Endothelin A Receptor Blockade Reduces Diabetic Renal Injury via an Anti-Inflammatory Mechanism

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Endothelin (ET) receptor blockade delays the progression of diabetic nephropathy; however, the mechanism of this protection is unknown. Therefore, the aim of this study was to test the hypothesis that ETA receptor blockade attenuates superoxide production and inflammation in the kidney of diabetic rats. Diabetes was induced by streptozotocin (diabetic rats with partial insulin replacement to maintain modest hyperglycemia [HG]), and sham rats received vehicle treatments. Some rats also received the ETA antagonist ABT-627 (sham+ABT and HG+ABT; 5 mg/kg per d; n = 8 to 10/group). During the 10-wk study, urinary microalbumin was increased in HG rats, and this effect was prevented by ETA receptor blockade. Indices of oxidative stress, urinary excretion of thiobarbituric acid reactive substances, 8-hydroxy-2-deoxyguanosine, and H2O2 and plasma thiobarbituric acid reactive substances were significantly greater in HG rats than in sham rats. These effects were not prevented by ABT-627. In addition, renal cortical expression of 8-hydroxy-2-deoxyguanosine and NADPH oxidase subunits was not different between HG and HG+ABT rats. ETA receptor blockade attenuated increases in macrophage infiltration and urinary excretion of TGF-β and prostaglandin E2 metabolites in HG rats. Although ABT-627 did not alleviate oxidative stress in HG rats, inflammation and production of inflammatory mediators were reduced in association with prevention of microalbuminuria. These observations indicate that ETA receptor activation mediates renal inflammation and TGF-β production in diabetes and are consistent with the postulate that ETA blockade slows progression of diabetic nephropathy via an anti-inflammatory mechanism.


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macrophage infiltration in the renal cortex in nondiabetic nephropathies (26) and attenuates the increase in TGF-β mRNA in glomeruli from diabetic rats (27).

Because ET-1 stimulates the production of ROS and inflammatory mediators, we proposed that enhanced activation of the ETA receptor promotes renal injury in type 1 diabetes via increased production of ROS and inflammatory mediators. Therefore, the aim of this study was to test the hypothesis that ETA receptor blockade attenuates oxidative stress and inflammation in the kidney of diabetic rats.

**Materials and Methods**

**Animal Model**

All experiments were performed using male Sprague-Dawley rats (200 to 250 g; Harlan Laboratories, Indianapolis, IN) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the Medical College of Georgia Institutional Animal Care and Use Committee. Blood glucose levels were measured (Accu-Check III model 766; Boehringer Mannheim, Indianapolis, IN), and each rat was weighed and assigned randomly to one of four groups: Normal rats that received vehicle treatments (sham), diabetic rats with partial insulin replacement to maintain moderate hyperglycemia (HG), sham rats that received the ETA receptor antagonist ABT-627 (sham + ABT), and HG rats that received ABT-627 (HG+ABT). ABT-627 (5 mg/kg per d) was administered via the drinking water to facilitate continuous administration (28). The concentration of ABT-627 in the water was adjusted weekly to maintain appropriate dosing and to account for the increased water intake in STZ-treated rats. ABT-627 has 1000-fold selectivity for the ETA receptor over ETB receptors (28).

Rats were anesthetized on day 0 with isoflurane (IsoFlo; Abbott Laboratories, North Chicago, IL) to facilitate intravenous injection of either 65 mg/kg STZ (Sigma Chemical, St. Louis, MO; HG and HG+ABT rats) or vehicle sham + ABT rats. The next day, blood glucose levels were measured and the rats were anesthetized to facilitate subcutaneous insertion of a 2.3 × 2.0-mm sustained-release insulin implant (HG and HG+ABT rats; Linshin Canada, Scarborough, ON, Canada) or a 2.3 × 2.0-mm palmitic acid implant (vehicle; sham and sham + ABT rats). Blood glucose and body weight were measured at 3- to 4-d intervals for 10 wk. Periodically, rats were placed in metabolic cages for 24-h urine collection and food and water intake measurements. After 10 wk, rats were anesthetized with pentobarbital sodium (Nembutal, 65 mg/kg intraperitoneally; Abbott Laboratories), and blood and kidneys were harvested.

**Telemetric Measurements**

Rats were anesthetized with pentobarbital sodium anesthesia, and telemetry transmitters (Data Sciences, St. Paul, MN) were implanted in the abdominal aorta according to the manufacturer’s specifications. Rats were allowed to recover from surgery and returned to individual housing for 1 wk before initiation of data acquisition. Arterial pressure and heart rate (HR) waveforms were recorded continuously for 10 s every 10 min.

**Immunoblotting**

The renal cortex was pulverized while frozen and placed in ice-cold homogenization buffer (vol/wt ratio 10; 50 mmol/L Tris-HCl [pH 7.4], 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 250 mmol/L sucrose, 0.1% BME, and 10% glycerol) in the presence of protease inhibitors (1 mmol/L PMSF, 2 μmol/L leupeptin, 1 μmol/L pepstatin A, and 0.1% aprotinin) and homogenized on ice with a glass-Teflon homogenizer. Protein concentrations were determined by standard Bradford assay, and Western blotting was performed as described previously (7). Bands were detected, and densitometric analysis was performed with the Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE). Primary antibodies included rabbit polyclonal antibodies for cyclooxygenase-2 (COX-2; Cayman Chemical, Ann Arbor, MI) and the NADPH oxidase subunits p22phox, p47phox, and p67phox (Santa Cruz Biotechnology, Santa Cruz, CA). Equal protein loading was verified by probing for β-actin (Sigma Chemicals).

**Immuno histochemical Analysis**

Kidneys were perfused with 4% paraformaldehyde in 100 mmol/L dibasic sodium phosphate buffer and immersed in 4% paraformaldehyde solution overnight at room temperature, transferred to 70% ethanol for 24 h, and paraffin embedded. The kidneys were sectioned at a thickness of 4 μm onto Superfrost plus slides and processed as described previously (7). Slides were incubated in the absence or presence of primary antibodies to COX-2 (Cayman Chemical), 8-hydroxy-2′-deoxyguanosine (8-OHdG; Oxis International, Portland, OR), or ED-1 (CD68; Serotec, Kidlington, Oxford, UK) in humidity chambers overnight at 4°C, followed by incubation with peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) or goat anti-mouse IgG (Serotec) for 30 min at room temperature. To assess renal injury, kidney sections were stained using a periodic acid-Schiff stain kit, Gomori’s Trichrome Stain Kit, blue collagen (Richard Allen Scientific, Kalamazoo, MI), or Picro-Sirius Red according to standard methods (29). The stained sections were viewed with an Olympus BX40 microscope (Olympus America, Melville, NY) on bright-field setting fitted with a digital camera (Olympus DP12; Olympus America). For semiquantitative evaluation, Dr. Yamamoto examined histologic sections in a blind manner for mesangial proliferation, mesangial expansion, interstitial mononuclear cell infiltration, and arteriolar thickening. For quantification of ED-1, positive cells were counted in 425 × 320-mm sections of kidney cortex (magnification ×40).

**Urine and Plasma Assays**

Plasma and urine creatinine concentrations were measured by the picric acid method adapted for microtiter plates (30). Creatinine and picric acid were obtained from Sigma Chemical Co. Urinary and plasma immunoreactive ET concentrations were measured by RIA (Amersham Pharmacia Biotech, Arlington Heights, IL) and chemiluminescent immunoassay (R&D Systems, Minneapolis, MN), respectively. Plasma and urinary measures of oxidative stress were measured using an OXiTek thiobarbituric acid reactive substances (TBARS) assay kit (ZeoToMetrix, Buffalo, NY), an Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes, Eugene, OR), and a BIOXYTECH 8-hydroxy-2′-deoxyguanosine (8-OHdG)-EIA Kit (Oxis International). Urinary concentrations of TGF-β1 were determined by a TGF-β1 Max Immunoassay System (Promega Corp., Madison, WI). Urinary concentrations of albumin and the metabolites of prostaglandin E2 (PGE2), prostacyclin (6-keto-prostaglandin F1α [6-keto-]), and thromboxane A2 (TxB2) were measured using enzyme immunoassay kits from Cayman Chemical.

**Statistical Analyses**

Values are expressed as mean ± SEM. P < 0.05 was considered significant. The significance of differences between groups for BP data were evaluated with an ANOVA for repeated measures, followed by Duncan multiple-range post hoc tests. Between-group comparisons for
other measurements were performed using an ANOVA combined with post hoc contrasts (Statistica; StatSoft, Tulsa, OK).

**Results**

**Characteristics of Experimental Animals**

On day 0, the rats weighed 302 ± 2 g and had blood glucose levels that averaged 92 ± 2 mg/dl (n = 40). Twenty-four hours after STZ injection, blood glucose levels were elevated in HG and HG + ABT rats (465 ± 14 mg/dl; n = 24), confirming the onset of diabetes. As shown in Table 1, blood glucose levels were elevated in HG and HG + ABT rats to a similar degree, and blood glucose levels of sham rats were not changed by treatment with ABT-627. Relative to sham and sham + ABT rats, rats in the HG and HG + ABT groups gained less weight during the study period and displayed renal hypertrophy, hyperphagia, polypsisia, polyuria, and glomerular hyperfiltration (as estimated by creatinine clearance). None of these parameters differed significantly between HG and HG + ABT rats. Plasma ET-1 concentration was increased by ABT-627 treatment only in HG rats. Hyperglycemia increased urinary ET-1 excretion, and this was unaffected by ABT-627 treatment (Figure 1).

**BP and HR Measurements**

Figure 2 illustrates mean arterial pressure (MAP) and HR in sham, sham + ABT, HG, and HG + ABT rats during a 5-d baseline period and the 10-wk treatment period. ABT-627 significantly reduced MAP throughout the treatment period in sham and HG rats (P < 0.05) with no effect on HR. Hyperglycemia had no effect on MAP or HR for the first 7 wk of treatment, but MAP and HR were decreased in the HG group during the last 2 to 3 wk of treatment (P < 0.05). The sham group maintained a constant MAP throughout the 10-wk period.

**Measures of Renal Injury**

HG rats exhibit increased microalbumin excretion, compared with sham rats, as early as 2 wk after STZ injection, and this effect was sustained during the entire 10 wk of the study. ABT-627 treatment prevented microalbuminuria in diabetic rats (Figure 1). As shown in Figure 3, histologic examination using periodic acid-Schiff staining (basement membranes, glycoproteins), Gomori’s trichrome staining (collagen), and Picro-Sirius Red staining (connective tissue and fibers) revealed no remarkable changes in kidney structure of HG rats compared with sham rats or ABT-627–treated HG rats after 10 wk of diabetes.

**Measures of Oxidative Stress**

Urinary excretion of hydrogen peroxide and TBARS was elevated significantly after only 2 wk of hyperglycemia and remained at these levels for the duration of the study (Figure 4). Moreover, plasma TBARS and urinary excretion of 8-OHdG (measured only at the end of the 10-wk study) were significantly increased in HG rats compared with sham rats. ETA receptor blockade did not prevent the hyperglycemia-induced increase in any of these indices of oxidative stress. In addition, neither immunohistochemical staining of 8-OHdG nor expression of the NADPH oxidase subunits p22phox, p47phox, or p67phox was altered by ABT-627 treatment in the renal cortex of HG rats (Figure 4).

**Analysis of Inflammatory and Profibrotic Markers**

In the cortex of HG rats, the number of cells that stained positive for ED-1, a macrophage marker, was significantly greater than in sham rats (Figure 5). ABT-627 treatment reduced the hyperglycemia-induced increase in the number of ED-1–positive cells. Urinary excretion of TGF-β was increased in HG rats and ABT-627–treated HG rats compared with sham rats (Figure 5) during the early part of the study (2 to 6 wk). After 10 wk, there was a further increase in TGF-β excretion in the HG group, and ABT-627 attenuated this increase.

**Urinary Analysis of Prostanoid Production**

Urinary excretion rates were determined for PGEM, 6-keto, and TxB2. PGEM is a measure of PGE2 production. 6-keto is

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**Table 1.** Characteristics of sham, sham + ABT, HG, and HG + ABT rats after 10 wk of treatment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sham</th>
<th>Sham + ABT</th>
<th>HG</th>
<th>HG + ABT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>415 ± 12</td>
<td>434 ± 6</td>
<td>331 ± 7b</td>
<td>331 ± 7b</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.4 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.9 ± 0.1b</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Kidney:body weight (mg/g)</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.4</td>
<td>5.6 ± 0.3b</td>
<td>4.9 ± 0.2b</td>
</tr>
<tr>
<td>Blood glucose; mg/dl</td>
<td>82 ± 1</td>
<td>93 ± 2</td>
<td>461 ± 16b</td>
<td>454 ± 11b</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>14 ± 2</td>
<td>18 ± 1</td>
<td>38 ± 1b</td>
<td>38 ± 1b</td>
</tr>
<tr>
<td>Water intake (ml/d)</td>
<td>25 ± 2</td>
<td>27 ± 3</td>
<td>180 ± 12b</td>
<td>167 ± 16b</td>
</tr>
<tr>
<td>Urine flow (ml/d)</td>
<td>14 ± 1</td>
<td>18 ± 2</td>
<td>158 ± 10b</td>
<td>131 ± 17b</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.5 ± 0.1b</td>
<td>1.3 ± 0.1b</td>
</tr>
<tr>
<td>Plasma (ET-1; pg/ml)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.7 ± 0.2b,c</td>
</tr>
</tbody>
</table>

Excretory data were derived from 24-h urine collections in metabolic cages within 48 h of the rats’ being killed. Plasma samples were obtained under anesthesia immediately before the rats were killed. Data are means ± SEM (n = 6 to 11). ET-1, endothelin-1; HG, diabetic rats with partial insulin replacement to maintain modest hyperglycemia; HG + ABT, HG rats that were treated with ABT-627; sham + ABT, sham rats that were treated with ABT-627.

bP < 0.05 versus sham.

cP < 0.05 versus HG.
formed by the hydration of prostacyclin and is an estimate of prostacyclin production. TxA₂ is hydrolyzed rapidly to TxB₂.

Because of its transient nature, TxB₂ measurements reflect renal TxA₂ production (31). Urinary excretion of all three prostanoid metabolites was greater in HG rats compared with sham rats (*P < 0.01; Figure 6), with the increase in PGEM excretion evident beginning at the 2-wk time point. There were no differences in urinary excretion of 6-keto or TxB₂ between HG rats and HG rats that were treated with the ETₐ receptor antagonist; however, ABT-627 prevented the further rise in PGEM excretion that was observed in HG rats at the end of the 10-wk study (*P = 0.001). ABT-627 had no effect on prostanoid excretion in sham rats.

Expression of Cortical COX-2

Although there was a trend for higher expression of COX-2 in the cortex of HG rats, ABT-627 had no effect on cortical COX-2 expression in hyperglycemic animals (Figure 7). Fur-
thermore, immunohistochemical analysis revealed no difference in the localization or expression of COX-2 in the renal cortex of any group (Figure 7, C through F).

Discussion

In experimental models of diabetes, ET\(_A\) receptor antagonists as well as combined ET\(_A/ET_B\) receptor antagonists have been shown to delay the progression of diabetic nephropathy (1–3). Although the protective effects of ET receptor blockade were demonstrated previously, the mechanism of this protection is unknown. Therefore, the purpose of this study was to determine whether enhanced activation of the ET\(_A\) receptor promotes renal injury in STZ-induced diabetes via increased production of ROS and inflammatory mediators. Ten weeks after the induction of diabetes, HG rats exhibited glomerular hyperfiltration, as estimated by creatinine clearance, and had de-
creased BP compared with sham rats. These animals also exhibited increased urinary excretion of ET-1 but no change in plasma levels, observations that together indicate increased renal ET-1 production in diabetes. Blockade of the ETA receptor prevented the development of albuminuria in this model of type 1 diabetes, although no major structural alterations were evident in the kidneys of the HG rats at this relatively early stage of diabetes. Oxidative stress was increased in the diabetic rats; however, the production of ROS was not attenuated by treatment with the ETA receptor antagonist ABT-627. In contrast, ETA receptor blockade attenuated the macrophage infiltration and blunted the increase in urinary excretion of TGF-β and PGEM in diabetic rats. These data implicate ETA receptor activation in the renal inflammatory and profibrotic but not pro-oxidant events that arise during type 1 diabetes.

Inflammation plays a key role in the pathogenesis of diabetic nephropathy. Our data indicate that macrophage infiltration is increased in the renal cortex of diabetic rats and attenuated by treatment with ABT-627. This is consistent with reports that ETA receptor blockade suppresses macrophage infiltration in the renal cortex of rats with aldosterone-induced hypertension (25) and hypokalemia-induced renal tubulointerstitial injury (26). Previous studies have shown that macrophage infiltration precedes development of structural changes in the diabetic kidney (15,16). In a nondiabetic model of ET-dependent kidney fibrosis, an increase in the density of CD4-positive lymphocytes and macrophages was observed in the kidney well before the onset of fibrosis (32). These data and our own support the theory that chronic renal inflammation is one of the first detectable changes that can be observed during the progression of renal injury. ABT-627 treatment of sham rats also decreased ED-1–positive cells in the renal cortex; however, it is not clear whether the mechanism is similar in sham and hyperglycemic rats. Although renal hypertrophy was evident in HG rats, we did not observe significant structural changes in the kidney during this study, although some previous reports have shown glomerular enlargement, matrix expansion, interstitial fibrosis, and arteriolopathy during early diabetes in the STZ model (33–36). These differences may be due to the strain or gender of rats used or the use of insulin to maintain moderate hyperglycemia.

In addition, we found that urinary excretion of TGF-β, a profibrotic cytokine that is implicated in the development of diabetic nephropathy, is attenuated by blockade of the ETA receptor. This is consistent with the work of Nakamura et al. (21), which demonstrated that treatment with FR139317, another selective ETA receptor antagonist, reduced TGF-β mRNA in the glomeruli of diabetic rats and supports a role of ETA receptor activation in the progression of renal injury that is associated with hyperglycemia. The source of TGF-β in our experiments currently is unknown. However, it is known that the activation of macrophages stimulates TGF-β secretion (37).

Figure 3. Representative images of renal histology of sham (A, D, and G), HG (B, E, and H), and HG+ABT (C, F, and I) rats after 10 wk of treatment. Periodic acid-Schiff stain (A, B, and C), Gomori’s trichrome stain (D, E, and F), and Picro-Sirius Red staining (G, H, and I). Magnification, ×40.
It is possible that ET-1 stimulates the renal recruitment of macrophages that, in turn, promote the release of TGF-β. Such a mechanism may account for the attenuation of TGF-β excretion by ABT-627 in our studies.

PGE₂ is an important regulator of vascular tone, glomerular filtration, renin release, and sodium transport in the kidney (38). Inhibition of COX-2 (39) or the PGE receptor EP-1 (40) prevents proteinuria and reduces renal injury in diabetes. Furthermore, in genetically hypertensive rats, the PGE₂/EP-1 signaling pathway has been shown to play an important role in the development of renal injury (41). Therefore, our study tested the hypothesis that the beneficial effects of ETA receptor blockade are due in part to a reduction in prostanoid production. We found that excretion of PGEM was reduced in diabetic rats that were treated with ABT-627, although excretion of prostacyclin and thromboxane metabolites was unchanged. Our data suggest that ETA receptor activation contributes to PGE₂ production in diabetic rats. ET-1 can regulate the renal production of PGE₂ by influencing the expression of the enzymes that are responsible for the synthesis of PGE₂ in the kidney, namely COX-2 and PGE synthase. Although activation of ETA receptors results in stimulation of COX-2 to produce PGE₂ in renal mesangial cells (42), our results suggest that the decrease in PGE₂ excretion during ETA receptor blockade in diabetes is not due to a reduction of COX-2 expression in the renal cortex. Alternatively, macrophages can release PGE₂ in response to inflammatory signals (43,44) and can also stimulate PGE₂ release from neighboring fibroblasts (45). Therefore, it is possible that blockade of ETA receptors could reduce PGE₂ production in diabetic rats by reducing inflammation and macrophage recruitment in the kidney.

Our finding that BP and HR are decreased in HG rats compared with sham rats is consistent with previous studies in STZ-induced diabetic rats (46–48). These changes may reflect the decline in cardiac function that arises during this stage of the disease (49). In contrast, some groups have reported no change (50,51) or an increase in BP in diabetic rats (52). It is possible that the discrepancies in these studies may be due to differences in the severity or the duration of diabetes, use of anesthetics, or method of BP measurement. In any case, the diabetes-induced alterations in these cardiovascular parameters in our study were unaffected by ABT-627 and, therefore, seem to arise independent of ETA receptor activation.

Based on findings that ETA receptor activation stimulates NADPH oxidase to produce superoxide (10,13,53) and that the NADPH oxidase inhibitor apocynin prevents proteinuria and reduces renal injury in diabetes (8), we proposed that blockade of ETA receptors would reduce oxidative stress in the kidney of diabetic rats and therefore reduce renal injury. To address this postulate, we measured lipid peroxidation, hydrogen peroxide excretion, DNA damage, and expression of NADPH oxidase subunits as indices of oxidative stress. Although widely used and accepted, these are indirect measures of ROS production that do not reveal the sites of ROS production or action and have significant limitations. The TBARS assay may overestimate malondialdehyde (MDA) levels in the urine as a result of...
cross-reactivity with other aldehydes, sugars, and amino acids (54). It is not clear whether urinary excretion of MDA, hydrogen peroxide, and 8-OHdG reflects renal or systemic production of ROS. Therefore, oxidative stress in the renal cortex was targeted by immunostaining for 8-OHdG (an indicator of oxidative DNA damage) and Western blot analysis of NADPH oxidase subunits in renal cortical homogenates. Increased plasma, urinary, or tissue 8-isoprostane levels also are a widely accepted measure of oxidative stress. We did not use this analysis because our data also demonstrated increased prostaglandin metabolites that were associated with induction of diabetes; therefore, an increase in 8-isoprostane levels may reflect an increase in substrate for oxidation rather than an increase in ROS production. Although it is possible that ET_A

Figure 5. Profibrotic and inflammatory markers in sham, sham + ABT, HG, and HG + ABT rats. (A) Urinary excretion of TGF-β. (B) Quantification of ED-1⁺ cells in the renal cortex. Data are means ± SEM. *P < 0.05 versus sham; †P < 0.05 versus HG + ABT (n = 4 to 5).
receptor blockade had localized effects that were not detected by the aforementioned analyses, our data revealed no effect of ABT-627 on these systemic or renal measures of oxidative stress in STZ-induced diabetic rats, even though it attenuated the

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**Figure 6.** Prostaglandin excretion by sham, sham+ABT, HG, and HG+ABT rats. (A) Time course of prostaglandin E2 metabolite (PGEM) excretion. (B) 6-Keto PGF₁α and thromboxane A₂ (TxB₂) excretion at the 10-wk time point. Data are means ± SEM. *P < 0.05 versus sham; †P < 0.05 versus HG (n = 4 to 5).
development of albuminuria. These observations refute the postulate that oxidative stress results from ET_A receptor activation in diabetes. Although ET_A receptor blockade reduces oxidative stress in ET-1–dependent models of hypertension, it may not be effective in diabetes because hyperglycemia can generate ROS through multiple pathways (55). Alternatively, it is possible that ET-1 enhances oxidative stress via activation of ET_B receptors in the diabetic rat. ET-1 stimulates the production of ROS in endothelial cells (56) and in the rat aorta (53) via an ET_B receptor–dependent mechanism. We also cannot exclude the possibility that hyperglycemia-induced renal oxidative stress in diabetes provides the stimulus for increased ET-1 production. Indeed, studies have shown that superoxide and hydrogen peroxide can increase ET-1 production in human mesangial cells and diabetic rat glomeruli (57,58). It is interesting that plasma ET-1 levels are unaffected by ABT-627 treatment in sham rats yet ABT-627–treated diabetic rats have elevated plasma ET-1 levels. The underlying mechanism of this increase is unknown; however, we speculate that hyperglycemia renders the ET_B receptors dysfunctional, leading to increased activation of ET_A receptors. Furthermore, renal production of ET-1 is increased (as indicated by urinary excretion of ET-1) in diabetes without a concomitant increase in plasma ET-1. Furthermore, a recent report showed that the combination of diabetes and ET_B receptor deficiency causes severe low-renin hypertension via ET_A receptor activation with increasing plasma ET-1 levels (59), thereby supporting the concept that hyperglycemia increases tissue ET-1 levels.

**Conclusion**

Most studies agree that the effects of ET-1 include remodeling, vasoconstriction, vascular smooth muscle cell proliferation, glomerulosclerosis, and induction of cytokine expression. Excessive ET-1 production or ET_A receptor activation in the kidney of diabetic rats contributes to the development of diabetic nephropathy; however, the specific sites of ET-1 action in the diabetic kidney and the pathways by which ET-1 induces renal injury have not been elucidated fully. Our study suggests that ET-1 via the ET_A receptor may induce renal injury, at least in part, by promoting macrophage infiltration and stimulating production of TGF-β and PGE_2. The roles of inflammatory mediators and PGE_2 in the regulation of ET-1–induced diabetic renal injury remain to be investigated. By identifying signaling pathways that are necessary for the pathologic effects of ET-1, we can gain a better understanding of the development of diabetic nephropathy.

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**Disclosures**

None.

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