Role of nitric oxide deficiency in the development of hypertension in hydronephrotic animals

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Carlström M, Brown RD, Edlund J, Sällström J, Larsson E, Teerlink T, Palm F, Wåhlin N, Persson AE. Role of nitric oxide deficiency in the development of hypertension in hydronephrotic animals. Am J Physiol Renal Physiol 294: F362–F370, 2008. First published November 21, 2007; doi:10.1152/ajprenal.00410.2007.—Hydronephrotic animals develop renal injury and hypertension, which is associated with an abnormal tubuloglomerular feedback (TGF). The TGF sensitivity is coupled to nitric oxide (NO) in the macula densa. The involvement of reduced NO availability in the development of hypertension in hydronephrosis was investigated. Hydronephrosis was induced by ureteral obstruction in young rats. Blood pressure and renal excretion were measured in adulthood, under different sodium conditions, and before and after chronic administration of either Nω-nitro-L-arginine methyl ester (L-NNAME) or L-arginine. Blood samples for ADMA, SDMA, and L-arginine analysis were taken and the renal tissue was used for histology and determination of NO synthase (NOS) proteins. TGF characteristics were determined by stop-flow pressure technique before and after administration of 7-nitroindazole (7-NI) or L-arginine. Hydronephrotic animals developed salt-sensitive hypertension, which was associated with pressure natriuresis and diuresis. The blood pressure response to L-NNAME was attenuated and L-arginine supplementation decreased blood pressure in hydronephrotic animals, but not in the controls. Under control conditions, reactivity and sensitivity of the TGF response were greater in the hydronephrotic group. 7-NI administration increased TGF reactivity and sensitivity in control animals, whereas, in hydronephrotic animals, neuronal NOS (nNOS) inhibition had no effect. L-Arginine attenuated TGF response more in hydronephrotic kidneys than in controls. The hydronephrotic animals displayed various degrees of histopathological changes. ADMA and SDMA levels were higher and the renal expressions of nNOS and endothelial NOS proteins were lower in animals with hydronephrosis. Reduced NO availability in the diseased kidney in hydronephrosis, and subsequent resetting of the TGF mechanism, plays an important role in the development of hypertension.

Hypertension is the most common chronic disorders worldwide and secondary forms of hypertension are found in 5–10% of the hypertensive population, of which most can be linked to renal disease (24). In humans, as well as in experimental models of salt-sensitive hypertension, there is a growing body of evidence pointing at a close relationship between nitric oxide (NO) deficiency and development of hypertension (37).

Hydronephrosis due to obstruction at the level of the pelvic-ureteric junction is a common condition in children, with an incidence in newborns of ~1%. The obstruction is mostly partial and congenital of origin. Unilateral hydronephrosis causes salt-sensitive hypertension in both rats (5) and mice (7).

The renal function in hydronephrotic animals, measured as renal blood flow and glomerular filtration rate (GFR), is rather well preserved for a long period (31, 58). However, micropuncture experiments (41) showed that the tubuloglomerular feedback (TGF), which is an important mechanism in the control of GFR and blood pressure regulation, is reset during volume expansion in a paradoxical way to a much higher sensitivity and activity in the hydronephrotic kidney. The regulation of the sensitivity and reactivity of the TGF is intimately coupled to NO production in the macula densa (4) and the same kind of TGF resetting has been described in animal models for hypertension (11, 49, 59).

NO is synthesized from a family of NO synthases [neuronal NO synthase (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS)] that utilize L-arginine as a substrate (48). Most evidence indicates that NO production is reduced in renal disease (1). There are many ways in which NO deficiency develops and reduced NO availability can be the consequence of L-arginine limitation and/or increased levels of endogenous NOS inhibitors (1). Furthermore, an increased NO consumption as the result of oxidative stress may lead to reduced NO levels (46). Reduced levels of L-arginine have been observed in renal failure, but the clinical relevance is uncertain, as the measured serum concentrations are well in excess of the Km for eNOS (1, 38). Nevertheless, both clinical (20, 47, 52, 60) and experimental studies (12, 14, 18, 22, 23, 36, 44) have demonstrated that substrate supplementation stimulates NO production and attenuates renal damage and salt-sensitive hypertension. Furthermore, chronic NO inhibition with Nω-nitro-L-arginine methyl ester (L-NNAME), an antagonist for L-arginine, causes salt-sensitive hypertension and the development of renal injury (62). It has been shown in models of experimental hypertension (e.g., Dahl salt-sensitive rats) that the NOS activity in the renal medulla is reduced, which is associated with decreased protein expression of all three NOS isoforms (55).

In the normal rat, NO activity increases as an adaptive response to volume expansion in a paradoxical way to a much higher sensitivity and reactivity in the hydronephrotic kidney. The regulation of the sensitivity and reactivity of the TGF is intimately coupled to NO production in the macula densa (4) and the same kind of TGF resetting has been described in animal models for hypertension (11, 49, 59).

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Based on earlier observations, it was hypothesized that NO deficiency, especially in the renal tissue, is involved in the development of salt-sensitive hypertension in hydronephrosis. In the present study, blood pressure and renal function were examined, before and after chronic inhibition or stimulation of the NO system, in controls, and hydrenephrotic animals treated with different sodium diets. The plasma levels of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine and L-arginine were determined and the renal tissue was used for histological evaluation and determination of NOS proteins.

**METHODS**

Male Sprague-Dawley rats (Møllegaard, Copenhagen, Denmark) were divided into three experimental groups: unilateral and bilateral hydrenephrotic animals and sham-operated controls. The experiments were performed 4–6 wk after ureteral obstruction, and in four separate series.

**Creation of Partial Unilateral and Bilateral Ureteral Obstruction**

A partial unilateral (PUUO) or bilateral (PBUO) ureteral obstruction was created in 3-wk-old rats to induce hydrenephrosis, as described earlier (5, 6). In short, anesthesia with spontaneous inhalation of isoflurane (Forene, Abbot Scandinavia AB, Kista, Sweden) was used and the abdomen was opened sterile through a midline incision and the left, or both, ureters were isolated and embedded in the underlying psoas muscle. Sham operations in control animals were performed in the same way, but without dissecting the ureter. All animals were then left to grow with free access to normal-sodium (NS) diet for 4–6 wk.

**Classification of Hydrenephrosis**

The degree of hydrenephrosis was visually examined before insertion of the telemetric equipment as described earlier (5). In those animals not receiving a telemetric device, palpation of the kidney was performed during anesthesia with isoflurane. The obstructed kidneys were categorized as having a normal appearance (I) or as having mild (II), moderate (III), or severe (IV) hydrenephrosis. Grossly enlarged, sacculated kidneys were categorized as nonfunctioning (V). Animals allocated to categories I and V and those with abnormalities in the contralateral kidney were excluded from this study. All series consisted of an equal number of animals from the different groups. At the time of death, the estimated hydrenephrotic degrees were confirmed in all animals.

**Telemetric Measurements**

The telemetric device (PA-C40, DSI, St. Paul, MN) was implanted in the adult animals, and telemetric measurements of mean arterial blood pressure (MAP) were conducted as previously described (5, 6).

**Experimental Protocols**

In **series I**, renal excretion of electrolytes and fluid was studied during NS conditions (0.7% NaCl, SD389-R36, Lactamin) and high-sodium (HS) conditions (4% NaCl, SD312-R36, Lactamin). Plasma samples were obtained for ADMA, SDMA, and L-arginine analysis. Renal histology was evaluated and the nNOS and eNOS levels were determined in both hydrenephrotic and control animals.

In **series II**, blood pressure and renal excretion were studied during NS and HS conditions, and before and after administration of l-NAME (Sigma, St. Louis, MO), and in **series III** before and after L-arginine (l-arginine monohydrochloride, Sigma). None of the experiments in **series II and III** were started earlier than 10 days after the probe implantation.

In **series IV**, characteristics of the TGF were determined in micropuncture experiments by stop-flow technique before and after administration of 7-nitroindazole (7-NI; Sigma) or l-arginine. Non-treated controls and unilateral obstructed animals were used.

An equilibration period of 10 days was allowed on both the NS and HS diet before any measurements were performed.

**Series I**

**Renal excretion measurements. Series I** consisted of 15 controls, 12 PUUO, and 9 PBUO animals, which were housed individually in metabolism cages for 24 h. Urine production, sodium and potassium concentrations were determined as previously described (5, 6).

L-Arginine, ADMA, and SDMA measurements. Withdrawals of 1 ml blood were made from anesthetized controls, PUUO and PBUO animals and plasma concentrations of L-arginine, ADMA, and SDMA were determined simultaneously by high-performance liquid chromatography, as described previously (56) but with modified chromatographic separation conditions (9).

**Western blotting for nNOS and eNOS**. Infusion of cold PBS was started once the right renal vein was cut to remove the blood: the kidneys were then explanted for Western blotting or histology analysis. Renal cortex and medulla were separated and homogenized in lysis buffer (1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM NaF, 80 mM Tris, pH 7.5) containing enzyme inhibitors (Complete Mini; 1 tablet/1.5 ml; Roche Diagnostics, Mannheim, Germany). Samples were run on 7.5% Tris-HCl gels with Tris/glycine/SDS buffer. The proteins were detected, after transfer to nitrocellulose membranes, with rabbit anti-nNOS (1 μg/ml; Zymed Laboratories; Invitrogen, Carlsbad, CA) and mouse anti-eNOS (1 μg/ml; Zymed Laboratories) and horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit and goat anti-mouse, 1:5,000; Sigma) by an ECL camera (Kodak image station 2000; New Haven, CT). β-Actin was detected with mouse anti-rat β-actin antibody (1:10,000; Sigma) and secondary HRP-conjugated goat anti-mouse antibody (1:60,000; Kirkegaard and Perry Laboratories, Gaithersburg, MD).

**Histology**. The kidneys were explanted and prepared for a blinded histopathological evaluation (8). Sagittal slices of the renal tissue were fixed in formalin (4% in PBS) and embedded in paraffin. Embedded tissue blocks were cut into 5-μm-thick sections and stained with periodic acid Schiff (PAS) and Picro-Sirius. PAS was used to stain basal membranes and Picro-Sirius for visualizing interstitial fibrosis, especially collagen. The renal cortex, medulla, and the papilla were investigated for fibrosis, inflammation (i.e., infiltration of plasma cells and lymphocytes), tubular changes (i.e., hyaline material, atrophy), and glomerular changes (i.e., sclerosis, mesangial matrix increase, and shrunken glomeruli). The tissues evaluated were given a score of 0–4, depending on the severity of change (0 = no changes, 1 = detectable changes, 2 = mild, 3 = moderate, and 4 = severe changes). The lowest score was given if the renal histoarchitecture was normal, with no changes in any of the investigated parameters. The highest score represented major distortion of the normal histoarchitecture in both cortex and medulla.

**Series II**

**Effect of L-NAME on blood pressure and renal function. Series II** consisted of 8 controls, 11 PUUO, and 11 PBUO animals. Telemetric measurements commenced in the following order of diets: NS, NS + l-NAME, HS, and finally HS + l-NAME. L-NAME was administered in the drinking water (0.5 mg/ml) for 1 wk. For the NS and HS diets, blood pressure was measured continuously for 48 h and throughout the whole period when l-NAME was administered. Once the telemetric measurements were completed at each dietary period, renal excretion was measured in a selection of animals with variable degrees of hydrenephrosis from each group.

**Series III**

**Effect of l-arginine on blood pressure and renal function. Series III** consisted of 12 controls, 14 PUUO, and 10 PBUO animals. Telemetric measurements commenced in the following order of diets: NS, NS + l-NAME, HS, and finally HS + l-NAME. L-NAME was administered in the drinking water (0.5 mg/ml) for 1 wk. For the NS and HS diets, blood pressure was measured continuously for 48 h and throughout the whole period when l-NAME was administered. Once the telemetric measurements were completed at each dietary period, renal excretion was measured in a selection of animals with variable degrees of hydrenephrosis from each group.
measurements commenced in the following order: NS, NS + L-arginine, HS, and HS + L-arginine. L-Arginine was administered in the drinking water (1 mg/ml) for 30 days to half of the animals in each group (i.e., 6 groups). Blood pressure was measured for at least 48 h. In the L-arginine-treated animals, blood pressure was measured after 15 and 30 days. Once the telemetric measurements were completed, renal excretion measurements were performed as described for series I.

Series IV

Effect of 7-NI or L-arginine on TGF characteristics. The animals were anesthetized and prepared for micropuncture experiments. TGF characteristics were determined, before and after administration of 7-NI or L-arginine, by stop-flow technique (4).

Group 1: normovolemic, nNOS inhibition with 7-NI. A single intraperitoneal dose (25 mg/kg) of the selective nNOS inhibitor 7-NI, dissolved in heated peanut oil (Sigma), was administered and the stop-flow measurements were taken after a minimum of 15 min.

Group 2: normovolemic, intratubular L-arginine infusion. With addition of L-arginine (10^{-2} M) to the artificial ultrafiltrate, the stop-flow measurements were taken 15 min after intratubular administration at a low, 10 nl/min, non-TGF-activating perfusion rate.

Stop-flow measurements. The left kidney was exposed through a subcostal flank incision, dissected free from surrounding tissue and isolated fixed in a plastic cup with 3% agar solution. TGF characteristics were determined by stop-flow technique. Randomly chosen proximal tubular segments on the surface were punctured with a sharpened glass pipette (OD 3–5 μm) filled with lissamine green-stained 1 M NaCl solution. The pipette was connected to a servo-nulling pressure system (World Precision Instruments, New Haven, CT) to determine the proximal tubular free-flow pressure (P_{PSF}). A second pipette (OD 7–9 μm), filled with artificial ultrafiltrate and connected to a microperfusion pump (Hampel, Frankfurt, Germany), was inserted in the last accessible segment of the proximal tubule. A solid wax block was placed in between with a third pipette (OD 7–9 μm). The proximal tubular stop-flow pressure (P_{SFF}) upstream to the block was determined at different perfusion rates (0–35 nl/min) in the loop of Henle. The maximal change in stop-flow pressure (ΔP_{SFF}) was used to indicate the TGF reactivity and the tubular flow rate, eliciting half-maximal ΔP_{SFF} [i.e., the turning point (TP)], indicated the TGF sensitivity. To create TGF-response curves, normalized data were used in a nonlinear least-squares curve-fitting program (4).

Calculations and Statistics

Values are presented as means ± SE. Single comparisons between normally distributed parameters were tested for significance with Student’s paired or unpaired t-test. For multiple comparisons, ANOVA followed by the Fisher’s posttest was used. For the stop-flow pressure measurements, multiple groups were compared by one-way ANOVA. The Bonferroni posttest for paired multiple comparisons was used to allow for more than one comparison with the same variable. This states a significance level of P/M, where M is the number of comparisons to be made. Scored data for the histological evaluation were analysed by the Kruskal-Wallis test followed by the Mann-Whitney U-test. Statistical significance was defined as P < 0.05.

Ethics

The experiments were approved by The Uppsala Ethical Committee for Animal Experiments.

RESULTS

All animals used in this study were in good condition, and at the beginning of the experiments there were no differences in body weight (BW) between the different groups (controls 376 ± 12 g, PUUO 374 ± 11 g, and PBUO 346 ± 9 g).

Series I

Renal excretion measurements. Renal excretion data, for the controls and hydronephrotic animals, are summarized in Table 1. The urine excretion rate was higher and the urine concentrating ability reduced in the hydronephrotic groups, than in the controls, for both diets. On NS diet, both PBUO and PUUO animals displayed increased excretion of osmoles, sodium and potassium. During HS condition, PUUO animals had a tendency for increased electrolyte excretion, whereas PBUO animals had a reduced ability for excreting electrolytes.

L-Arginine, ADMA, and SDMA measurements. The results from the measurements of L-arginine, ADMA, and SDMA in plasma are summarized in Table 2. The ADMA and SDMA levels were higher in the hydronephrotic animals than in the controls, but for SDMA it did not reach statistical significance for the PUUO animals. A significant difference was also found between the PUUO and PBUO animals for ADMA and SDMA concentrations, whereas plasma concentrations of L-arginine were similar in the three groups.

Table 1. Renal excretion data on normal- and high-salt diets

<table>
<thead>
<tr>
<th></th>
<th>Normal-Sodium Diet</th>
<th>High-Sodium Diet</th>
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<tbody>
<tr>
<td></td>
<td>Controls (n = 15)</td>
<td>PUUO (n = 8)</td>
</tr>
<tr>
<td>Water intake, μl·h^{-1}·g body wt^{-1}</td>
<td>61±2</td>
<td>69±3*</td>
</tr>
<tr>
<td>Food intake, mg·h^{-1}·g body wt^{-1}</td>
<td>51±2</td>
<td>52±2</td>
</tr>
<tr>
<td>Diuresis, μl·h^{-1}·g body wt^{-1}</td>
<td>24±2</td>
<td>35±4*</td>
</tr>
<tr>
<td>Na^+ excretion, μmol·h^{-1}·g body wt^{-1}</td>
<td>3.2±0.14</td>
<td>3.72±0.22*</td>
</tr>
<tr>
<td>K^+ excretion, μmol·h^{-1}·g body wt^{-1}</td>
<td>5.39±0.21</td>
<td>5.88±0.33*</td>
</tr>
<tr>
<td>Osmolality, mosmol/kg H_2O</td>
<td>1,629±66</td>
<td>1,389±110*</td>
</tr>
<tr>
<td>Osmolar excretion, μmol·h^{-1}·g body wt^{-1}</td>
<td>40.0±2.1</td>
<td>45.2±2.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE. PUUO, partial unilateral ureteral obstruction; PBUO, partial bilateral ureteral obstruction. *P < 0.05 compared with controls on same diet.

Table 2. Plasma L-arginine, ADMA, and SDMA in controls, PUUO, and PBUO animals

<table>
<thead>
<tr>
<th></th>
<th>L-Arginine, μmol/l</th>
<th>ADMA, μmol/l</th>
<th>SDMA, μmol/l</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>PUUO</td>
<td>PBUO</td>
</tr>
<tr>
<td></td>
<td>132.4±5.9</td>
<td>143.4±8.8</td>
<td>128.2±8.5</td>
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<tr>
<td></td>
<td>0.482±0.020</td>
<td>0.560±0.028*</td>
<td>0.747±0.063*</td>
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<tr>
<td></td>
<td>0.291±0.007</td>
<td>0.319±0.020</td>
<td>0.675±0.146*</td>
</tr>
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</table>

Values are means ± SE. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine. *P < 0.05 compared with controls. †P < 0.05 compared with PUUO.
Western blotting for NOS. The protein expression of nNOS was reduced in both cortex and medulla for the hydronephrotic groups (Fig. 1). The expression of eNOS was lower for both hydronephrotic groups, but was only found significant for the PBUO animals.

Histology. The results from the histological evaluation of the kidneys are summarized in Table 3. The PBUO group displayed the most severe changes among all the parameters investigated.

All the hydronephrotic kidneys, from animals with PUUO and PBUO, displayed variable degrees of dilatation of the pelvic area, with flattening of the renal papilla. Moreover, hydronephrotic kidneys exhibited areas with subepithelial fibrosis, infiltration of inflammatory cells (i.e., plasma cells and lymphocytes), predominantly localized to the medulla and pelvic region, and glomerular changes (i.e., sclerosis, mesangial matrix increase, and collapsed glomeruli). Tubular changes (i.e., hyaline material in the lumen, atrophy, and thickening of the basal membrane) and vascular changes (i.e., hypertrophy of the media, hyperplasia in arterioles and arteries) were only detected in animals with severe hydronephrosis. Fibrotic and glomerular changes were also identified in the contralateral kidneys of animals with PUUO. The sham-operated controls displayed normal histoarchitecture, with no changes in any of the sections investigated, with the exception of detectable fibrosis in four kidneys.

Series II

Effect of L-NAME on blood pressure and renal function. The effects of L-NAME on blood pressure are shown in Fig. 2. Under NS and HS conditions, both with and without L-NAME,
the blood pressure level was higher in the hydronephrotic animals than in the controls. Furthermore, PBUO animals had a higher blood pressure than the PUUO animals during all experimental periods. There was a significant increase in blood pressure for all three groups after the administration of L-NAME (both during NS and HS).

Without L-NAME, the salt sensitivity was more pronounced in PBUO (23 ± 2%) and PUUO (13 ± 4%) animals than in controls (4 ± 2%). L-NAME treatment increased the salt sensitivity only in the controls, and differences in salt sensitivity were no longer present between the PBUO (18 ± 5%), PUUO (13 ± 3%), and control (12 ± 2%) animals.

On NS diet, the blood pressure response to L-NAME treatment was more pronounced in the controls (26 ± 1%) than in the PUUO (18 ± 1%) and PBUO (16 ± 2%) animals. When a HS diet was given, this response became even more pronounced in the controls (34 ± 3%), whereas no significant differences were found for the PUUO (18 ± 2%) and PBUO (13 ± 2%) animals when compared with the NS diet.

An analysis of the relationship between the blood pressure and the steady-state sodium excretion in controls, PUUO, and PBUO animals under the different sodium conditions is presented in Fig. 3. The hydronephrotic groups displayed abnormal renal function curves with pressure natriuresis.

The effects of L-NAME on renal excretion are summarized in Table 4. On both diets after L-NAME treatment, the controls had a reduced ability for excreting sodium, potassium, and total osmoles. The effects of L-NAME were less pronounced in the hydronephrotic animals and reduced excretion was only found, for sodium and potassium, during NS diet.

**Series III**

**Effect of L-arginine on blood pressure and renal function.** The effects of chronic L-arginine supplementation on blood pressure are displayed in Fig. 4. As in *series I*, the hydronephrotic animals displayed salt-sensitive hypertension. Before L-arginine was given, the blood pressure levels were similar in the two groups of PBUO, PUUO, and control animals.

In animals not receiving L-arginine, blood pressure increased in both PBUO and PUUO animals on HS diet during the
both NS and HS conditions. In the controls, L-arginine supplementation decreased blood pressure only in PBUO animals during NS conditions or after L-arginine treatment reduced blood pressure during the experimental period and only in PBUO animals during NS conditions (Fig. 4).

In animals receiving L-arginine, blood pressure only increased in the PBUO animals during HS conditions. In PUUO animals, L-arginine treatment reduced blood pressure during both NS and HS conditions. In the controls, L-arginine supplementation had no effect on the blood pressure (Fig. 4).

The renal excretion did not change significantly after L-arginine supplementation; however, the hydronephrotic groups had a tendency for increased electrolytes and water excretion, which was not found in the controls (data not shown).

**Series IV**

Effect of 7-NI or L-arginine on the TGF characteristics. The influence of 7-NI or L-arginine on the characteristics of the TGF is shown in Table 5 and in Fig. 5. Throughout the stop-flow pressure measurements, the blood pressure remained stable. There were no differences in Pr or PsF between the controls and hydronephrotic animals during control conditions or after 7-NI or L-arginine administration. During control conditions, the reactivity of the TGF response, as indicated by the ΔPsF, was greater in the hydronephrotic group (14.4 ± 1.0 mmHg) than in control animals (9.4 ± 0.8 mmHg). Furthermore, the flow rate eliciting a half-maximal PsF response (i.e., TP) was lower in the hydronephrotic animals (14.9 ± 1.4 nl/min) than

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Table 4. Effects of L-NAME on renal excretion in controls and hydronephrotic animals treated with normal- or high-salt diets

<table>
<thead>
<tr>
<th></th>
<th>Normal-Sodium Diet</th>
<th>Normal-Sodium Diet + L-NAME</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>PUUO</td>
</tr>
<tr>
<td>Diuresis, µl·24 h⁻¹·g body wt⁻¹</td>
<td>25±1</td>
<td>33±1</td>
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<tr>
<td>Na⁺ excretion, µmol·24 h⁻¹·g body wt⁻¹</td>
<td>3.09±0.08</td>
<td>3.35±0.20</td>
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<tr>
<td>K⁺ excretion, µmol·24 h⁻¹·g body wt⁻¹</td>
<td>5.68±0.30</td>
<td>5.70±0.15</td>
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<tr>
<td>Osmolar excretion, µosmol·24 h⁻¹·g body wt⁻¹</td>
<td>50.1±0.3</td>
<td>51.5±1.5</td>
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</table>

High-Sodium Diet

<table>
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<tr>
<td></td>
<td>Controls</td>
<td>PUUO</td>
</tr>
<tr>
<td>Diuresis, µl·24 h⁻¹·g body wt⁻¹</td>
<td>130±3</td>
<td>160±9</td>
</tr>
<tr>
<td>Na⁺ excretion, µmol·24 h⁻¹·g body wt⁻¹</td>
<td>49.00±0.72</td>
<td>50.30±1.1</td>
</tr>
<tr>
<td>K⁺ excretion, µmol·24 h⁻¹·g body wt⁻¹</td>
<td>8.00±0.28</td>
<td>8.53±0.29</td>
</tr>
<tr>
<td>Osmolar excretion, µosmol·24 h⁻¹·g body wt⁻¹</td>
<td>149±1.5</td>
<td>150±2.47</td>
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</table>

Values are means ± SE. *P < 0.05 compared with the same group, on same diet, but without N⁶-nitro-L-arginine methyl ester (L-NAME).

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Table 5. TGF characteristics in control and hydronephrotic animals during control conditions and after the administration of 7-NI or L-arginine

<table>
<thead>
<tr>
<th></th>
<th>Control Condition</th>
<th>7-NI</th>
<th>L-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td>MAP, mmHg</td>
<td>111±3</td>
<td>199±5</td>
</tr>
<tr>
<td></td>
<td>Pr, mmHg</td>
<td>12.2±0.3</td>
<td>11.4±0.6</td>
</tr>
<tr>
<td></td>
<td>PsF, mmHg</td>
<td>39.9±0.3</td>
<td>39.8±0.5</td>
</tr>
<tr>
<td></td>
<td>ΔPsF, mmHg</td>
<td>9.4±0.8</td>
<td>14.1±0.8</td>
</tr>
<tr>
<td></td>
<td>TP, nl/min</td>
<td>19.1±1.2</td>
<td>14.6±1.0*</td>
</tr>
<tr>
<td></td>
<td>m/n</td>
<td>9/9</td>
<td>5/7</td>
</tr>
<tr>
<td>Hydronephrotic animals</td>
<td>MAP, mmHg</td>
<td>128±4†</td>
<td>131±2</td>
</tr>
<tr>
<td></td>
<td>Pr, mmHg</td>
<td>13.3±0.9</td>
<td>12.1±0.9</td>
</tr>
<tr>
<td></td>
<td>PsF, mmHg</td>
<td>41.7±0.9</td>
<td>41.0±0.8</td>
</tr>
<tr>
<td></td>
<td>ΔPsF, mmHg</td>
<td>14.5±0.8†</td>
<td>15.2±0.6</td>
</tr>
<tr>
<td></td>
<td>TP, nl/min</td>
<td>14.9±1.4†</td>
<td>13.7±1.4</td>
</tr>
<tr>
<td></td>
<td>m/n</td>
<td>9/9</td>
<td>4/7</td>
</tr>
</tbody>
</table>

Values are means ± SE. TGF, tubuloglomerular feedback; 7-NI, 7-nitroindazole; PT, proximal tubular pressure; PSF, stop-flow pressure; ΔPSF, maximal stop-flow pressure response; TP, turning point; m, animals; n, nephrons. *P < 0.05 compared with control values of same group. †P < 0.05 compared with values of control animals under similar conditions.

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Fig. 4. MAP in controls and hydronephrotic animals, treated with NS (dashed line) or HS diets (solid line) for 30 days, with or without L-arginine supplementation. *P < 0.05 compared with the control period, within same group and diet. †P < 0.05 decreased compared with the control period, within same group and diet.
in the controls (19.1 ± 1.2 nl/min), indicating a higher sensitivity of the TGF response in hydronephrosis.

After 7-NI administration, the reactivity and sensitivity of the TGF response in the control animals increased to a similar level as observed for the hydronephrotic animals during control conditions: this is seen as a leftward shift in the response curve in Fig. 5. Inhibition of nNOS with 7-NI had no effect in the hydronephrotic animals, as shown by the flattening of the pressure natriuresis curves, in hydronephrotic animals, could be caused by an increase in distal or collecting tubule reabsorption, due to reduced NO levels. It has been suggested (17) that resetting of the renal pressure natriuresis as well as decreased ANG II formation are important mechanisms in maintaining sodium and water homeostasis and in minimizing hypertension during HS intake in animals with a reduced number of nephrons. Furthermore, NO in the renal medulla appears to play an important role in regulating fluid and electrolyte homeostasis (46). In animals with hydronephrosis, especially those with bilateral hydronephrosis, these compensating mechanisms are not sufficient, at least not during HS intake.

Both hydronephrotic groups exhibited various degrees of histopathological alterations with fibrosis and inflammation, as well as glomerular and tubular changes that correlated with the degree of hydronephrosis. Oxidative stress is coupled to histopathological alterations and may contribute to the salt sensitivity. This is in accordance with the finding that the PBUO animals have the highest blood pressure and the most pronounced salt sensitivity.

The abnormal renal function curves in hydronephrotic animals with pressure natriuresis have been described in other forms of chronic hypertension (10, 15, 17). It is clear that essential hypertension is associated with abnormal pressure natriuresis. The causes for salt sensitivity have been discussed and are thought to involve reduced numbers of functional nephrons, decreased glomerular capillary filtration coefficient, and/or increased tubular reabsorption (16). Resetting of the TGF to a higher sensitivity increases the afferent arteriole resistance with subsequent hypertension but does not cause salt sensitivity. The flattening of the pressure natriuresis curves, in hydronephrotic animals, could be caused by an increase in distal or collecting tubule reabsorption, due to reduced NO levels. It has been suggested (17) that resetting of the renal pressure natriuresis as well as decreased ANG II formation are important mechanisms in maintaining sodium and water homeostasis and in minimizing hypertension during HS intake in animals with a reduced number of nephrons. Furthermore, NO in the renal medulla appears to play an important role in regulating fluid and electrolyte homeostasis (46). In animals with hydronephrosis, especially those with bilateral hydronephrosis, these compensating mechanisms are not sufficient, at least not during HS intake.

DISCUSSION

This study demonstrated that salt-sensitive hypertension in hydronephrosis was associated with abnormal changes of the NO system. Chronic NO blockade increased blood pressure more in controls than in hydronephrotic animals. Supplementation of NO substrate restored abnormal TGF and attenuated blood pressure development in hydronephrotic animals, but not in controls. Furthermore, increased plasma concentrations of endogenous NOS inhibitors, and reduced NOS proteins in the kidney tissue, indicated a close relationship between NO deficiency and the development of salt-sensitive hypertension in hydronephrosis.

Unilateral hydronephrosis is reported to cause a deficient urine concentrating ability in both rats (25) and mice (7), probably caused by reduction of the renal medulla and down-regulation of aquaporins-2 and sodium transporters in the obstructed kidney (34, 53). The present study supported earlier findings and demonstrated that increased excretion of water and electrolytes in bilateral hydronephrosis was even more pronounced during NS conditions. However, during HS conditions, bilateral hydronephrotic animals had a reduced osmolar excretion compared with the controls despite that no differences were found regarding their sodium intake. Reduced kidney function in animals with bilateral hydronephrosis may prolong the time to reach steady state on the new diet. This would indicate that PBUO animals are retaining salt, which would contribute to the salt sensitivity. This is in accordance with the finding that the PBUO animals have the highest blood pressure and the most pronounced salt sensitivity.

The abnormal renal function curves in hydronephrotic animals with pressure natriuresis have been described in other forms of chronic hypertension (10, 15, 17). It is clear that essential hypertension is associated with abnormal pressure natriuresis. The causes for salt sensitivity have been discussed and are thought to involve reduced numbers of functional nephrons, decreased glomerular capillary filtration coefficient, and/or increased tubular reabsorption (16). Resetting of the TGF to a higher sensitivity increases the afferent arteriole resistance with subsequent hypertension but does not cause salt sensitivity. The flattening of the pressure natriuresis curves, in hydronephrotic animals, could be caused by an increase in distal or collecting tubule reabsorption, due to reduced NO levels. It has been suggested (17) that resetting of the renal pressure natriuresis as well as decreased ANG II formation are important mechanisms in maintaining sodium and water homeostasis and in minimizing hypertension during HS intake in animals with a reduced number of nephrons. Furthermore, NO in the renal medulla appears to play an important role in regulating fluid and electrolyte homeostasis (46). In animals with hydronephrosis, especially those with bilateral hydronephrosis, these compensating mechanisms are not sufficient, at least not during HS intake.

Both hydronephrotic groups exhibited various degrees of histopathological alterations with fibrosis and inflammation, as well as glomerular and tubular changes that correlated with the degree of hydronephrosis. Oxidative stress is coupled to hypertension in several models and demonstrated in kidneys with ureteral obstruction (28), where it might impair renal NO production. Treatment modalities that increase NO formation (40) or inhibit oxidative stress (54) are beneficial to the progression of cellular and molecular parameters of tubulointerstitial fibrosis caused by obstruction of the ureter, whereas NO deficiency increases renal damage (21).

Long-term blockade of the NO system results in salt-sensitive hypertension (26, 39, 51, 57, 61). Acute blood pressure response to NOS inhibition appears to be due to generalized arterial vasoconstriction, whereas renal sodium and water handling are more important for the sustained blood pressure elevation and salt sensitivity. In the present study, the effects of chronic l-NAME administration on blood pressure and salt sensitivity were more pronounced in the controls than in the hydro-
nephrotic animals. After l-NAME administration, the blood pressure levels were higher in the hydronephrotic groups, but blood pressure elevation was still highest in the controls. Salt sensitivity increased only in the control animals, and after NO blockade, no differences in salt sensitivity were found between the groups. This indicated that animals with hydronephrosis had reduced NO availability, which could contribute to the development of salt-sensitive hypertension. The reduced effect of NO blockade in hydronephrosis was also apparent from the renal excretion measurements, where only the controls displayed a reduction in osmolar excretion after l-NAME administration. The suggestion that reduced NO availability explains salt-sensitive hypertension was further supported by reduced NOS protein expressions in the kidney cortex and medulla of the hydronephrotic animals.

The l-arginine-NO pathway has been ascribed an important role in the development of systemic hypertension and progressive renal disease (30). NOS substrate supplementation has beneficial effects on both blood pressure and renal disease in clinical trials (35, 47, 52) and in experimental models (12, 14, 27, 32, 40, 42, 50). In animals with ureteral obstruction, the administration of l-arginine increases GFR and renal blood flow to the postobstructed kidney and decreases the infiltration of macrophages of the renal parenchyma (29). In the present study, chronic l-arginine supplementation reduced blood pressure in PUUO animals during NS and HS conditions. In the PBUO animals, l-arginine did not reduce the blood pressure but protected from further increase during NS, but not during HS conditions, which could be due to volume retention. The PBUO animals increased fluid intake more than other groups, but urinary output did not increase to the same extent. Despite blood pressure increase, this did not result in matched sodium excretion.

The limitation of l-arginine and increased levels of endogenous NOS inhibitor (ADMA) are two possible causes of NO deficiency (1) in renal and cardiovascular disease (2, 3). The closely related compound SDMA does not inhibit NOS; however, arginine, ADMA, and SDMA share a common pathway for entry into the cell. High plasma levels of SDMA may indirectly reduce NO production by competing with arginine for cellular uptake (13).

In this study, l-arginine levels were normal, but plasma concentrations of ADMA and SDMA were elevated in the hydronephrotic animals. Endogenous inhibition of NOS and competition between SDMA and arginine within the kidney may be possible explanations for renal injury and hypertension in hydronephrosis.

TGF was reset to a higher sensitivity and reactivity in the hydronephrotic kidney during control conditions, indicating a low macula densa NO concentration. The same kind of TGF resetting is seen in both Milan hypertensive strain rats before they develop hypertension (49) and spontaneous hypertensive rats (SHR) (11). Furthermore, chronic blockade of nNOS, located in the macula densa cells, increases TGF sensitivity and leads to the development of hypertension (43).

It has been demonstrated that NOS expression does not always correlate with enzyme activity (19). In a study by Herrera et al. (19), upregulation of the NOS enzymes did only correlate with increased NO release in the beginning of chronic HS treatment. Possible explanations include that the upregulation of the enzyme expression is a compensatory mechanism for abnormally low NO release. However, in our study a reduced nNOS expression (PUUO and PBUO) and eNOS expression (PBUO) were found. The reduced protein expression of nNOS in the renal medulla further strengthens the idea of NO deficiency in the juxtaglomerular apparatus. The administration of 7-NI did not change TGF response in the hydronephrotic animals, whereas in the controls, increased reactivity and sensitivity were observed. This diminished role of NO from nNOS in blunting of TGF is also seen in SHR (59). Conversely, during intratubular infusion of l-arginine, decreased reactivity and sensitivity of the TGF were only determined in hydronephrotic animals. These observations indicated that l-arginine stimulated NO formation in hydronephrotic animals, which resets the TGF to the right, leading to increased GFR and electrolyte excretion.

In conclusion, hydronephrotic animals have a reduced response to NOS blockade, whereas no stimulation can reduce or protect from progressive blood pressure increase. Reduced NO availability in the diseased kidney, and subsequent resetting of the TGF mechanism, appears to play an important part in contributing to the development of hypertension in hydronephrosis.

GRANTS

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REFERENCES

HYDRONEPHROSIS, HYPERTENSION, AND NITRIC OXIDE


