Nitric oxide originating from NOS1 controls oxygen utilization and electrolyte transport efficiency in the diabetic kidney

Fredrik Palm,1,2 Angelica Fasching,1 Peter Hansell,1 and Örjan Källskog1

1Division of Integrative Physiology, Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; and 2Division of Nephrology and Hypertension, Department of Medicine, Georgetown University, Washington, District of Columbia

Submitted 27 April 2009; accepted in final form 11 November 2009

Palm F, Fasching A, Hansell P, Källskog Ö. Nitric oxide originating from NOS1 controls oxygen utilization and electrolyte transport efficiency in the diabetic kidney. Am J Physiol Renal Physiol 298: F416–F420, 2010. First published November 18, 2009; doi:10.1152/ajprenal.00229.2009.—Nitric oxide (NO) is a potent regulator of both vascular tone and cellular oxygen consumption (QO2). Diabetic kidneys have reduced NO availability and increased QO2. However, the exact nitric oxide synthase (NOS) isoform regulating QO2, hemodynamics, and excretory function in the diabetic kidney remains unclear. We therefore investigated the effects of both selective neuronal NOS (NOS1) inhibition and nonselective NOS inhibition. Oxygen utilization, electrolyte transport efficiency [tubular Na+/H+ (TNa/H+), renal blood flow (RBF), glomerular filtration rate (GFR), and mean arterial pressure (MAP)] were measured in vivo in control and streptozotocindiabetic rats before and after administration of the selective NOS1 inhibitor S-methyl-L-thiocitrulline (SMTC) or the nonselective NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). Diabetic rats had higher baseline QO2 and GFR than control rats, although RBF was similar in the groups. SMTC and L-NAME increased QO2 and reduced TNa/H+ only in the diabetic animals, whereas both inhibitors increased MAP and reduced RBF in both groups. GFR was reduced by L-NAME, but SMTC had no effect in either group. Carbachol increased RBF and decreased MAP in SMTC-treated rats, whereas it had no effect in L-NAME-treated rats, indicating that SMTC selectively inhibited NOS1. In conclusion, NO regulates RBF and GFR similarly in both control and diabetic rats. However, selective NOS1 inhibition increased QO2 and reduced TNa/H+ in the diabetic rat kidney, indicating a pivotal role of NO produced by NOS1 in maintaining control of QO2 and tissue oxygenation in these kidneys.

MATERIALS AND METHODS

All chemicals were from Sigma-Aldrich (St. Louis, MO) and of the highest grade available if not otherwise stated.

Animals and induction of diabetes. Age-matched male Wistar-Furth rats weighing 250–300 g were purchased from B&K (Sollentuna, Sweden). Animals had free access to water and standard rat chow (0.3% Na, 0.8% K, 21% protein; R3, Ewos, Södertälje, Sweden) throughout the study. All experiments were performed in accordance with the National Institutes of Health guidelines for use and care of laboratory animals and were approved by the Animal Care and Use Committee for Uppsala University. Diabetes was induced by an injection of streptozotocin (STZ; 45 mg/kg body wt; Sigma-Aldrich) in the tail vein. Animals were considered diabetic if blood glucose concentrations increased to >18 mmol/l within 24 h after STZ injection and remained elevated. Blood glucose concentrations were determined with test reagent strips (MediSense, Bedford, MA) in blood samples obtained from the cut tip of the tail in all animals.

Surgical procedures. Two weeks after allocation to the study, the animals (n = 10–12/group) were anesthetized with an intraperitoneal injection of thiobutabarbital (120 mg/kg body wt; Inactin, Sigma-Aldrich), placed on a thermo-controlled operating table at 37°C, and tracheotomized. Polyethylene catheters were placed in the right femoral vein for infusion of Ringer solution (5 ml · kg body wt−1 · h−1 for normoglycemic control animals, 10 ml · kg body wt−1 · h−1 for...
diabetic animals), the right femoral artery for blood pressure measurements (Statham P23DB, Statham Laboratories, Los Angeles, CA), and the left renal vein and carotid artery for blood samplings. The left ureter was catheterized to collect urine for subsequent analysis, and the urinary bladder was catheterized to allow urinary drainage. The left kidney was exposed by a left subcostal flank incision, immobilized in a plastic cup, and embedded in pieces of saline-soaked cotton wool, and the surface was covered with paraffin oil (Aptoketsbolaget, Gothenburg, Sweden).

**Simultaneous measurements of total renal QO₂, GFR, and RBF.** Animals were allowed a 45-min recovery period after surgery followed by 30 min of baseline measurements. Thereafter, either the NOS1-selective inhibitor S-methyl-l-thiocitrulline (SMTC; 1 mg/kg body wt bolus + 1 mg · kg body wt⁻¹ · h⁻¹ continuous infusion) (6) or the nonselective NOS inhibitor N⁵-nitro-l-arginine methyl ester (l-NAME; 10 mg/kg body wt bolus + 10 mg · kg body wt⁻¹ · h⁻¹ continuous infusion) (22) was administered, followed by a 15-min stabilization period. All parameters were thereafter measured for another 30-min period.

GFR was estimated by measurements of clearance of [³H]inulin. The [³H] was given initially as a bolus dose of 185 kBq (American Radiolabeled Chemicals, St. Louis, MO) and then continuously infused in Ringer solution (185 kBq · kg body wt⁻¹ · h⁻¹). The [³H] activities in urine and plasma were measured with a standard liquid scintillation technique, and GFR was estimated from inulin clearance and calculated according to GFR = U · V/P, where U and P denote the activity of [³H]inulin in the urine and plasma, respectively, and V denotes the urine flow in milliliters per minute.

Total RBF was measured with an ultrasound probe placed around the left renal artery (Transonic Systems, Ithaca, NY), and cortical RBF was measured with laser-Doppler flowmetry (PF 4001-2, Perimed, Stockholm, Sweden) (10). All measured parameters were continuously recorded with a Power Lab instrument (AD Instruments, Hastings, UK). Blood gas parameters were analyzed (iSTAT, Abbott, Princeton, NJ) on samples drawn from the left renal vein and carotid artery before and after administration of the NOS inhibitors at the end of each period. Kidney weights were determined at the end of the experiments. Urine volumes were measured gravimetrically, and urinary Na⁺ concentrations were measured by flame spectrophotometry (IL543, Instrumentation Lab, Milan, Italy).

**Verification of NOS1-selective inhibition of SMTC.** In a separate set of experiments (n = 5/group), Inactin-anesthetized rats were tracheotomized and catheters were placed in the right femoral artery for monitoring blood pressure, in the right femoral vein for infusion of drugs, and in the bladder. One ultrasound flow probe (Transonic Systems) was placed around the left renal artery and a second ultrasound flow probe (Transonic Systems) around the left femoral artery. The 30-min recovery period after surgery was followed by 10 min of baseline recordings before administration of vehicle, SMTC (1 mg/kg body wt bolus + 1 mg · kg body wt⁻¹ · h⁻¹ continuous infusion), or l-NAME (10 mg/kg body wt bolus + 10 mg · kg body wt⁻¹ · h⁻¹ continuous infusion). Fifteen minutes thereafter, the acetylcholine analog carbachol (1 mg · kg body wt⁻¹ · h⁻¹) was continuously infused for 5 min. Renal vascular resistance (RVR) and femoral vascular resistance were calculated.

**Calculations.** The filtration fraction (FF) was estimated as FF = (GFR/MBF) · (1 − Hct). RVR was calculated as mean arterial pressure (MAP) divided by RBF. In vivo renal QO₂ (μmol · min⁻¹ · kidney⁻¹) was estimated from the arteriovenous difference in O₂ content with a standard equation (O₂ct = [Hb] · O₂ saturation · 1.34 + PO₂ · 0.003) multiplied by total RBF. Tubular Na⁺ transport (TNa) per QO₂ was calculated from TNa/QO₂ with TNa = plasma Na⁺ concentration · GFR.

**Statistical evaluation.** All statistical analyses were performed with GraphPad Prism software (GraphPad Software, San Diego, CA). Multiple comparisons between different groups were performed by analysis of variance (ANOVA) followed by Tukey’s post hoc test. Multiple comparisons within the same group were performed by repeated-measures ANOVA followed by Dunnett’s or Tukey’s post hoc tests for paired comparisons. When comparing before and after a treatment within the same animals, a paired Student’s t-test was used. FF data were analyzed with the Mann-Whitney test for unpaired or paired comparisons. Descriptive statistics are presented as mean values ± SE. For all comparisons, P < 0.05 was considered statistically significant.

**RESULTS**

All diabetic animals had hyperglycemia compared with normoglycemic control animals [20.2 ± 0.6 (n = 22) vs. 4.5 ± 0.1 mM (n = 20)]. Diabetic animals weighed less (293 ± 4 g; n = 22) compared with the age-matched normoglycemic control animals (346 ± 9 g; n = 20). Kidney weights increased in diabetic animals compared with normoglycemic control animals (left 1.43 ± 0.02 and right 1.46 ± 0.02 g vs. 1.13 ± 0.02 and 1.13 ± 0.03 g; n = 22 and n = 20, respectively).

Diabetic kidneys had higher baseline QO₂ compared with controls when all baseline values from the diabetic groups were compared with those of the control groups [10.9 ± 1.4 (n = 22)] vs. 7.4 ± 0.8 μmol · min⁻¹ · kidney⁻¹ (n = 20), respectively; P < 0.05] (Fig. 1A). TNa/QO₂ was similar in both groups (Fig. 1B). Both SMTC and l-NAME selectively affected QO₂ and TNa/QO₂ only in the diabetic kidneys (Fig. 1).

Baseline MAP and RVR were similar and increased after administration of both SMTC and l-NAME in both groups, although SMTC caused a smaller blood pressure increase in the diabetic group compared with the corresponding control group (Fig. 2). Total and cortical blood flow decreased after both SMTC and l-NAME (Fig. 1B). Both SMTC and l-NAME selectively affected QO₂ and TNa/QO₂ only in the diabetic kidneys (Fig. 1).

**Fig. 1. Total oxygen consumption (A) and transported sodium per consumed oxygen (B) in control and diabetic rats during baseline (open bars) and after respective treatment (gray bars). *P < 0.05 vs. baseline within the same group; #P < 0.05 vs. corresponding control group at corresponding time. Values are means ± SE. SMTC, S-methyl-l-thiocitrulline; l-NAME, N⁵-nitro-l-arginine methyl ester.
l-NAME decreased GFR in both groups. Baseline FF was increased in diabetic animals compared with control animals when all baseline values from the diabetic groups were compared with those of the control groups \(0.53 \pm 0.03 \text{ (n = 22) vs. } 0.43 \pm 0.02 \text{ (n = 20) \; respectively; } P < 0.05\). SMTC and l-NAME increased FF in control, whereas neither NOS inhibitor affected FF in diabetic rats (Fig. 3B).

Urinary flow was higher in the diabetic animals than in control animals and was only significantly altered in diabetic rats after administration of SMTC (Table 2). Baseline urinary Na\(^+\) excretion was similar in both groups, and only SMTC administered to diabetic rats affected Na\(^+\) excretion.

Carbachol alone decreased MAP, RVR, and femoral vasculature distal to the femoral artery and decreased blood carbachol induced vasodilatation in both renal vasculature and aortic arch. NOS1 AND OXYGEN UTILIZATION IN DIABETIC KIDNEY

Fig. 2. Mean arterial blood pressure (A) and renal vascular resistance (B) in control and diabetic rats during baseline (open bars) and after respective treatment (gray bars). *\(P < 0.05\) vs. baseline within the same group; †\(P < 0.05\) vs. corresponding control group at corresponding time. Values are means ± SE.

DISCUSSION

The main new findings from the present study are that QO\(_2\) in the diabetic kidney is closely regulated by NO released from NOS1. It appears that NO generated from NOS1 acts as a “brake” to slow metabolism in an effort to normalize or constrain QO\(_2\) in the diabetic kidney. Selective NOS1 blockade did not affect GFR in either control or diabetic rats, whereas it significantly increased RVR. However, GFR decreased drastically when nonselective NOS inhibition was applied, implying a significant involvement of NO released from NOS3 in the control of GFR.

In this study, we applied selective NOS1 inhibition similar to what has previously been described (6) and we confirmed that the dose of SMTC in the present study selectively inhibits NOS1. Importantly, we showed that the acetylcholine analog carbachol induced vasodilation in both renal vasculature and muscle distal to the femoral artery and decreased blood pressure to a similar degree in animals administered either

![Fig. 3. Glomerular filtration rate (A) and filtration fraction (B) in control and diabetic rats during baseline (open bars) and after respective treatment (gray bars). *\(P < 0.05\) vs. baseline within the same group; †\(P < 0.05\) vs. corresponding control group at corresponding time. Values are means ± SE.](http://ajprenal.physiology.org/)

### Table 1. Total and cortical renal blood flows before and after respective treatment in control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Total Renal Blood Flow, ml·min(^{-1})·kidney(^{-1})</th>
<th>Cortical Renal Blood Flow, laser units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Before</td>
<td>After</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMTC</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>l-NAME</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMTC</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>l-NAME</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for \(n\) animals. SMTC, S-methyl-thiocitrulline; l-NAME, N\(^o\)-nitro-l-arginine methyl ester. *\(P < 0.05\) compared with baseline within the same group.

### Table 2. Urinary flow and urinary extraction of sodium before and after respective treatment in control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Urinary Flow Rate, (\mu l)·min(^{-1})·kidney(^{-1})</th>
<th>Na(^+) Excretion, pmol·min(^{-1})·kidney(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Before</td>
<td>After</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMTC</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>l-NAME</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMTC</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>l-NAME</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE for \(n\) animals. *\(P < 0.05\) compared with baseline within the same group; †\(P < 0.05\) compared with corresponding control group at corresponding time.
We (24) and others (12) have reported increased QO2 by the diabetic kidney. This is in agreement with previous studies in both rats and humans (27, 28, 31). Carbachol had no effect when administered to L-NAME-treated rats. However, SMTC caused a similar increase in RVR and MAP but did not affect the vascular response to carbachol, indicating a fully working NOS3 system. Interestingly, Deng et al. (6) reported only minor effects of a similar SMTC dose on MAP, RBF, GFR, and QO2, although TNa/QO2 significantly decreased. Our results are somewhat deviating from this previous report, since we found a significant effect of SMTC on MAP and RBF, the latter measured by two separate techniques. However, we confirm the previous report that a low dose of SMTC does not affect GFR or QO2. These discrepancies might be attributed to strain differences (Wistar-Furth vs. Sprague-Dawley rats) but were not investigated further.

We (24) and others (12) have reported increased QO2 by the diabetic kidney and in isolated cells from diabetic kidneys. Increased QO2 by the diabetic kidney results in reduced tissue oxygen availability (21, 26), which may contribute to the development of progressive kidney dysfunction ultimately leading to chronic renal failure (29). Several factors are likely to contribute to the increased QO2 by the diabetic kidney, including reduced NO bioavailability (22, 24), increased mitochondria uncoupling (7), and increased tubular electrolyte transport due to the elevated GFR (12). Although the diabetic kidney utilizes more oxygen, there are still mechanisms present that regulate QO2 and mitochondrial efficiency. In this study, we show that NO released from NOS1 is a major regulator for maintaining control of QO2 in the diabetic kidney. Interestingly, the Na\(^+\) transport mediated by both Na\(^+\)/H\(^+\) exchange and the Na\(^+\)/K\(^+\)/ATPase is inhibited by NO (8, 20). Indeed, we found increased urinary Na\(^+\) excretion but also decreased TNa/QO2 after NOS1 blockade. However, it is likely that NO mainly influences total kidney QO2 and the calculated TNa/QO2 by affecting mitochondrial oxygen usage. A possible explanation for the increased influence of NOS1 on QO2 in the diabetic animals might be the generally lower NO concentrations found in the kidney cortex of these animals. NOS1 might provide a greater portion of the total NO produced, which results in higher NO levels in the tubular cells responsible for the major part of QO2 and electrolyte transport. It should be noted that NOS1 is mainly localized to macula densa cells and efferent arteriole in the kidney cortex (2), but Deng et al. (6) demonstrated that NOS1 possesses inhibitory properties on QO2 by isolated proximal tubular cells. NO effectively inhibits mitochondria respiration in a dose-dependent manner (9, 10) and has been shown to have this effect in the kidney, especially during situations of low oxygen tension (1) as is the situation in the diabetic kidney.

RVR increased whereas RBF decreased to the same extent after both NOS1 and nonselective NOS inhibition in both control and diabetic rats, indicating a pivotal role for NOS1 in the regulation of intrarenal hemodynamics. Most previous studies have shown that both NOS1 and nonselective NOS inhibition reduce RBF and/or increase RVR in both control and diabetic rats (3, 6, 11, 22, 24, 32). In contrast, NOS1 inhibition did not alter GFR in either control or diabetic rats, whereas nonselective NOS inhibition decreased GFR in the present study. Bachmann and coworkers (2) described the precise intrarenal location of NOS1 and reported massive NOS1 staining preferentially in the efferent arteriole in the renal vasculature. Thus selective NOS1 inhibition will decrease RBF by constriction of the efferent arteriole, but without decreasing GFR since the glomerular filtration pressure is maintained. The FF can give some insight into the site of vasoconstriction. The increased FF and reduced RBF after both NOS1 and nonselective NOS blockade in control animals confirm previous reports (3, 6, 11, 32) and indicate a preferential vasoconstriction of the efferent arteriole. In addition to any direct vascular effect of nonselective NOS inhibition, the reduction in renal plasma flow will also limit GFR. The hemodynamic response to NOS blockade was generally similar in the diabetic rats although there were no significant changes in FF. The reason for discrepancy between the present study and the previous reports is not known, but the increased baseline FF in Wistar-Furth compared with Sprague-Dawley rats (18) might explain the difference. Nevertheless, it should also be noted that GFR can be regulated by mechanisms dissociated from direct hemody-
namic effects (15), but this is beyond the scope of the present study.

In conclusion, NO regulates RBF and GFR similarly in both control and diabetic rats. However, selective NOS1 inhibition increased QO2 and reduced TNa/QO2 in the diabetic rat kidney, indicating a pivotal role of NO produced by NOS1 in maintaining control of QO2 and tissue oxygenation in these kidneys. These findings further highlight the pivotal role of the NO system in maintaining kidney function in diabetes.

NOTE ADDED IN PROOF

The authors discovered that the filtration fraction was calculated using an incorrect formula when the manuscript was proofread. The correct filtration fraction values are now reported in RESULTS and in Fig. 3B, showing substantially higher values than originally reported. Furthermore, the paragraph in DISCUSSION regarding the filtration fractions has been adjusted to reflect the corrected results. These changes were made after the review process was completed but do not alter the conclusions of this study.

GRANTS

This study was supported by the Swedish Medical Research Council (9940, 72XD-15043, 10840, 14X-2553, and K2003-04X-03522-32), the Swedish Society for Medical Research, the Fredrik and Ingrid Thuring Foundation, the Marcus and Amalia Wallenberg Foundation, the Magnus Bergvall Foundation, and a National Institute of Diabetes and Digestive and Kidney Diseases K99/R00 Grant (DK-077858 to F. Palm).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


