Reduced nitric oxide in diabetic kidneys due to increased hepatic arginine metabolism: implications for renomedullary oxygen availability

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Palm F, Friederich M, Carlsson P-O, Hansell P, Teerlink T, Liss P. Reduced nitric oxide in diabetic kidneys due to increased hepatic arginine metabolism: implications for renomedullary oxygen availability. Am J Physiol Renal Physiol 294: F30–F37, 2008. First published October 17, 2007; doi:10.1152/ajprenal.00166.2007.—Nitric oxide (NO) is a potent regulator of both vascular tone and oxygen utilization. Diabetes is commonly associated with both NO deficiency and reduced renomedullary oxygen availability. Arginine availability as regulator of NO production has gained growing interest. We hypothesized that arginine limitation causes diabetes-induced renomedullary NO deficiency, which directly influences renomedullary oxygen tension (PO2). Medullary NO, PO2, and blood flow were measured in control and streptozotocin-induced diabetic rats, which were treated or not treated with α-tocopherol, and administered l-arginine followed by Nω-nitro-l-arginine methyl ester. Major components of arginine metabolism were also investigated. Diabetic rats had reduced renomedullary NO levels compared with controls. Arginine selectively increased NO levels in diabetic rats and totally restored NO levels in α-tocopherol-treated animals. Tocopherol prevented the reduction in medullary PO2 in the diabetic animals. Although blood flow increased equally in all groups, arginine increased PO2 exclusively in the diabetic groups. Diabetes decreased plasma arginine and asymmetric dimethylarginine concentrations, but increased hepatic CAT-2A and plasma ornithine independently of α-tocopherol treatment. In conclusion, diabetic rats had reduced renomedullary NO due to decreased plasma arginine following increased hepatic arginine uptake and degradation. This was unrelated to oxidative stress. The diabetes-induced reduction in renomedullary PO2 was restored by either acute arginine administration, which also restored NO levels, or long-term antioxidant treatment. Arginine increased medullary NO and PO2 independently of altered hemodynamics in the diabetic groups. This reveals a direct regulatory function of NO for renomedullary PO2 especially during situations of elevated oxidative stress.

diabetes mellitus; blood flow; plasma arginine; asymmetric dimethylarginine; oxidative stress; vationic amino acid transporter

THE INTRARENAL METABOLISM is highly heterogeneous and is influenced by several factors, including local metabolic demand, electrolyte transport, and blood supply, to match the requirements of that particular region. Recently, the renal medulla has been ascribed increasing importance in the development of several pathological conditions, including diabetic nephropathy (32), hypertension (24), and contrast media-induced nephropathy (5).

The renal medulla receives a limited blood supply, which is not proportional to the energy demand (10). This is in part compensated by a substantial anaerobic metabolism, especially in the inner medulla (10). Another consequence of the lower blood flow and relatively high energy demand is that the renal medulla operates on the brink of hypoxic conditions already during normal physiological conditions (5, 31, 34). High concentrations of nitric oxide (NO) are constantly present in the renal medulla (18, 45). NO effectively inhibits mitochondrial respiration in a dose-dependent manner (20, 21) and has been shown to this effect in the kidney, especially during situations of low oxygen tension (PO2) (1). Several pathological conditions, including insulin-deficient diabetes mellitus, are closely associated with decreased basal renal PO2 (31, 32, 42), which makes NO inhibition of the mitochondrial respiration an important mechanism to avoid complete oxygen depletion. It is believed that the diabetes-induced reduction in renal tissue PO2 per se, especially in the medulla, can result in a hypoxic gene response, contributing to the development of renal dysfunction (29).

NO is produced by the intracellular NO synthase (NOS), which converts l-arginine to citrulline and NO. The production rate is dependent on several factors, including the availability of l-arginine (17), cofactors such as tetrahydrobiopterin (43), the presence of endogenous NOS inhibitors (41), PO2 (6), but also on the amount of NOS protein (16). Furthermore, NO can be stored and released from non-NOS-dependent sources, such as S-nitrosothiols and myoglobins (36), and the potential importance of this has been reported (37).

The bioavailability of NO is determined by the interplay between the production and the degradation. Degradation is predominately accomplished by NO being scavenged by hemoglobin, but also to a high degree by interactions with superoxide radicals to form peroxynitrite, as modeled by Buerk et al. (7).

We have previously observed reduced NO bioavailability in the kidney cortex of diabetic rats, which was accompanied by altered hemodynamic response to alterations in the NO system (30). However, little effect was observed on oxygen availability when NO levels in the kidney cortex were increased, which might have contributed to the relatively high PO2 in this region of the kidney. The renal medulla has a lower PO2 during normal physiological conditions, which might be more influenced by alterations in bioavailable NO.

In the present study, we focused on NO and oxygen availability in the diabetic renal medulla and tested the following hypothesis: 1) reduced renomedullary NO levels in the diabetic kidney cortex of diabetic rats, which was accompanied by altered hemodynamic response to alterations in the NO system (30). However, little effect was observed on oxygen availability when NO levels in the kidney cortex were increased, which might have contributed to the relatively high PO2 in this region of the kidney. The renal medulla has a lower PO2 during normal physiological conditions, which might be more influenced by alterations in bioavailable NO.

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animals are due to substrate (i.e., arginine) limitation. If so, which are the potential mechanisms? 2) NO has a regulatory role for oxygen availability in the diabetic renal medulla, which is blood flow independent. 3) The observed alterations in renomedullary NO and oxygen availability in the diabetic animals are due to increased oxidative stress, which can be reversed by antioxidant treatment with \( \alpha \)-tocopherol.

**MATERIALS AND METHODS**

**Animals.** Inbred male Wistar-Furth rats (Scanbur, Sollentuna, Sweden), weighing 250–310 g, were randomly divided into age-matched control (\( n = 8 \)) and diabetic animals (\( n = 10 \)) with free access to water and standard rat chow (R3, Ewos, Södertälje, Sweden). Additional control (\( n = 7 \)) and diabetic animals (\( n = 7 \)) were supplemented with chow containing 5% (wt/wt) DL-\( \alpha \)-tocopherol (Merck Eurolab, Stockholm, Sweden), which previously has been shown to have pronounced antioxidant effects especially in the renal medulla of diabetic rats (31).

In a separate set of animals (\( n = 5–6 \)/group), plasma urea, ornithine, and citrulline concentrations were measured. Kidney cortex and hepatic arginine activity, mRNA expression of CAT-1 and -2A, and arginine I and II protein expression were also analyzed. The local animal ethics committee at Uppsala University approved all experiments.

**Diabetes induction and surgical procedures.** Diabetes was induced by an injection of streptozotocin (STZ; 45 mg/kg, Sigma-Aldrich, St. Louis, MO) in the tail vein. Animals were considered diabetic if blood glucose increased to \( \geq 15 \) mmol/l within 24 h and remained elevated. Two weeks thereafter, all animals were anesthetized with an intra-peritoneal injection of thiobutabarbital (Inactin; 120 mg/kg nondiabetic, 80 mg/kg diabetic animals, Sigma-Aldrich) and prepared for measurements, as previously described (31).

Plasma arginine, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). Four hundred microliters of blood was taken, mixed with 10 \( \mu \)l of heparin, centrifuged, and the plasma was stored at \(-20^\circ\)C. Arginine, ADMA, and SDMA concentrations were determined by HPLC, as described previously (11, 39). In brief, solid-phase extraction on polymeric cation-exchange extraction columns was performed after addition of monomethylarginine as an internal standard. After derivatization with ortho-phthalaldehyd reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection. Interassay coefficients of variation were <3% for arginine and ADMA and <4% for SDMA.

**Renal medullary NO concentration, blood flow, and \( P_{O_2} \).** The animals were allowed a 60-min recovery period after surgery followed by 6 × 30 min of measurements. After 2 × 30 min of control measurements, a bolus injection of \( l \)-arginine (50 mg/kg iv, Sigma-Aldrich) followed by infusion of \( l \)-arginine dissolved in Ringer solution (150 mg kg\(^{-1}\) h\(^{-1} \)) was given. After another 2 × 30 min, a bolus injection of \( l \)-NAME (10 mg/kg iv, Sigma-Aldrich) followed by infusion of \( l \)-NAME (3 mg kg\(^{-1}\) h\(^{-1} \)) iv was administered. GFR was estimated by inulin clearance. \( [\text{H}] \)inulin (185 kBq/ml, American Radiolabeled, St. Louis, MO) was initially given as a bolus dose of 185 kBq and then infused (5 mg kg\(^{-1}\) h\(^{-1} \) iv). Urine and arterial blood samples were taken for subsequent analysis.

**Renomedullary NO concentrations were measured by a hemoglobin-trapping technique,** which utilizes that NO stoichiometrically interacts with oxyhemoglobin, forming methemoglobin (2, 18, 44, 45), which is the method of choice for renomedullary NO measurements (18, 45). In brief, oxyhemoglobin [3.0 \( \mu \)mol/l, human A0 hemoglobin (ferrous), Sigma-Aldrich] was dissolved fresh daily in modified Krebs buffer (5.0 mmol/l KCl, 1.7 mmol/l KHPO\(_4\), 2.5 mmol/l CaCl\(_2\), 2.6 mmol/l NaHCO\(_3\), osmolality adjusted to 800 mosmol/ kgH\(_2\)O by addition of NaCl and pH adjusted to 7.35) (32, 44). The microdialysis probe (PES membrane, length 1.0 mm, diameter 0.5 mm and cut off 6 kDa; Angthos, Stockholm, Sweden) was perfused at 2.0 \( \mu \)l/min. The absorbance maximum for oxyhemoglobin was 414.3 nm and for methemoglobin 406.8 nm (44). Spontaneous hemoglobin degradation was corrected by using the absorbance at 411 nm as reference zero (44). The NO concentration was calculated by integrating the areas of net increase in methemoglobin (394–411 nm) and net decrease in oxyhemoglobin (411–430 nm) spectrophotometrically (Lambda 2 UV/VIS Spectrophotometer, PerkinElmer, Boston, MA) (44). Calibration was performed using known concentrations of NO (10, 25, 50, 100, 200 nmol/l) in deoxygenized Krebs buffer. The in vitro detection limit was below 10 nmol/l and the autooxidation was <5% of the total signal at 10 nmol/l. The experiment-specific autooxidation was determined and subtracted from the measurement.

Blood flow was measured using laser-Doppler flowmetry (probe 411, Permire, Stockholm, Sweden) (31) and \( P_{O_2} \) by modified Clark-type microelectrodes (Unisense, Aarhus, Denmark) (23, 35). Probes were inserted into the renal medulla (~4 mm from the surface) with micromanipulators and recorded continuously using MacLab (AD Instruments, Hastings, UK).

The left kidney was weighed and dissected to verify the sites of measurements. Incorrectly located measurements were discarded.

**Urine parameters.** The urine volumes were measured gravimetrically, the osmolality by a freezing-point technique (model 3MO, Advanced Instruments, Norwood, MA), and urinary sodium and potassium concentrations by flame spectrophotometry (IL543, Instrumentation Lab, Milan, Italy).

**Quantification of mRNA for CATs.** Total RNA was isolated with the guanidinium-based lysis buffer method with an RNAquous-4 PCR Kit (Ambion, Austin, TX) and treated with DNaseI. RT reactions were performed using Superscript III first-strand cDNA synthesis (Invitrogen, Carlsbad, CA). For real-time PCR (ABI PRISM 7700, ABI, Foster City, CA) of mRNA CAT-1, the Taqman probe set used was forward 5'-CCAGAACCACCGTGAGCT-3' and reverse 5'-TGATGCTGTG- CACCAGCAT-3' (GenBank accession no. U70476), and of mRNA CAT-2A forward 5'-GGATGCCTAGTGGTATG-3' and reverse 5'-AGAATCAGAAAAAGTAGCC-3' (accession no. AF158025). Endogenous 18S ribosomal RNA was used as a reference.
Arginase activity. Arginase activities in liver and kidney cortex homogenates were analyzed, as previously described (19). Protein expression of arginase in kidney cortex and liver. Samples were homogenized in 700 μl buffer (1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM NaF, 80 mM Tris, pH 7.5) containing enzyme inhibitors (phosphatase inhibitor cocktail-2, 10 μl/ml, Sigma-Aldrich, and Complete Mini; 1 tablet/1.5 ml, Roche Diagnostics, Mannheim, Germany). Samples were run on 12.5% Tris-HCl gels with Tris/glycine/SDS buffer, and the proteins were detected after transfer to nitrocellulose membranes using goat anti-rat arginase I antibody for liver tissues (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) and goat anti-rat arginase II antibody for kidney tissues (1:1,000; Santa Cruz Biotechnology) and HRP-conjugated secondary antibody (rabbit anti-goat, 1:10,000; Kirkegaard and Perry Laboratories). -Actin was detected using mouse anti-rat-actin antibody (1:10,000; Sigma Aldrich) and secondary HRP-conjugated goat-anti mouse antibody (1:60,000; Kirkegaard and Perry Laboratories).

Plasma urea, ornithine, and citrulline. Urea were analyzed using the colorimetric reaction of urea with 1-phenyl-1,2-propanedione-2-oxime (19). Ornithine and citrulline were analyzed using capillary electrophoresis (P/ACE System 5000 with LIF detector, Beckman Instruments, Fullerton, CA) with homoarginine as an internal standard, as previously described (40). In brief, 10 μl of deproteinized
plasma was mixed with 10 μl borate buffer (193 mmol/l) and 30 μl 4-fluor-7-nitrobenzofurazan dissolved in anhydrous acetonitrile (6.8 mmol/l), heated to 60°C for 6 min and separated using a 36-cm capillary with an internal diameter of 30 μm. Separation conditions were 5-s injection by pressure, 15 kV, excitation at 488 nm, fluorescence detection at 520 nm, running buffer consisting of 193 mmol/l borate + 100 mmol/l deoxycholic acid, and 20°C.

Statistics. All values are means ± SE. Multiple comparisons between different groups were performed using ANOVA followed by Fisher’s least significant difference test. Multiple comparisons within the same group were performed using repeated-measures ANOVA followed by a post hoc test for paired comparisons (Statview, Abacus Concepts, Berkeley, CA). P < 0.05 was considered statistically significant.

RESULTS

Blood glucose concentrations were 5.2 ± 0.1 mmol/l in the control animals (n = 8) and 20.7 ± 0.6 mmol/l in the 2-wk diabetic animals (n = 10, P < 0.05). The kidney weights increased in the 2-wk diabetic animals compared with the control animals (1.29 ± 0.04 and 1.10 ± 0.03 g, respectively; P < 0.05). Both blood glucose and kidney weight were unaltered by treatment with α-tocopherol (data not shown).

Nitric oxide. The baseline renomedullary NO concentration was lower in the diabetic animals compared with the control animals and was unaffected by long-term α-tocopherol treatment in both control and diabetic animals (Fig. 1). L-Arginine administration increased the NO concentration more in diabetic animals than in control animals. Furthermore, there was a larger effect of L-arginine administration in the diabetic animals treated with α-tocopherol than in untreated diabetic animals (P < 0.05). Nω-nitro-L-arginine methyl ester (L-NAME) caused a pronounced decrease in renomedullary NO concentration in all investigated groups, but a NO signal corresponding to ~17 nmol/l was still present after L-NAME administration in all groups.

PO2. The baseline renomedullary PO2 was lower in the diabetic animals (104.0 ± 8 vs. 103.8 ± 7.3 laser units, n = 10, respectively) and was unaffected by α-tocopherol treatment (105 ± 9.5, n = 7, and 108.4 ± 7.3 laser units, n = 7, for α-tocopherol-treated control and diabetic animals, respectively). L-Arginine increased blood flow in all groups compared with baseline, while L-NAME caused a pronounced decrease in blood flow, which was similar in all groups (Fig. 2).

l-Arginine, ADMA, arginine/ADMA ratio, and SDMA. Diabetic animals had lower plasma arginine (−49%) and ADMA (−17%) compared with controls (Figs. 4, A and B). α-Tocopherol treatment did not affect either of these parameters. The calculated arginine/ADMA ratios were higher in both control groups (Fig. 4C), whereas SDMA was similar in all investigated groups (Fig. 4D).

Glomerular filtration rate and urinary excretion of electrolytes. The 2-week diabetic animals had higher a baseline glomerular filtration rate (GFR) than control animals, which was unaffected by α-tocopherol treatment (Table 1). L-Arginine administration resulted in increased GFR in the α-tocopherol-treated control animals, whereas the GFR in untreated control animals and both groups of diabetic animals was unaffected by L-arginine. L-NAME caused a pronounced decrease in GFR in all investigated groups. Baseline urinary flow rate was increased sevenfold in all diabetic animals compared with control animals (Table 1). L-Arginine selectively decreased the urinary flow rate in the diabetic animals. L-NAME increased urinary flow rate in both untreated and α-tocopherol-treated controls, but it decreased the flow rate in the diabetic animals. The baseline sodium excretion was not statistically significant between any of the groups (Table 1). L-Arginine increased sodium excretion in the diabetic animals, but not in control animals. L-NAME increased sodium excretion in all animals compared with baseline, except for α-tocopherol-treated control animals. All diabetic animals had higher potas-

| Table 1. Renal parameters and blood pressure obtained from control and diabetic animals with or without α-tocopherol treatment during baseline and after administration of l-arginine followed by l-NAME |
|----------------|----------------|----------------|----------------|----------------|----------------|
|                | Glomerular Filtration Rate, ml/min | Urinary Flow Rate, μl/min | Sodium Excretion, pmol/min | Potassium Excretion, pmol/min | Mean Blood Pressure, mmHg |
| Control (n = 8) |                             |                             |                             |                             |                             |
| Baseline       | 0.92 ± 0.09                 | 1.29 ± 0.20                 | 72 ± 9                      | 316 ± 124*                 | 113 ± 7                     |
| L-Arginine     | 1.02 ± 0.07                 | 1.94 ± 0.35                 | 123 ± 36*                  | 799 ± 356*                 | 108 ± 7                     |
| L-NAME         | 0.71 ± 0.07*                | 4.34 ± 1.22*                | 59 ± 10                    | 554 ± 122*                 | 120 ± 6*                    |
| Control + α-tocopherol (n = 7) |                             |                             |                             |                             |                             |
| Baseline       | 1.03 ± 0.06                 | 1.09 ± 0.20                 | 44 ± 12                    | 102 ± 44                   | 109 ± 4                     |
| L-Arginine     | 1.15 ± 0.06*                | 1.57 ± 0.32                 | 60 ± 16                    | 280 ± 117                  | 109 ± 4                     |
| L-NAME         | 0.73 ± 0.09*                | 3.61 ± 0.57*                | 59 ± 10                    | 554 ± 122*                 | 120 ± 6*                    |
| Diabetes (n = 10) |                             |                             |                             |                             |                             |
| Baseline       | 1.17 ± 0.08†                | 8.38 ± 1.99†                | 126 ± 36                   | 464 ± 61†                  | 123 ± 3†                    |
| L-Arginine     | 1.08 ± 0.06                 | 4.96 ± 0.62‡                | 355 ± 51†                  | 751 ± 122‡                 | 123 ± 5                    |
| L-NAME         | 0.62 ± 0.06*                | 3.14 ± 0.54*                | 290 ± 55†                  | 537 ± 77                   | 140 ± 3‡                    |
| Diabetes + α-tocopherol (n = 7) |                             |                             |                             |                             |                             |
| Baseline       | 1.28 ± 0.08†                | 8.76 ± 2.45†                | 144 ± 35†                  | 407 ± 96†                  | 114 ± 3                     |
| L-Arginine     | 1.27 ± 0.07                 | 6.65 ± 1.72‡                | 331 ± 43†                  | 609 ± 74                   | 111 ± 2                     |
| L-NAME         | 0.90 ± 0.10*                | 4.12 ± 0.92*                | 332 ± 58†                  | 585 ± 118                  | 123 ± 6†                    |

Values are means ± SE of the 60-min sampling period. L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 compared with baseline within the same group. †P < 0.05 compared with the corresponding period for control animals.

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sium excretion compared with control animals (Table 1). L-arginine increased potassium excretion in both untreated control and untreated diabetic animals. L-NAME increased potassium excretion in all control animals.

Blood pressure. Mean arterial blood pressure was higher in the untreated diabetic animals compared with the corresponding control group (Table 1). Arginine did not affect blood pressure, whereas L-NAME increased blood pressure in all investigated animals.

CAT mRNAs, ornithine, citrulline, arginase activities, and arginase protein expression. Hepatic mRNA expression of CAT-2A and plasma ornithine concentrations were increased in both diabetic groups compared with controls, whereas hepatic arginase activities and plasma citrulline concentrations were similar in all groups (Figs. 5, A and B, and Table 2). Plasma urea concentration, hepatic arginase I protein expression, and cortical arginase activity were elevated in diabetic animals compared with controls (Fig. 5C and Tables 2 and 3). Cortical CAT-1 mRNA and arginase II protein expressions were similar in all groups (Table 3).

**DISCUSSION**

The main finding of the present study was that renomedullary NO bioavailability was markedly decreased in STZ-induced diabetic rats and was not prevented by long-term antioxidant treatment. The reduced NO concentration could instead be related to substrate limitation, manifested as decreased plasma concentration of l-arginine. Interestingly, intravenous administration of l-arginine only restored the bioavailable renomedullary NO concentration in diabetic animals that had been treated with the reactive oxygen species (ROS) scavenger α-tocopherol, suggesting at least two separate mechanisms for the reduced NO. Furthermore, increasing the renomedullary NO concentration directly increased medullary PO2 in diabetic animals. These findings indicate that STZ-induced diabetic rats have reduced plasma arginine and increased ornithine levels due to increased hepatic arginine uptake via CAT-2A, which results in decreased NO bioavailability in the renal medulla. Furthermore, these results also demonstrate that reduced renomedullary NO concentration has important implications for oxygen availability in this region of the kidney, independently of any hemodynamic alteration. Increased oxidative stress can only in part explain these alterations.

Arginine availability and renomedullary NO availability. NO synthesis by intracellularly located NOS is highly dependent on the cellular transport of arginine and therefore dependent on the extracellular arginine availability. The plasma arginine concentration is regulated by de novo arginine synthesis predominantly by the proximal tubular cells, cellular arginine transport, and degradation rate by hepatic arginase (25). It has been shown that arginine synthesis depends on the plasma citrulline level (13). It is therefore unlikely that the reduced arginine levels in the diabetic animals reflect decreased arginine synthesis, since all investigated animals had similar citrulline levels. However, the diabetic rats had increased hepatic CAT-2A mRNA expression. This suggests increased hepatic uptake, which is the rate-limiting step in hepatic arginine metabolism (9). The presence of increased plasma ornithine levels further confirmed increased arginine degradation as the main cause of reduced plasma arginine levels in the diabetic animals. Diabetic rats had increased protein expression of hepatic arginase I, although the hepatic in vitro arginase activity was similar in all groups. The reason...
adaptation to reduced O2 availability. Interestingly, NO regulation (20, 21), and the results from the present study support this conclusion that the diabetic animals have reduced renomedullary NO concentration independently of increased oxidative stress. Similar reductions in plasma arginine levels have been reported from salt-restricted rats, which also was linked to increased hepatic arginine metabolism (19). These findings resulted in the conclusion that the diabetic animals have reduced renomedullary P02 due to increased oxidative stress, but also due to the reduced NO availability.

**Endogenous NOS inhibition.** Interestingly, the lower NO concentration in the diabetic animals is unlikely to be a result of competitive NOS inhibition by endogenous ADMA, since the plasma level of ADMA in the diabetic animals was lower than in control animals. However, the arginine/ADMA ratio concentration in the diabetic animals is unlikely to be a result of increased oxidative stress, but also due to the reduced NO availability.

for this discrepancy is presently unknown but might include different posttranslational regulation of the enzymatic activity during the sustained hyperglycemia. The reduced plasma levels of arginine occurred despite the fact that diabetic animals have a higher food intake (34), which potentially could counteract these reductions. Indeed, plasma urea levels were increased in untreated diabetics, and antioxidant treatment prevented this increase. A plausible explanation includes that fact that the reduced oxidative stress in the treated diabetic animals improved kidney function. Since the kidneys are the major source of urea elimination from the plasma, an improved kidney function might counteract any increase in plasma urea levels.

We also investigated the compartment of the arginine-degrading system in the kidney cortex. Although diabetic animals displayed increased in vitro arginase activity in the kidney cortex, but not arginase II protein expression, these alterations are most likely insignificant for regulating plasma arginine levels. The insignificant role of the kidney cortex in the regulation of plasma arginine levels in these diabetic animals is further supported by the findings that all investigated groups had similar cortical CAT-1 mRNA levels and that antioxidant treatment normalized both cortical arginase II protein expression and activity, whereas it failed to normalize plasma arginine.

From the above findings, we conclude that diabetic animals have increased hepatic arginine degradation resulting in reduced plasma arginine levels, which limited the renomedullary NO levels.

**Interaction among renomedullary NO, oxygen availability, and oxidative stress.** NO synthesis by NOS is also dependent on oxygen availability, and the Kox for oxygen of the different NOS isoforms (6). In the present study, the diabetic animals had 22% lower average basal renomedullary tissue P02, which was prevented by long-term α-tocopherol treatment. We have previously shown that reduced renomedullary P02 is related to increased cellular oxygen consumption in diabetic animals (31). Increased ROS production increases the scavenging of NO, which directly stimulates the mitochondrial oxygen utilization (20, 21), and the results from the present study support this as being an important regulator of oxygen availability in the renal medulla of diabetic rats. Indeed, several reports have shown that reducing the NO bioavailability results in increased oxygen consumption (8, 12, 22). Possible sources of oxidative stress in diabetes are NADPH oxidase, xanthine oxidase, uncoupled NO synthase, and mitochondria (14). Normalizing mitochondrial ROS formation has been shown to prevent the activation of several classic pathways known to contribute to the development of diabetic nephropathy (27).

Hypoxia-inducible factor (HIF)-1 plays a pivotal role in the adaptation to reduced O2 availability. Interestingly, NO regulates the activities and/or levels of the HIF-1α-degrading proteins prolyl hydroxylase domain-containing enzymes (PHD1, -2, and -3). Berchner-Pfannschmidt and coworkers (3) showed that NO initially, both during normoxic and hypoxic conditions, inhibits PHD with subsequent increased HIF-1α levels. However, long-term NO exposure resulted in decreased HIF-1α levels due to de novo synthesis of PHD2, further demonstrating an important role of NO in maintaining O2 homeostasis.

The α-tocopherol-treated diabetic animals had a larger NO increase in response to l-arginine administration, which in part might have contributed to the increased oxygen availability. Cellular arginine transport in the renal medulla is predominantly accomplished by CAT-1 (18), and the diabetic animals had indeed increased CAT-1 mRNA expression in the kidney cortex. However, the increased NO concentration after l-arginine administration in the diabetic animals suggests that the cellular arginine transporters operate at a subsaturated condition and that any alteration in CAT-1 expression only has a minor effect on overall NO production. A more plausible explanation is that the antioxidant treatment prevented NOS uncoupling and reduced the superoxide levels, both contributing to increased NO bioavailability after arginine stimulation. Furthermore, our results suggest that reduced arginine availability is a major determinant of renomedullary NO concentration independently of increased oxidative stress. Similar reductions in plasma arginine levels have been reported from salt-restricted rats, which also was linked to increased hepatic arginine metabolism (19). These findings resulted in the conclusion that the diabetic animals have reduced renomedullary P02 due to increased oxidative stress, but also due to the reduced NO availability.
factors, including NO release from inhibition. Several possible mechanisms might be contributing between control and diabetic animals after NOS inhibition with there were no differences in the medullary blood flow response mediated alterations in blood flow in the renal cortex (30), been shown to be able to release NO in the renal medulla for alterations. Reducing the oxidative stress by interacting with medullary NO and PO2. The NO deficiency is linked to alterations. Glomerular hyperfiltration was present in the STZ-treated animals, as commonly reported during the initial phase after the onset of diabetes (33). Notable is that α-tocopherol prevented the decrease in renomedullary PO2 without affecting hyperfiltration, which we also previously have reported (31). These findings resulted in the conclusion that renomedullary blood flow is regulated similarly in control and diabetic rats and that any differences observed in oxygen availability between the two groups are largely independent of blood flow alterations.

In conclusion, diabetic animals have reduced renomedullary NO concentration and PO2. The NO deficiency is linked to decreased arginine availability following increased hepatic arginine degradation. Administration of arginine concomitantly increased medullary NO and PO2 in diabetic animals independently of altered hemodynamics, inferring metabolic alterations. Reducing the oxidative stress by α-tocopherol treatment augmented the NO response to arginine administration in diabetic animals but did not restore basal NO deficiency per se. These findings demonstrate that NO regulates oxygen availability and that reduced NO may cause hypoxia in the renal medulla. These mechanisms can be important factors in the onset and progression of renal metabolic and hemodynamic alterations commonly associated with sustained hyperglycemia.

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