Superoxide anion curbs nitric oxide modulation of afferent arteriolar ANG II responsiveness in diabetes mellitus

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Schooomaker, Gwynn C., Rachel W. Fallet, and Pamela K. Carmines. Superoxide anion curbs nitric oxide modulation of afferent arteriolar ANG II responsiveness in diabetes mellitus. Am. J. Physiol. Renal Physiol. 278: F302–F309, 2000.—Experiments were performed to test the hypothesis that the impact of endogenous nitric oxide (NO) on ANG II-induced renal arteriolar constriction is reduced in rats with insulin-dependent diabetes mellitus (65 mg/kg streptozotocin; STZ). Arteriolar diameter responses to exogenous ANG II were quantified before and during NO synthase inhibition (100 µM N-nitro-L-arginine; L-NNA) by using the in vitro blood-perfused juxtamedullary nephron technique. Afferent arteriolar lumen diameter averaged 20.7 ± 2.0 µm in Sham kidneys and 25.9 ± 1.3 µm in STZ kidneys (P < 0.05). Efferent arteriolar diameter did not differ between Sham and STZ rats. In kidneys from Sham rats, afferent and efferent arterioles to ANG II (0.1–10.0 nM) were exaggerated significantly by L-NNA. L-NNA also augmented afferent arteriolar ANG II responses in kidneys from STZ rats (high-glucose bath) but did not alter ANG II responses in afferent arterioles from STZ rats. L-NNA also accentuated efferent, but not afferent, arteriolar ANG II responses in STZ kidneys during acute restoration of bath glucose to normal levels. Superoxide dismutase (150 U/ml) restored the ability of L-NNA to allow exaggerated afferent arteriolar responses to ANG II in kidneys from STZ rats. These observations indicate that superoxide anion suppresses the modulatory influence of endogenous NO on ANG II-induced afferent arteriolar constriction in diabetes. Efferent arteriole; N-nitro-L-arginine; rat; streptozotocin; superoxide dismutase

RENAH HEMODYNAMIC DYSFUNCTION plays a central role in the pathophysiological consequences of insulin-dependent diabetes mellitus (IDDM). Specifically, glomerular hyperfiltration (and/or consequent maladaptations to this process) is believed to ultimately engender reductions in glomerular filtration rate and the development of diabetic glomerulopathy. Although some investigators have advanced the postulate that increased nitric oxide (NO) production contributes to the renal hemodynamic maladaptations of IDDM (6, 23, 28, 39) other studies have failed to discern any effect of IDDM on the renal hemodynamic response to nitric oxide synthase (NOS) inhibition (17, 22, 26, 34). Data from our laboratory indicate that NOS inhibition exerts a diminished impact on renal arteriolar baseline diameter in streptozotocin (STZ)-treated rats and that superoxide dismutase (SOD) normalizes the diameter response to NOS inhibition (30). These observations suggest that superoxide anion accelerates NO degradation in IDDM, thus reducing the functional impact of endogenous NO on the renal microvasculature in IDDM.

In addition to its direct vasodilator effect, endogenously produced NO normally tempers renal vascular responsiveness to a variety of constrictor stimuli (7, 27, 33, 36). In particular, the counterbalancing influences of ANG II and NO on the renal microvasculature appear to represent primary determinants of renal perfusion and glomerular filtration. It is easy to envision that an imbalance in the vasoactive actions of these paracrine substances could contribute to the disruption of renal hemodynamic function in IDDM. Indeed, a recent analysis of glomerular filtration rate responses to NOS inhibition and/or ANG II receptor blockade suggested that combined alterations in ANG II and NO may contribute to the hyperfiltration in STZ rats (26). However, functional interactions between ANG II and NO have not yet been probed by direct means in renal arterioles from diabetic animals. The purpose of the present study was to test the postulate that IDDM is associated with an impaired modulatory impact of NO on ANG II-induced renal arteriolar vasoconstriction. Additional experiments assessed the ability of SOD to restore defective NO modulation of afferent arteriolar ANG II responsiveness in kidneys from diabetic rats.

METHODS

Induction of Diabetes Mellitus

Male Sprague-Dawley rats were anesthetized with methohexitol sodium (50 mg/kg; Brevipal ip; Eli Lilly, Indianapolis, IN) to facilitate intravenous injection of STZ (65 mg/kg; Sigma Chemical, St. Louis, MO). Sham rats received vehicle treatment. The rats recovered from anesthesia and were housed overnight with ad libitum access to food and water. The following morning, blood glucose levels were measured (Accu-Check III model 766, Boehringer Mannheim, Indianapolis, IN) and the rats were anesthetized again to facilitate subcutaneous insertion of a 2.3 × 2.0-mm sustained-release insulin implant (Linplant; Linshin Canada, Scarborough, Ontario, Canada) into STZ rats via a 16-G needle. Sham rats received vehicle implants (microcryrstallized palmitic acid). Blood glucose concentration and body weight were measured twice weekly thereafter. Some animals were housed individually in
metabolic cages (Nalgene, Nalge, Rochester, NY) for 2 days immediately before the terminal experiment. The total volume of urine collected during the final 24-h period was centrifuged (to remove sediment) and stored at −70°C. These samples were assayed for creatinine concentration by a picric acid-based microplate assay (35).

In Vitro Blood-Perfused Juxtamedullary Nephron Technique

Two to three weeks after induction of diabetes, experiments were performed by using the in vitro blood-perfused juxtamedullary nephron technique (11). The rat was anesthetized with pentobarbital sodium (50 mg/kg Nembutal ip; Abbott Laboratories, North Chicago, IL), and an angiotensin-converting enzyme inhibitor (enalaprilat, 2 mg/ia; Merck Research Laboratories, Rahway, NJ) was administered to suppress endogenous ANG II formation and its impact on vascular tone. Thirty minutes later, the right renal artery was cannulated via the superior mesenteric artery. This procedure initiated in situ perfusion of the kidney with Tyrode solution containing 52 g/l dialyzed BSA and D-glucose at a concentration of either 90 mg/dl (Sham rats) or 300 mg/dl (STZ rats). The rat was then exsanguinated via a carotid arterial cannula into a heparinized syringe, and the kidney was harvested for in vitro study. Renal perfusion was maintained throughout the dissection procedure needed to reveal the tubules, glomeruli, and vasculature of juxtamedullary nephrons. Ligatures were placed around the distal segments of the large arterial branches that supplied the exposed microvasculature.

When necessary to obtain an adequate volume of blood for renal perfusion, an additional blood-donor rat was anesthetized, treated with enalaprilat, subjected to acute bilateral nephrectomy, and exsanguinated via a carotid arterial cannula into a heparinized syringe. The collected blood was pooled with that harvested from the kidney donor and processed to remove leukocytes and platelets (19). The resulting perfusate was stirred continuously in a closed reservoir that was pressurized under 95% O2-5% CO2, thus providing both oxygenation and the driving force for perfusion of the dissected kidney at a constant renal arterial pressure of 110 mmHg. Kidneys from STZ rats were perfused with blood from STZ rats, and kidneys from Sham rats were perfused with blood from Sham rats.

The renal perfusion chamber was warmed, and the tissue surface was superfused with Tyrode solution containing 10 g/l BSA at 37°C. Except when otherwise noted, this bathing solution contained 300 mg/dl glucose for experiments utilizing STZ kidneys and 90 mg/dl glucose for experiments utilizing Sham kidneys. The renal microvasculature was transilluminated on the stage of a compound microscope, and a single afferent or efferent arteriole was selected for study on the basis of visibility and blood flow. Video images of each microvessel were generated continuously and stored on videotape for later analysis. In one experiment in which two vessels could be visualized within the same field of view, responses of both vessels were recorded simultaneously and analyzed separately during videotape playback.

Experimental Protocols

Effect of NOS inhibition on ANG II-induced arteriolar vasconstriction. Arteriolar diameter responses to increasing concentrations of ANG II (Sigma Chemical) were evaluated by exposing kidneys from Sham and STZ rats to the following superfusate bathing solutions: 1) Tyrode solution alone (5–10 min); 2) Tyrode solution containing 0.1, 1.0, and 10 nM ANG II (3 min at each concentration); and 3) Tyrode solution alone (10 min). After this recovery period, endogenous NOS inhibition was achieved by addition of 100 µM N’-nitro-L-arginine (L-NNA; Aldrich Chemical, Milwaukee, WI) to the Tyrode bathing solution, as described previously (31). Juxtamedullary afferent arteriolar diameter responses to 100 µM L-NNA are apparent within 5 min, maximal at 10 min, and stable for >30 min thereafter (19). Accordingly, a 15-min L-NNA treatment period preceded initiation of the second ANG II exposure sequence (0.1, 1.0, and 10 nM ANG II; 3 min each) in the continued presence of L-NNA. This was followed by a recovery period during which the tissue was exposed to Tyrode solution containing L-NNA alone. Imposition of two consecutive ANG II exposure sequences according to this protocol (but in the absence of L-NNA) evokes indistinguishable juxtamedullary arteriolar diameter responses in normal kidneys, even at peptide concentrations 10-fold higher than those employed in the present study (8).

Effect of reduced extracellular glucose concentration on ANG II-NO interactions in IDDM. Additional experiments addressed the possibility that an acute decrease in extracellular glucose toward normal levels might restore NO modulation of ANG II responses in STZ rats. Afferent or efferent arteriolar diameter responses to ANG II were documented in STZ kidneys before and during L-NNA treatment according to the protocol described above, except that all bath solutions contained 90 mg/dl glucose.

Effect of SOD on ANG II-NO interactions. The contribution of endogenous superoxide anion to the altered NO modulation of arteriolar ANG II responses in IDDM was explored through the use of acute SOD treatment. SOD from bovine erythrocytes (Sigma Chemical; 3,100–5,100 U/mg) was added to the perfusate blood to achieve a final concentration of 150 U/ml. This concentration of SOD has been reported to achieve complete scavenging of extracellular superoxide anion (16). Experiments were performed by using kidneys from both Sham rats and STZ rats perfused with SOD-supplemented blood (treatment groups designated Sham+SOD and STZ+SOD, respectively). Afferent arteriolar diameter responses to ANG II were obtained in these kidneys before and during L-NNA treatment, according to the protocol described above.

Data Analysis

Arteriolar lumen diameter was measured from videotaped images at 12-s intervals from a single point along the length of the vessel. The average diameter during the final minute of each treatment period was utilized for statistical analysis by repeated-measures ANOVA or Friedman repeated-measures ANOVA on ranks, as appropriate, followed by a Newman-Keuls test. Simple between-group comparisons were performed by using the unpaired t-test. All statistical computations were performed utilizing the SigmaStat 2.03 software package (SPSS, Chicago, IL). Statistical significance was defined as P values <0.05. All data are reported as means ± SE (n = number of arterioles, unless specified otherwise).

RESULTS

Animal Characteristics

Table 1 summarizes the salient characteristics of Sham and STZ rats utilized in the present study. At the time of injection of either STZ or vehicle, the rats receiving STZ weighed somewhat more than those receiving vehicle (Sham rats). However, because Sham rats gained more weight (80 ± 7 g; n = 20 rats) than STZ rats (24 ± 5 g; n = 31 rats; P < 0.05) during the
Arteriolar Vasoconstriction

Effect of NOS Inhibition on ANG II-Induced Arteriolar Vasocostrictron

Table 2 summarizes arterial diameters in the various treatment groups before (untreated) and during NOS inhibition (100 µM L-NNA). As reported previously (32), baseline afferent arterial diameter was significantly increased in kidneys from STZ rats, but efferent arteriolar diameter did not differ significantly between Sham and STZ rats. The afferent arteriolar dilation associated with IDDM remained evident in SOD-treated STZ kidneys but did not achieve statisti-
cal significance in STZ kidneys bathed in low-glucose (90 mg/dl) solution.

Figure 1A illustrates the effect of l-NNA on ANG II-induced constriction of afferent arterioles in kidneys from Sham rats. Addition of 1 and 10 nM ANG II to the bathing solution reduced lumen diameter to 17.2 ± 1.6 and 9.9 ± 2.2 µm, respectively (n = 6), and removal of ANG II from the bathing solution restored diameter to 19.8 ± 1.4 µm [not significant (NS) vs. baseline]. Addition of L-NNA to the bathing solution decreased afferent arteriolar diameter by 6.0 ± 1.4 µm (Table 2). During continued L-NNA treatment, the same concentrations of ANG II reduced afferent arteriolar lumen diameter to 9.7 ± 1.5 µm (1 nM) and 4.1 ± 1.4 µm (10 nM). These responses significantly exceeded those observed in the same vessels before L-NNA treatment. Thus L-NNA treatment amplified afferent arteriolar vasoconstrictor responses to exogenous ANG II in kidneys from Sham rats. After removal of ANG II from the bathing solution, but in the continued presence of L-NNA, afferent diameter was restored to 14.0 ± 2.0 µm (NS vs. L-NNA baseline). This observation confirms that the responses to ANG II during L-NNA treatment were not merely a time-related decline in arteriolar diameter.

Figure 1B illustrates the impact of NOS inhibition on ANG II-induced constriction of afferent arterioles from STZ rats. Addition of ANG II (1 and 10 nM) to the solution bathing STZ kidneys reduced afferent diameter to 19.6 ± 1.8 and 10.7 ± 2.0 µm, respectively. These responses did not differ significantly (either as absolute change in diameter or as % change in diameter) from those observed in Sham kidneys. On removal of ANG II from the bathing solution, afferent diameter was re-
stored to 24.7 ± 1.4 µm (NS vs. baseline). Addition of 100 µM L-NNA to the bathing solution reduced afferent arteriolar diameter in STZ kidneys by 6.4 ± 1.0 µm (Table 2), a response that did not differ from that observed in Sham kidneys. During continued exposure to L-NNA, ANG II reduced afferent arteriolar diameter by 21.2 ± 2.7 µm (10 nM). These responses to ANG II in the presence of L-NNA did not differ from those observed in the same arterioles before L-NNA treatment. Removal of ANG II from the L-NNA-containing bath, with lumen diameter averaging 14.5 ± 3.7 µm under these conditions. Removal of ANG II from the L-NNA-containing bathing solution restored afferent arteriolar diameter to 22.9 ± 2.2 µm (NS vs. L-NNA baseline). Thus the modulatory impact of L-NNA on effenter arteriolar ANG II responsiveness was sustained in kidneys from STZ rats.

Effect of Reduced Extracellular Glucose Concentration on ANG II-NO Interactions in IDDM

In experiments performed exclusively using kidneys from STZ rats, effenter arteriolar diameter was unaffected by 0.1 and 1 nM ANG II before L-NNA treatment but decreased significantly to 21.1 ± 2.7 µm on exposure to 10 nM ANG II (Fig. 2B). These responses did not differ significantly from those observed in effenter arterioles from Sham rats. Removal of ANG II from the bathing solution restored effenter arteriolar diameter to 27.0 ± 2.7 µm (100 ± 3% of baseline). The tendency for L-NNA to decrease effenter diameter failed to achieve statistical significance in kidneys from STZ rats (−2.4 ± 1.3 µm; Table 2). However, effenter arteriolar responses to 10 nM ANG II were exaggerated significantly by L-NNA treatment, with lumen diameter averaging 14.5 ± 3.7 µm under these conditions. Removal of ANG II from the L-NNA-containing bathing solution restored effenter diameter to 27.0 ± 4.4 µm (10 nM ANGII) vs. L-NNA baseline). Thus the inability of L-NNA on effenter arteriolar ANG II responsiveness was sustained in kidneys from STZ rats.

In kidneys from STZ rats, effenter arteriolar diameter was unaffected by 0.1 and 1 nM ANG II before L-NNA treatment but decreased significantly to 21.1 ± 2.7 µm on exposure to 10 nM ANG II (Fig. 2B). These responses did not differ significantly from those observed in effenter arterioles from Sham rats. Removal of ANG II from the bathing solution restored effenter arteriolar diameter to 27.0 ± 2.7 µm (100 ± 3% of baseline). The tendency for L-NNA to decrease effenter diameter failed to achieve statistical significance in kidneys from STZ rats (−2.4 ± 1.3 µm; Table 2). However, effenter arteriolar responses to 10 nM ANG II were exaggerated significantly by L-NNA treatment, with lumen diameter averaging 14.5 ± 3.7 µm under these conditions. Removal of ANG II from the L-NNA-containing bathing solution restored effenter diameter to 22.9 ± 2.2 µm (NS vs. L-NNA baseline). Thus the modulatory impact of L-NNA on effenter arteriolar ANG II responsiveness was sustained in kidneys from STZ rats.

Effect of Reduced Extracellular Glucose Concentration on ANG II-NO Interactions in IDDM

In experiments performed exclusively using kidneys from STZ rats, effenter and effenter arteriolar function was assessed by using bath solutions containing 90 mg/dl glucose. Under these conditions that impose on STZ kidneys an acute restoration of extracellular glucose concentration toward normal levels, arteriolar baseline diameters did not differ significantly from those observed in either Sham kidneys or STZ kidneys studied in the high-glucose environment (Table 2). Figure 3 illustrates the effects of L-NNA on arteriolar ANG II responsiveness in STZ kidneys studied using 90 mg/dl glucose bath solutions. Afferent arteriolar diameter averaged 21.4 ± 2.6 µm during exposure to 1 nM ANG II and further declined to 10.9 ± 2.3 µm when exposed to 10 nM ANG II (P < 0.05 vs. baseline). Removal of ANG II from the bath restored diameter to 22.3 ± 1.3 µm (NS vs. baseline). Subsequent L-NNA treatment reduced arteriolar diameter by 4.4 ± 1.4 µm (Table 2). During continued exposure to L-NNA in the 90 mg/dl glucose bath, ANG II reduced arteriolar lumen diameter in STZ kidneys to 13.3 ± 1.7 µm (1 nM) and 7.2 ± 0.9 µm (10 nM). These responses to ANG II in the presence of L-NNA did not differ from those observed in the same arterioles before L-NNA treatment (Fig. 3A). Thus the inability of L-NNA treatment to allow exaggerated effenter arteriolar ANG II responsiveness remained evident in STZ kidneys bathed in solutions containing 90 mg/dl glucose.

The effect of reduced bath glucose concentration on effenter arteriolar responses in STZ kidneys is shown in Fig. 3B. Effenter arteriolar diameter averaged 23.2 ± 2.2 µm during exposure to 1 nM ANG II (NS vs. baseline) and further declined to 16.8 ± 3.3 µm when exposed to 10 nM ANG II (P < 0.05 vs. baseline). Removal of ANG II from the bath restored effenter diameter to 23.3 ± 2.4 µm (NS vs. baseline). Subsequent L-NNA treatment only reduced effenter arteriole diameter by 2.1 ± 0.7 µm (Table 2). During L-NNA
treatment, 1 and 10 nM ANG II decreased efferent arteriolar diameter to 17.3 ± 3.0 and 11.2 ± 2.6 µm (both P < 0.05 vs. L-NNA baseline), respectively. Efferent arteriolar responses to 1 and 10 nM ANG II during L-NNA treatment significantly exceeded those observed in the same vessels under untreated conditions. After removal of ANG II from the bath, efferent arteriolar diameter was restored to 19.5 ± 2.7 µm (NS vs. L-NNA baseline). Thus the modulatory influence of L-NNA on efferent arteriolar ANG II responsiveness was sustained in STZ kidneys bathed in solutions containing low (normal) glucose concentrations.

Effect of SOD on NO-ANG II Interactions

Figure 4 depicts the effects of L-NNA on afferent arteriolar responses to ANG II in kidneys perfused with blood containing 150 U/ml SOD. In Sham+SOD kidneys, afferent arteriolar responsiveness to ANG II was similar to that observed in kidneys from Sham rats studied without SOD supplementation, with 10 nM ANG II decreasing arteriolar diameter to 10.0 ± 1.4 µm. After recovery of kidneys from the initial ANG II challenge (20.6 ± 0.8 µm; NS vs. baseline), L-NNA evoked a 4.2 ± 0.9-µm decline in arteriolar diameter (Table 2; NS vs. Sham without SOD or Sham). During continued L-NNA treatment, the same concentrations of ANG II (1 and 10 nM) reduced arteriolar lumen diameter to 20.5 ± 2.1 and 10.9 ± 1.2 µm, respectively. These responses to 1 and 10 nM ANG II significantly exceeded those observed in the same vessels before L-NNA treatment. After removal of ANG II from the bathing solution, but in the continued presence of L-NNA, afferent arteriolar diameter was restored to 23.6 ± 2.0 µm (NS vs. L-NNA baseline). Therefore, SOD supplementation restored the ability of L-NNA to amplify afferent arteriolar vasoconstrictor responses to exogenous ANG II in kidneys from STZ rats.

DISCUSSION

Considerable attention has focused on elucidating the role of ANG II in the maladaptive renal hemodynamic events accompanying IDDM. Conventional wisdom asserts that the renin-angiotensin system is sup-
pressed during the early stages of IDDM, as evidenced by the frequent observation of reduced plasma renin activity under these conditions. However, ACE inhibition retards development of glomerular injury in IDDM (25, 43), and most evidence indicates that this occurs through an ANG II-dependent mechanism (27, 37, 40). These observations suggest that the renal hemodynamic impact of endogenous ANG II may be exaggerated in IDDM (21, 42), despite normal or reduced renal tissue ANG II levels. Increased ANG II sensitivity may occur as a consequence of the hyperfiltration process (or may arise despite the development of hyperfiltration) and could contribute to the subsequent development of diabetic glomerulopathy and end-stage renal disease. The results of the present study support the contention that renal arteriolar ANG II responsiveness is retained during the early stage of a IDDM in a moderately hyperglycemic rat model characterized by polydipsia, polyuria, and increased creatinine excretion (consistent with hyperfiltration). Moreover, our data reveal that the normal modulatory influence of NO on afferent arteriolar ANG II responsiveness is absent under these conditions (but is restored by superoxide dismutase), whereas NO modulation of ANG II-induced efferent arteriolar constriction remains intact.

Numerous studies have revealed that the renal hemodynamic impact of ANG II is exaggerated by NOS inhibition, suggesting that endogenous NO tempers the renal vasoconstrictor impact of ANG II under normal conditions. In accord with this contention, juxtamedullary afferent and efferent arteriolar diameter responses to ANG II are exaggerated by treatment with L-NNA (18, 19). In the present study, the modulatory impact of 100 µM L-NNA on ANG II responsiveness was confirmed in both afferent and efferent arterioles from Sham rats. At this concentration, L-NNA is maximally effective in reducing afferent arteriolar baseline diameter and abolishing the afferent arteriolar vasodilator response to acetylcholine in our experimental setting (18, 31). The modulatory impact of L-NNA on ANG II responsiveness cannot be attributed to the L-NNA-induced decrease in diameter per se, because preconstriction with norepinephrine does not exaggerate juxtamedullary afferent arteriolar ANG II responsiveness (19). Thus the increased ANG II responsiveness observed during L-NNA treatment indicates that endogenous NO normally tempers ANG II-induced renal vasoconstrictor responsiveness.

IDDM is generally reported to impair renal vasodilator responses to acetylcholine and other endothelium-dependent agents (14, 15, 20, 41). However, the effect of IDDM on the increase in renal vascular resistance evoked by NOS inhibition remains controversial (6, 17, 22, 23, 26, 28, 34, 39). We previously observed markedly diminished afferent and efferent arteriolar vasoconstrictor responses to L-NNA in kidneys from STZ rats (30), suggesting that the tonic functional impact of NO on the renal microvasculature is decreased in IDDM. In the present study, however, the effect of NOS inhibition on baseline afferent arteriolar diameter remained intact in STZ kidneys, whereas efferent arteriolar responses were abated. At least two phenomena may contribute to this discrepancy with our previous data. First, the present study employed acute enalaprilat treatment to facilitate evaluation of arteriolar responsiveness to exogenous ANG II, whereas our previous study was performed in the setting of an intact renin-angiotensin system. In light of the complex interplay between tissue ANG II and NO levels under normal conditions, and our incomplete understanding of the status of the intrarenal renin-angiotensin system in IDDM (1), the impact of enalaprilat treatment on NO-dependent baseline tone in the present studies cannot be reasonably estimated. Second, our earlier studies specifically targeted the tonic influence of endogenous NO on baseline diameter by generating L-NNA concentration-response relationships in kidneys from Sham and STZ rats, whereas the present study employed a complex experimental design that optimized our ability to detect NO modulation of ANG II responses through paired observations. This latter design is not a powerful means of detecting between-group differences in the effect of NOS inhibition on baseline diameter. Nevertheless, coupled with the use of enalaprilat-treated tissue to provide a functional baseline of reduced tissue ANG II formation, the design of the present studies provides a powerful assessment of the modulatory influence of NO on contractile responses to exogenous ANG II.

Short-term exposure to high-glucose media exaggerates ANG II-induced constriction of rabbit afferent arterioles by inhibiting the modulatory action of NO (4). The results of the present study extend this observation by demonstrating that the usual effect of L-NNA to allow enhanced afferent arteriolar ANG II responsiveness is absent in kidneys from diabetic rats. NO modulation of afferent arteriolar ANG II responsiveness was also absent in STZ kidneys studied during an acute reduction of extracellular glucose concentration toward normal levels, indicating that this phenomenon is not osmotic in origin. Pfleger and co-workers (34) have documented loss of the ability of L-NNA to promote renal vasoconstrictor responses to adenosine in IDDM. Thus it appears that neither basal nor agonist-stimulated NO levels are sufficient to provide the normal modulatory impact of NO on renal vasoconstrictor events in IDDM. Although this phenomenon can be expected to promote exaggerated renal vasoconstrictor responsiveness to ANG II in IDDM, afferent arteriolar ANG II responses observed in the absence of NOS inhibition did not differ between Sham and STZ kidneys in the present study. The multiple paracrine influences on the renal microvasculature, as well as alterations in endogenous ANG II synthesis, AT1 receptor density, and/or intracellular signaling events are all potential mechanisms through which the IDDM might influence the impact of ANG II on the renal microvasculature. We suspect that the loss of NO-dependent modulation of afferent arteriolar ANG II responsiveness in IDDM is offset by the diminished response of this arteriolar segment to membrane depolarization and opening of voltage-gated Ca2+ channels (9), pro-
cesses that are critical in eliciting the constrictor response to ANG II. Baseline afferent arteriolar tone is also heavily dependent on membrane potential and Ca$^{2+}$ influx through these channels (10). Hence, decreased functional expression of voltage-gated Ca$^{2+}$ channels and/or a hyperpolarizing shift in membrane potential (e.g., increased K$^{-}$ conductance) could engender the reduced preglomerular resistance characteristic of diabetic hyperfiltration, despite a reduced microvascular impact of endogenous NO.

Ichihara and co-workers (18) have provided evidence that both NOS-1 and NOS-3 provide NO that influences afferent arteriolar basal tone, whereas NOS-3 is the primary source of NO that modulates afferent arteriolar ANG II responsiveness. Hence, one could postulate that reduced NOS-3 expression might underlie the decreased impact of NO on afferent tone (30) and the absence of NO modulation of afferent arteriolar contractile responsiveness evident in IDDM. This scenario is improbable in light of the increase in afferent arteriolar NOS-3 immunostaining and NADPH-diaphorase staining that has been described at 1, 2, and 4 wk after induction of IDDM (38). It is more likely that increased degradation, rather than decreased synthesis, underlies the reduced bioavailability of NO in IDDM. Chronic exposure of cultured endothelial cells to a high-glucose environment promotes production of superoxide anion (12) which, in a virtually diffusion-limited reaction, reacts with NO to form peroxynitrite. Although not representing a significant pathway for NO degradation under normal conditions, peroxynitrite formation gains functional significance as a means of reducing the half-life of NO in states of oxidant stress such as hypertension or IDDM. Accordingly, although not altering responses in normal vessels, treatment with SOD or free radical scavengers ameliorates the impaired endothelium-dependent vasodilator function accompanying IDDM in a variety of nonrenal vascular beds (3, 14, 24, 29) and in renal interlobar arteries (13). We previously reported the ability of exogenous SOD to restore normal afferent and efferent arteriolar diameter responses to NOS blockade in IDDM (30). This observation suggests that superoxide anion accumulation promotes a reduction in ambient NO concentration and its impact on the renal microvasculature in IDDM, even in the face of normal or increased NO synthesis. A deleterious impact of superoxide anion on the renal microvascular actions of NO in IDDM is further implicated by our present observation that exogenous SOD unmasked a modulator effect of endogenous NO on ANG II-induced afferent arteriolar constriction in STZ kidneys, while not altering ANG II responses in kidneys from Sham rats. Thus superoxide anion acting in the vicinity of the afferent arteriole appears responsible for the impaired availability of NO to temper ANG II-induced constriction of that vascular segment.

Although the influence on NO on efferent arteriolar baseline diameter is suppressed in STZ rats (30), the results of the present study reveal that the modulatory impact of NO on efferent arteriolar ANG II responsiveness is preserved. This surprising observation may relate to the distinct sources of the NO that influences juxtamedullary arteriolar baseline diameter and constrictor responsiveness. Through the use of inhibitors that are selective for NOS-1 or nonselective (affecting all 3 NOS isoforms), Ichihara et al. (18) provided evidence that baseline diameters of juxtamedullary efferent arterioles are maintained under the tonic influence of NO derived from both NOS-3 (from endothelial sources) and NOS-1 [expressed in macula densa cells and the efferent arteriolar endothelium (5)]. Moreover, their work indicated that juxtamedullary efferent arteriolar ANG II responsiveness is modulated solely by NO produced through the enzymatic activity of NOS-1 (18). It is possible that the NO produced by NOS-1 is either anatomically or physiologically protected from reaction with superoxide anion in IDDM, or that superoxide anion production in IDDM is relatively compartmentalized at endothelial sites and thus reacts predominantly with NO derived from endothelial sources (mainly NOS-3). Further experiments are necessary to address these postulates.

In summary, the results of the present study confirm that NOS inhibition allows exaggerated juxtamedullary arteriolar diameter responses to ANG II, reflecting the modulatory impact of endogenous NO on agonist-induced vasoconstriction. NO modulation of afferent arteriolar ANG II responsiveness was absent in afferent arterioles from diabetic rats but was unmasked by acute treatment with superoxide dismutase. NO modulation of efferent arteriolar ANG II responsiveness was retained in kidneys from diabetic rats, perhaps due to compartmentalization of either NO or superoxide anion production. These observations support the contention that the renal microvascular impact of endogenous NO is suppressed in IDDM, largely as a result of NO degradation via reaction with superoxide anion.

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