone. The rats were maintained on a normal diet and water ad libitum. In general, rats were diabetic within 48 h, as assessed by plasma glucose levels, and they were studied 8 weeks after STZ or buffer treatment.

Renal cortical slices (0.5 mm thick) from normal or diabetic rats were prepared by a previously described method (8,9). Slices (15–30) were incubated in 2 ml standard Krebs-Ringer bicarbonate with glucose (KRBG) medium containing 0.2% fatty acid–free bovine serum albumin (BSA) at 37°C in a shaking water bath for two consecutive 30-min intervals. Each slice was incubated for a 30-min baseline period, after which various agents were added, and response to an agent was observed for the next 30-min period. Thus, each slice served as its own control. After incubations, tissue dry weight was determined.

The standard KRBG medium contained the following (in mmol/l): 120 NaCl; 4.7 KCl; 1.2 $MgSO_4$; 2.5 $CaCl_2$; 1.2 KH_2PO_4; 26.8 $NaHCO_3$; and 10 glucose, pH 7.4. Iloprost, a stable analog of prostacyclin (2K36,374), was a gift from Berlex (Cedar Knolls, NJ). 3-Amino-*m*-trifluoromethyl-phenyl-2-pyrazoline (BW755c) was a gift from Burroughs Wellcome (Beckenham, Kent, U.K.). 12-HETE and baicalein were purchased from

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AA, arachidonic acid; ANG, angiotensin; BSA, bovine serum albumin; BW755c, 3-amino-*m*-trifluoromethyl-phenyl-2-pyrazoline; CO, cyclooxygenase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HPLC, high-performance liquid chromatography; JG, juxtaglomerular; KRBG, Krebs-Ringer bicarbonate with glucose; LO, lipoxygenase; PG, prostaglandin; RIA, radioimmunoassay; STZ, streptozotocin.

Biomol (Philadelphia, PA) and stored at −70°C. Pentex BSA (fatty acid–free, fraction V) was obtained from Miles (Elkhart, IN), and human insulin was from Lilly (Indianapolis, IN). ANG II was obtained from Peninsula (Belmont, CA) and dissolved in KRBG buffer with 0.2% BSA. 12-HETE was dissolved in ethanol and prepared on ice just before use. The final concentration of ethanol in KRBG medium was 0.05%. This concentration of ethanol was also added to control incubations and did not influence renin production. Since the HETEs, BW755c, and baicalein are all sensitive to light, preparation of these agents and the experiments themselves were conducted in the dark to avoid any significant loss of potency (8,9).

Measurement of renin activity. Renin activity in the plasma or renin concentration in the supernatant of the incubations was determined as described previously (8,9). In the incubations, renin release or 12-HETE level during the second 30 min of incubation was expressed as the percentage of basal release in the same slice during the first 30-min period.

Measurement of 12-HETE. 12-HETE was extracted with trace amounts of [^{3}H]12-HETE from the 2-ml incubates of rat renal cortical slices using 500 mg C_{18} Bond Elut minicolumns (Analytichem, Torrance, CA), in accordance with our previously published methods (7,9). A specific radioimmunoassay (RIA) technique was used to quantify 12-HETE (7,9). The sensitivity of the assay was 10 pg/ml, with 7% precision. All control and experimental samples were run in the same assay. A further validation of 12-HETE included the use of an independent antibody provided by Dr. L. Levine (Brandeis University, Waltham, MA), which gave an excellent correlation coefficient ($r = 0.9$). Some extracted samples were further purified by high-performance liquid chromatography (HPLC) using a series 4 high-performance liquid chromatograph (Perkin-Elmer/Cetus, Norwalk, CT) equipped with a C_{18} column (Shandon 25 cm, 3 μm) and an LC-35 ultraviolet detector. A combination of gradient and isocratic elution program was used with the solvents acetonitrile, 0.01% acetic acid (pH 3.7), and methanol, as described previously (7,9). The HPLC-purified samples were then quantified by RIA. The pre- and post-HPLC RIAs gave a good correlation ($r = 0.92$).

Data analysis. All results are expressed as means ± SE. To compare control with experimental values for renin and 12-HETE, analysis of variance with both unpaired Student's t test and Duncan's or Dunnett's multiple-range test (when appropriate) was used to assess the significance.

RESULTS

Plasma glucose levels in the diabetic rats at the time of death were elevated significantly compared with controls (23 ± 3 vs. 8 ± 0.6 mmol/l, $P < 0.001$). During the course of the study, the normal rats gained weight (370 ± 4 g), whereas the diabetic rats did not gain weight (210 ± 7 g). Despite their lack of weight gain overall, the kidney weight of the diabetic rats was twofold higher (4.7 ± 0.3 g) than the kidney weight of the normal rats (2.4 ± 0.2 g). Plasma renin activity and tissue renin concentrations in control and diabetic rats were determined at the time of killing. The plasma renin activity of the diabetic rats (0.8 ± 0.1 ng ANG I · ml^{-1} · h^{-1}) was significantly lower than that of control rats (3.9 ± 0.8 ng ANG I · ml^{-1} · h^{-1}, $n = 5$, $P < 0.01$). Similarly, the basal renal renin concentration per milligram tissue in the diabetic rats was lower than that in control rats (21 ± 2 vs. 38 ± 6 ng ANG I · mg tissue^{-1} · h^{-1}, $n = 8$, $P < 0.05$).

Effects of iloprost (a stable analog of prostacyclin) and 12-HETE on renin release. Renin release from individual cortical slices was relatively stable during two consecutive 30-min periods. In a representative experiment, the mean renin release during the first 30-min incubation was 11.2 ng ANG I · mg tissue^{-1} · h^{-1} (100%) and that released during the second 30-min incubation was 10.4 ng ANG I · mg tissue^{-1} · h^{-1} (93%). However, the absolute levels of renin release exhibit considerable variation between incubations even when corrected for slice weight, as indicated previously by us and by others (9,14,15), but values within the same slice do not greatly differ. This emphasizes the importance of using each slice as its own control. Iloprost (10^{-6} mol/l) produced a significant increase in renal renin secretion in normal rats (100 ± 3 vs. 127 ± 7%, $P < 0.01$) (Fig. 1 and Table 1). In diabetic renal tissue, iloprost caused a similar stimulation of renin secretion (100 ± 5 vs. 126 ± 2%, $P < 0.01$).

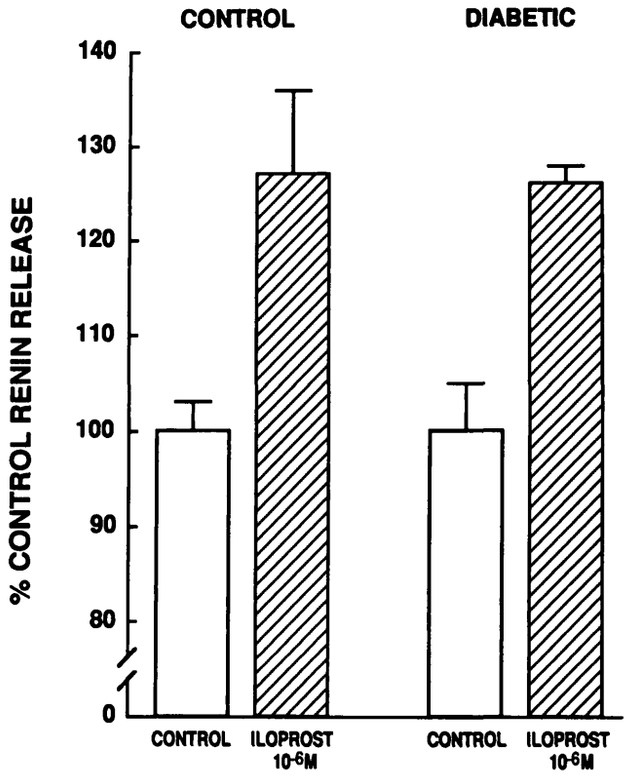

FIG. 1. Effects of iloprost, a stable analog of prostacyclin, on renin secretion by renal cortical slices from control and STZ-induced diabetic rats. Incubations were performed as indicated in the text. The effects of iloprost are expressed as a percentage of baseline at 30 min. Values are means ± SE representing seven to eight experiments. In both control and diabetic rats, iloprost had similar stimulatory effects on renin secretion. $P < 0.01$ compared with respective control values.

12-HETE (10^{-7} mol/l) added to control tissue caused a significant inhibition of renin release (100 ± 3 vs. 78 ± 2%, $P < 0.01$ [51 ± 4 vs. 40 ± 4 ng · mg tissue^{-1} · h^{-1}]). In contrast, the diabetic renal tissue was more sensitive to the action of 12-HETE at the same dose (99 ± 5 vs. 65 ± 4%, $P < 0.05$ [30.3 ± 2.2 vs. 19.8 ± 2 ng · mg tissue^{-1} · h^{-1}]) (Fig. 2).

Effects of LO blockers on basal and iloprost-induced renin release. To examine whether LO blockers would alter basal or iloprost-stimulated renin secretion in diabetes,

TABLE 1
Renin release in control slices and after addition of iloprost

Control renin release (ng/ANG 1 · mg tissue^{-1} · h^{-1})			Iloprost-induced renin release (ng/ANG 1 · mg tissue^{-1} · h^{-1})		
Slice no.	0 min	30 min	Slice no.	0 min	30 min
1	7.2	7.6	9	18.3	20.0
2	18.4	17.7	10	6.9	7.7
3	9.1	9.2	11	8.5	9.7
4	5.3	5.1	12	19.6	28.6
5	25.6	27.1	13	8.9	13.6
6	19.3	15.4	14	5.7	7.2
7	8.4	9.1	15	12.7	16.3
8	11.9	12.5			
Mean ± SE	13.2 ± 2.5	13.0 ± 2.5		11.5 ± 2.1	14.7 ± 2.9*

*$P < 0.02$, compared with respective control values.

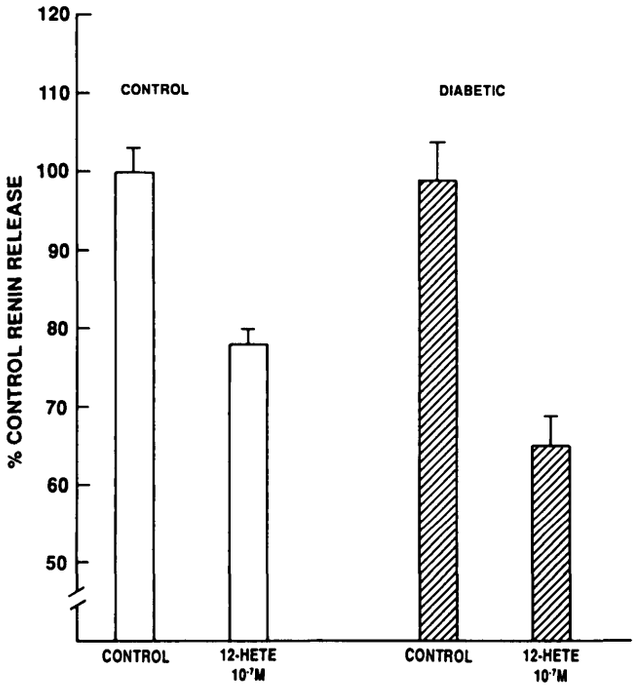

FIG. 2. Effects of 12-HETE (10^{-7} mol/l) on renin secretion by renal cortical slices from control and STZ-induced diabetic rats. Each value represents the mean ± SE of 8–10 experiments. 12-HETE had an exaggerated inhibitory effect on renin secretion in the diabetic rats. $P < 0.05$ vs. 12-HETE in control rats.

slices from normal and diabetic animals were incubated in the presence of two different LO blockers, BW755c (10^{-5} mol/l) and baicalein (10^{-6} mol/l). Neither LO blocker altered basal renin secretion in normal (control, 100 ± 4%; BW755c, 95 ± 4%; baicalein, 99 ± 4%) or diabetic rats (control, 100 ± 3%; BW755c, 98 ± 6%; baicalein, 93 ± 5%). However, both inhibitors significantly increased iloprost-induced renin secretion in diabetic rats (Table 2).

Effects of ANG II on 12-HETE formation and renin secretion. The presence of 12-HETE in renal cortical slices was confirmed by using two different 12-HETE antibodies and by comigration with authentic 12-HETE in the HPLC system. In normal animals, ANG II (10^{-8} mol/l) increased 12-HETE levels to 165 ± 15% of control ($P < 0.05$; control 207 ± 15 vs. ANG II 316 ± 28 pg/mg tissue). Similarly, in diabetic animals, ANG II increased 12-HETE levels (177 ± 33%) (Fig. 3). In contrast, ANG II (10^{-8} mol/l) inhibition of renin secretion was significantly enhanced in diabetic rats (100 ± 5 vs. 58 ± 3% [19 ± 3.6 vs. 11.1 ± 1.9 ng · mg tissue^{-1} · h^{-1}], $P < 0.01$) compared with controls (100 ± 6 vs. 79 ± 3% [47.9 ± 4.7 vs. 38 ± 4.8 ng · mg tissue^{-1} · h^{-1}]) (Fig. 4).

TABLE 2
Effects of LO inhibitors (BW755c and baicalein) on iloprost-induced renin secretion from control and STZ-induced diabetic rats

Agent or vehicle added	Percentage of control resin release	
	Control rats	STZ-induced diabetic rats
Control	100 ± 4	100 ± 3
Iloprost (10^{-6} mol/l)	126 ± 6	129 ± 6
Iloprost + baicalein (10^{-6} mol/l)	119 ± 2	155 ± 8*
Iloprost + baicalein (10^{-6} mol/l)	124 ± 5	156 ± 8*

Data are means ± SE, representing five to six experiments. Incubations were conducted as indicated in METHODS. In diabetic rats, both BW755c and baicalein further potentiated iloprost-induced renin secretion. *$P < 0.05$ vs. iloprost in diabetic rats.

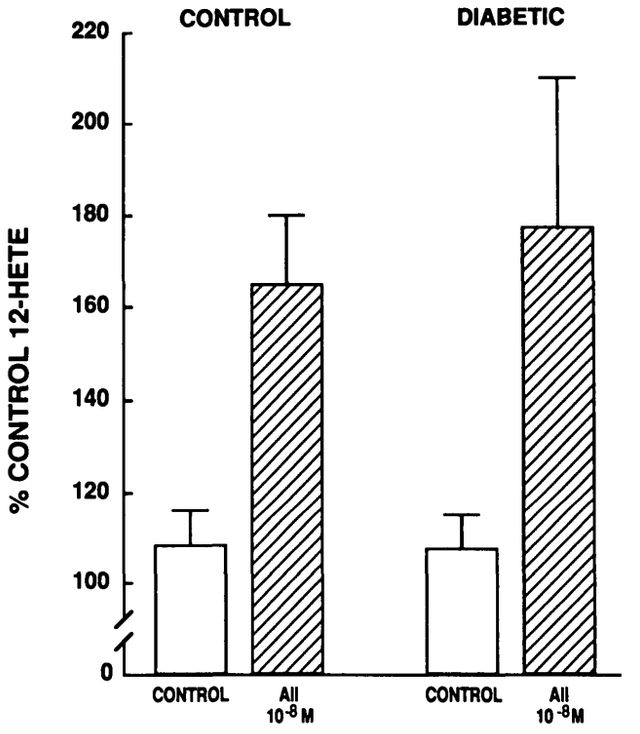

FIG. 3. Effects of ANG II (10^{-8} mol/l) on 12-HETE synthesis by renal cortical slices from control and diabetic animals. The effects of ANG II are expressed as a percentage of baseline. Each value represents the mean ± SE of five to eight experiments. In both control and diabetic rats, ANG II had similar stimulatory effects on 12-HETE synthesis. $P < 0.02$ compared with respective control values.

Effects of insulin on ANG II inhibition of renin in normal and diabetic rats. Insulin at 1.0 mU/ml (7×10^{-9} mol/l) significantly blocked the ANG II inhibition of renin in the control animals (control 100 ± 6%, ANG II 80 ± 3%, ANG II + insulin 112 ± 8%, $P < 0.05$ vs. ANG II). In diabetic

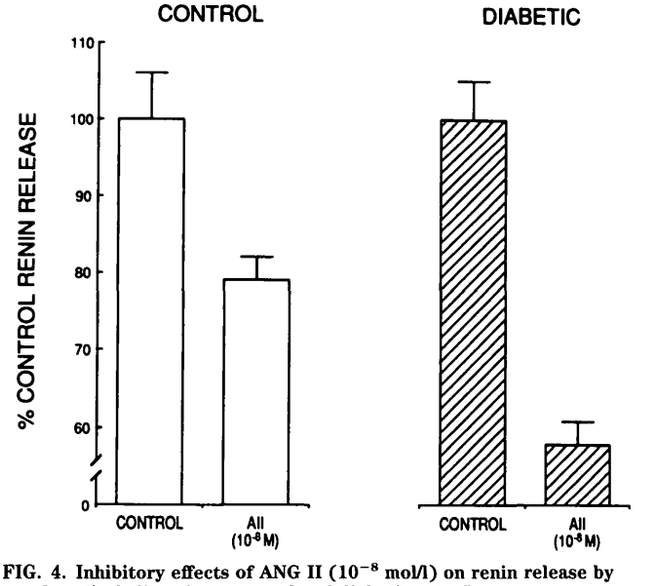

FIG. 4. Inhibitory effects of ANG II (10^{-8} mol/l) on renin release by renal cortical slices from control and diabetic rats. Results are expressed as means ± SE, representing five to eight experiments. Diabetic tissue was more sensitive to the action of ANG II, showing greater inhibition than control tissue. $P < 0.01$ vs. ANG II in control animals.

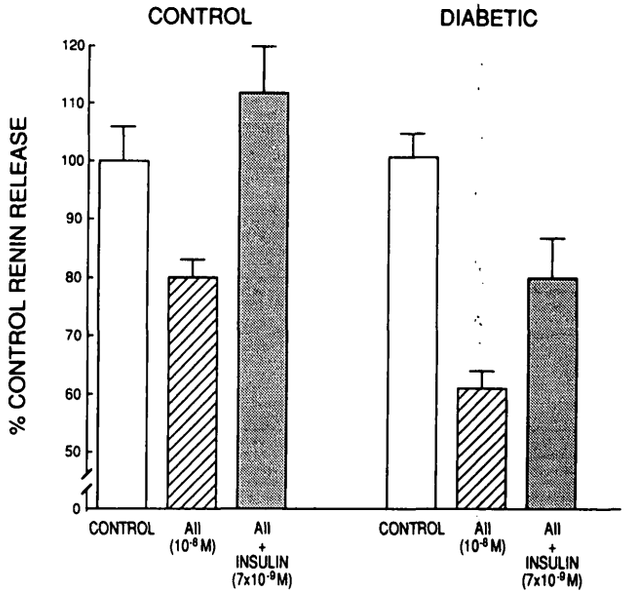

FIG. 5. Effects of insulin on ANG II inhibition of renin secretion in normal and diabetic rats. Results are expressed as means ± SE, representing five to seven experiments. In control rats, insulin significantly blocked the ANG II inhibition of renin secretion. $P < 0.05$ vs. ANG II in control rats. In diabetic rats, insulin blunts the effects of ANG II action on renin. $P < 0.05$ vs. ANG II in diabetic rats.

animals, insulin blunted the effects of ANG II action on renin (control 101 ± 4%, ANG II 61 ± 3%, ANG II + insulin 80 ± 7%, $P < 0.05$ vs. ANG II) (Fig. 5).

DISCUSSION

Altered eicosanoid production is among the many factors implicated in the pathogenesis of diabetic vascular disease. Both diminished vascular PG synthesis and elevated HETEs have been reported (10,11). Prostacyclin and HETEs not only exert opposite effects on renin secretion (2,8), but several studies have suggested that HETEs also inhibit the endogenous production of endothelial and vascular PGs (11,15) and are potent modulators of PG-induced renin release (15,16). It is also known that ANG II stimulates not only renal PG synthesis (17) but also the formation of 12-HETE, which functions as an important mediator of ANG II–induced renin inhibition in kidney JG cells (9,18).

The present studies were undertaken to address two issues. The first was to determine if ANG II increases 12-HETE formation in diabetic kidneys. The second was to see if there is an altered responsiveness to prostacyclin and 12-HETE.

The data presented in these studies indicate that both plasma and tissue renin activity (calculated per milliliter or milligram tissue, respectively) are significantly reduced in diabetic animals. This agrees with previous studies in both diabetic humans and animals (19,20). The kidney weights of our rats were significantly increased as reported in other animal models of diabetes (19,21).

A striking observation in these studies is the increased renin responsiveness to 12-HETE and ANG II. However, there was no difference in the ANG II–induced 12-HETE formation. Hypersensitivity to ANG II has also been noted in the vascular actions of ANG II (20,22,23). A number of observations also indicate that abnormalities in both vasoconstrictor and vasodilator responsiveness occur in both clinical and experimental diabetes.

We considered the possibility that lowered levels of renin or altered responsiveness to 12-HETE/ANG II was due to damage to glomeruli and JG cells in the diabetic animals. However, no pathological findings were noted under light or electron microscopy. Elevations of glucose in the media up to 500 mg/dl (27.8 mmol/l) also do not modify the renin response of normal or diabetic tissue (our unpublished observations). Similarly, STZ at the dose used does not cause detectable renal injury (24), and direct addition of STZ (10^{-6}–10^{-5} mol/l) to renal tissue had no effect on renin release.

Since diabetic renal tissue was more sensitive to the action of 12-HETE, we also examined whether LO inhibitors would alter basal or iloprost-mediated renin in diabetes. The basal level of renin did not increase with LO inhibition in diabetes. This may be the result of comparing the chronic low-renin state over an 8-week period with ex vivo exposure to LO inhibition for only 30 min. In contrast, LO inhibition enhanced the stimulatory response of iloprost. This observation provides further support for an imbalance between the effect on the CO versus LO pathway in the diabetic state.

Previous studies have shown that 12-HETE plays a key role in mediating several actions of ANG II, including renin and aldosterone secretion and vasopressor actions (7,9,25). Natarajan et al. (26) have recently shown that in porcine vascular smooth muscle cells, ANG II specifically increases 12-HETE concentrations when exposed to high glucose levels, such as those associated with diabetes. Under these conditions, ANG II also increases 12-LO mRNA and protein expression. Brown et al. (27) have similarly shown that in porcine aortic endothelial cells, elevated glucose induces increases in agonist (bradykinin, thrombin)-mediated 15-HETE formation. Others have shown decreased ANG II–induced PG release in the kidneys of diabetic rats (28). In diabetic patients with hyporeninemic hypoaldosteronism, a calcium gluconate infusion (which is a stimulus for prostacyclin production in normal subjects) does not increase urinary prostacyclin but significantly increases urinary 12-HETE levels in these patients (29). However, in the current study there was no evidence to support an additional increase in 12-HETE formation by ANG II in the diabetic renal tissue. It is possible, however, that other LO products may be involved in ANG II–induced LO action in the kidney. Our studies are in agreement with reports by Schambelan et al. (30), who were unable to show increases in 12- and 15-HETEs in the glomeruli of STZ-induced diabetic rats after incubation with labeled AA (30).

Rat renal cortical slices are not homogeneous, and although only JG cells release renin, it is possible that many cell types, such as tubular, vascular, and interstitial cells, could also contribute to the 12-HETE released into the medium. However, we have shown that ANG II–induced increases in 12-HETE levels are similar both in the isolated JG cells and in renal cortical slices (9).

Insulin is known to modulate ANG II action in isolated femoral vessels and inhibit the response to norepinephrine and ANG II. In diabetic rats, insulin partially normalizes renin levels and pressor responsiveness to ANG II (19). In the present studies, nanomolar concentrations of insulin reversed the inhibitory effects of ANG II on renin in the normal rats. ANG II action on renin was enhanced in diabetic rats and insulin partially reversed the exaggerated action of ANG II on renin.

The cellular mechanisms whereby ANG II or 12-HETE produces an enhanced renin inhibition are not clear. Both ANG II and 12-HETE actions are expressed through the calcium-protein kinase C messenger systems (9,15,26), and there is some evidence of increased intracellular calcium protein kinase C in diabetic renal vascular tissues (13,31). Therefore, the altered regulation of renin could be due to changes in these second messengers in diabetes.

In conclusion, our study demonstrates that the capacity of renal cortical tissue to synthesize 12-HETE in response to ANG II is not altered in diabetes. However, altered responsiveness to ANG II and 12-HETE may contribute to reduced renin secretion.

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REFERENCES

1. Larsson C, Anggard E: Increased juxtamedullary blood flow on stimulation of intrarenal prostaglandin biosynthesis. *Eur J Pharmacol* 25:326–331, 1974
2. Whorton A, Misono K, Hollifield J, Frolich J, Inagami R, Oates J: Prostaglandin and renin release: stimulation of renin release from rabbit renal cortical slices by PGI_2. *Prostaglandins* 14:1095–1104, 1977
3. Terragno N, Early J, Roberts M, McGiff J: Endogenous prostaglandin synthesis inhibitor effects on production of prostacyclin by renal blood vessels. *Clin Sci Mol Biol* 55 (Suppl. 4):199–202, 1978
4. Schlondorff D, Ardaillou R: Prostaglandins and other arachidonic acid metabolites in the kidney. *Kidney Int* 29:108–119, 1986
5. Sraer J, Rigaud M, Bens H, Rabinovitch H, Ardaillou R: Metabolism of arachidonic acid via the lipoxygenase pathway in human and murine glomeruli. *J Biol Chem* 258:4325–4330, 1983
6. Larruc J, Rigaud M, Razaka G, Daret D, Desmond-Itenri J, Bricaud H: Formation of monohydroxyeicosatetraenoic acids from arachidonic acid by cultured rabbit aortic smooth muscle cells. *Biochem Biophys Res Commun* 112:242–249, 1983
7. Nadler JL, Natarajan R, Stern N: Specific action of the lipoxygenase pathway in mediating angiotensin II–induced aldosterone synthesis in isolated adrenal glomerulosa cells. *J Clin Invest* 80:1763–1769, 1987
8. Antonipillai I, Nadler J, Robin EC, Horton R: The inhibitory role of 12- and 15-lipoxygenase products on renin release. *Hypertension* 10:61–66, 1987
9. Antonipillai I, Horton R, Natarajan R, Nadler J: A 12-lipoxygenase product of arachidonate metabolism is involved in angiotensin action on renin release. *Endocrinology* 125:2028–2034, 1989
10. Nadler JL, Lee FO, Hsueh W, Horton R: Evidence of prostacyclin deficiency in the syndrome of hyporeninemic hypoaldosteronism. *N Engl J Med* 314:1015–1020, 1986
11. Yamajasetty BN, Stuart MJ: 15-Hydroxy-5,8,11,13-eicosatetraenoic acid inhibits human vascular cyclooxygenase: potential role in diabetic vascular disease. *J Clin Invest* 77:202–211, 1986
12. Natarajan R, Gonzales N, Xu L, Nadler J: Vascular smooth muscle cells exhibit increased growth response in response to elevated glucose. *Biochem Biophys Res Commun* 187:552–560, 1992
13. Jost-Vu E, Horton R, Antonipillai I: Altered regulation of renin secretion by insulin-like growth factors and angiotensin II in diabetic rats. *Diabetes* 41:1100–1105, 1992
14. Naftilan AJ, Oparil S: The role of calcium in the control of renin release. *Hypertension* 4:670–675, 1982
15. Antonipillai I: 12-Lipoxygenase products are potent inhibitors of prostacyclin-induced renin release. *Proc Soc Exp Biol Med* 194:224–230, 1990
16. Chansel D, Bea ML, Ardaillou R: Modulation of renin synthesis by lipoxygenase products in cultured human mesangial cells. *Mol Cell Endocrinol* 62:263–271, 1989
17. McGiff JC, Crowshaw K, Terragno HA, Lonigro AJ: Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Cir Res* 26 (Suppl. 1):I125–I130, 1970
18. Antonipillai I, Nadler J, Horton R: Angiotensin feedback inhibition of renin is expressed via the lipoxygenase pathway. *Endocrinology* 122: 1277–1281, 1988
19. Christlieb AR: Renin, angiotensin and norepinephrine in alloxan diabetes. *Diabetes* 23:962–970, 1974
20. Christlieb AR: Vascular reactivity to angiotensin II and norepinephrine in diabetic subjects. *Diabetes* 25:268–274, 1976
21. Hostetter TH, Troy JL, Brown BM: Glomerular hemodynamics in experimental diabetes mellitus. *Kidney Int* 19:410–415, 1981
22. Weidman P, Beretta-Piccoli C, Trost BN: Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. *Hypertension* 7: (Suppl. II)II33–II42, 1985
23. Hill MA, Larkins RG: Altered microvascular reactivity in streptozotocin-induced diabetes in rats. *Am J Physiol* 257:H1438–H1445, 1989
24. Evan AP, Mong SA, Gattone VH, Connors BA, Aronoff GA, Luff FC: The effect of streptozotocin and streptozotocin-induced diabetes on the kidney. *Renal Physiol* 7:78–89, 1984
25. Stern N, Golub M, Nozawa K, Berger M, Knoll E, Yanagawa N, Natarajan R, Nadler JL, Tuck ML: Selective inhibition of angiotensin II–mediated vasoconstriction by lipoxygenase blockade. *Am J Physiol* 257:H434–H443, 1989
26. Natarajan R, Jia-Li G, Rossi J, Gonzales N, Lanting L, Xu L, Nadler J: Elevated glucose and angiotensin II increases 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. *Proc Natl Acad Sci USA* 90:4947–4951, 1993
27. Brown ML, Jakubowski JA, Leventis LL, Deykin D: Elevated glucose alters eicosanoid release from porcine aortic endothelial cells. *J Clin Invest* 82:2136–2141, 1988
28. Sarubbi D, McGiff JC, Guilley J: Renal vascular responses and eicosanoid release in diabetic rats. *Am J Physiol* 257:F762–F768, 1989
29. Antonipillai I, Jost-Vu E, Horton R, Nadler J: Altered production of 12-lipoxygenase product, 12-hydroxyeicosatetraenoic acid in diabetes (Abstract). In *The 8th International Conference on Prostaglandins and Related Compounds, Montreal, Canada, July 26–31, 1992.* Milan, Italy, Fondazione Giovanni Lorenzini Medical Science Foundation, A315.
30. Schambelan M, Blake S, Sraer J, Bens M, Nivez MP, Wahbe F: Increased prostaglandin production by glomeruli isolated from rats with streptozotocin-induced diabetes mellitus. *J Clin Invest* 75:404–412, 1985
31. DeRubertis FR, Craven PA: Activation of protein kinase C in glomerular cells in diabetes: mechanisms and potential links to the pathogenesis of diabetic glomerulopathy. *Diabetes* 43:1–8, 1994