Glomerular renin angiotensin system in streptozotocin diabetic and Zucker diabetic fatty rats

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Substantial evidence suggests that the intrarenal renin-angiotensin system (RAS) plays a role in the pathogenesis of diabetic nephropathy. Although the glomerular RAS is activated in the streptozotocin (STZ)-diabetic rat, the status of the glomerular RAS in the Zucker diabetic fatty (ZDF) rat, which is a commonly used genetic model of diabetes, is not known. Angiotensinogen (AGT), angiotensin II (Ang II), angiotensin converting enzyme (ACE), and angiotensin converting enzyme 2 (ACE2) were measured in glomeruli isolated from 4-week-old STZ-diabetic rats and 32-week-old ZDF rats. Glomerular injury was evaluated by histopathologic methods. Both STZ-diabetic and ZDF rats exhibited marked hyperglycemia and renal hypertrophy, but only ZDF rats demonstrated proteinuria and glomerulosclerosis. Glomerular AGT and Ang II levels were increased significantly in STZ-diabetic compared with nondiabetic control rats, accompanied by a reduction in ACE2 activity. In contrast, glomerular AGT, Ang II, and ACE2 were similar in ZDF rats and lean controls. ACE levels were not affected by diabetes in either diabetic model. In conclusion, the glomerular RAS is activated in the STZ diabetic rat but not in the ZDF rat despite a similar degree of hyperglycemia. The mechanism of nephropathy in the ZDF rat may involve factors other than hyperglycemia and RAS activation, such as hypertension and hyperlipidemia. (Translational Research 2008;151:208–216)

Abbreviations: ACE2 = angiotensin converting enzyme 2; AGT = angiotensinogen; Ang = angiotensin; ARB = angiotensin receptor blocker; ELISA = enzyme-linked immunosorbent assay; OZR = obese Zucker rat; PAS = periodic acid-Schiff; PBS = phosphate-buffered saline; RAS = renin-angiotensin system; SE = standard error of the mean; STZ = streptozotocin; TMB = 3,3′,5,5′-tetramethylbenzidine dihydrochloride; ZDF = Zucker diabetic fatty

Diabetic nephropathy is characterized by accumulation of mesangial matrix in the glomerulus that leads to glomerulosclerosis and renal failure. Although the mediators of mesangial matrix expansion in diabetic nephropathy have not been identified fully, a prominent role for the peptide angiotension (Ang) II has been suggested based on clinical trials in diabetic patients with nephropathy. Treatment with angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are beneficial in slowing the progression of glomerulosclerosis in patients with both type 1 and type 2 diabetes.[1-4] Activation of the intrarenal renin-angiotensin system (RAS) in diabetic patients is suggested by an increased renal vasodilator response to ACE inhibition and angiotensin blockade.[5]

The streptozotocin (STZ)-induced diabetic rat is a model of type 1 diabetes characterized by hyperglycemia and insulin deficiency. Even though proteinuria and glomerulosclerosis develops slowly in this model,

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AT A GLANCE COMMENTARY

Background

Determination of the role of the intrarenal renin-angiotensin system (RAS) in diabetic nephropathy requires appropriate animal models. Our results suggest that the glomerular RAS is activated in rats administered the islet cell toxin streptozotocin (STZ-diabetic rats) but not in Zucker diabetic fatty (ZDF) rats, a genetic model, despite a similar degree of hyperglycemia.

Translational Significance

The glomerular lesions in ZDF rats are those of focal and global glomerulosclerosis and thus are not typical of human diabetic nephropathy. Thus the ZDF rat may not be an appropriate model to investigate the role of the glomerular RAS in glomerular injury in diabetes.

treatment with Ang II blockers has been shown to normalize urinary protein excretion and renal structural changes,[6-8] which suggests activation of the RAS in this model. Indeed, previous studies have shown activation of the RAS within the STZ-diabetic rat kidney[9] as well as significantly increased Ang II levels in the glomerulus.[10] In comparison, the Zucker diabetic fatty (ZDF) rat, which is a type 2 diabetes model, manifests hyperglycemia, insulin resistance, and hypertension accompanied by proteinuria and progressive glomerulosclerosis.[11] The beneficial effects of Ang II blockers in reducing proteinuria and glomerulosclerosis in this model are well documented,[12,13] suggesting that activation of the RAS may occur. However, data on the effect of diabetes on the intrarenal RAS are still lacking in the ZDF rat. The current study investigates the status of the glomerular RAS in both STZ-diabetic (type 1 diabetes) and ZDF (type 2 diabetes) rats. The major components of the RAS that regulate Ang II levels are angiotensinogen (AGT), the precursor of Ang II, and the enzymes ACE and ACE2 that are responsible for the formation and breakdown of Ang II, respectively. Therefore, we have examined these components in glomeruli of STZ-diabetic and ZDF rats.

METHODS

STZ-diabetic rat. Male Sprague-Dawley rats (150–200 g) were made diabetic by a single intravenous injection of STZ (Sigma, St. Louis Mo; 60 mg/kg body weight) into the tail vein. Control rats matched for age and body weight received an equal volume of the vehicle. The diabetic state of the animal was confirmed by the demonstration of nonfasting blood glucose levels $>$ 250 mg/dL 24 h after STZ injection. Food and water intake were given *ad libitum*. Diabetic rats were assigned to either an untreated group or an insulin-treated group. In the insulin-treated group, rats were implanted with miniosmotic pumps to deliver 3 units of insulin/day. Animals were sacrificed after 4 weeks of diabetes. Principles of laboratory animal care (NIH Publication No. 85-23, revised 1985) were followed.

ZDF rat. Male ZDF rats (fa/fa) and lean control rats (+/?) were purchased from Charles River Laboratories, Inc. (Wilmington, Mass) at 21 weeks of age and maintained on a 5008 Purina diet (Purina, St. Louis, Mo). Hyperglycemia was confirmed by measurement of blood glucose, and no insulin was administered. Animals were sacrificed at 32 weeks of age. Principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Measurement of physiologic parameters. The body weights of control and diabetic rats were measured once a week, and blood glucose and urinary protein were measured every 2 weeks. For measurement of blood glucose levels, blood was collected in conscious rats via a needle prick in the tail vein and was analyzed using a glucometer. Twenty-four-hour urine samples were collected from rats housed individually in metabolic cages with free access to water and food. After 24-h urinary volume had been measured, the urine samples were centrifuged and the supernatant was stored at -20°C until assayed for protein using the BIO-RAD method.

Isolation of glomeruli. Animals were sacrificed, and kidneys were rapidly removed, weighed, and minced. Glomeruli were isolated by sequentially sieving at 4°C using ice-cold phosphate buffered saline (PBS) buffer. Glomerular suspensions were examined by light microscopy and were found to contain $>$95% of glomeruli. The suspensions were centrifuged, and the pellet was resuspended in ice-cold PBS, sonicated, and recentrifuged at 13,000 g for 20 min. The resulting supernatant was used for various measurements as outlined below.

Measurement of angiotensinogen. Angiotensinogen levels in glomerular extracts were measured by a competitive enzyme-linked immunosorbent assay (ELISA) described in our recent publication.[10] Briefly, a 96-well plate was coated overnight at 4°C with 4 μg/mL of angiotensinogen (1-14) (Sigma). In the wells, samples or standards were mixed with antiangiotensinogen antibody (1:10,000) and incubated at room temperature for 2 h followed by washings and incubation with a peroxidase-conjugated secondary antibody (1:1000) for 1 h. The reaction was developed using 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2, stopped with 2N HCl, and read at 450 nm using an ELISA reader (Molecular Devices, Sunnyvale, Calif). Angiotensinogen levels in samples were calculated from a standard curve using angiotensinogen (1-14) as the standard.

Measurement of Ang II. Ang II levels were measured by a competitive ELISA (Peninsula Laboratories, Belmont, Calif) as described by us previously.[10,14] In brief, samples were mixed with rabbit anti-Ang II antibody and biotinylated Ang II peptide, followed by incubation in 96-well plates coated with goat antirabbit IgG for 2 h at room temperature.