Neuronal nitric oxide synthase inhibition sensitizes the tubuloglomerular feedback mechanism after volume expansion

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Background. In the kidney the neuronal isoform of nitric oxide synthase (nNOS) is located in the macula densa cells. These cells are known to be the sensor in the tubuloglomerular feedback. During volume expansion (VE), the tubuloglomerular feedback response is attenuated, allowing increased water and salt excretion. This study addressed the question whether inhibition of nNOS reestablishes the tubuloglomerular feedback response caused by acute extracellular VE.

Methods. In rats, VE was achieved by infusion of isotonic saline solution at 50 mL/hour * kg body weight. When urine flow was stabilized, the tubuloglomerular feedback response was evaluated by measuring changes in proximal tubular stop-flow pressure (Psf) in response to various loop of Henle perfusion rates. The loop of Henle was perfused with artificial ultrafiltrate and with addition of 1 mol/L non-specific NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME). In additional rats the nNOS inhibitor, 7-nitro indazole (7-NI), was given intrapertitoneally. Single nephron glomerular filtration rate (SNGFR) was also measured. GFR was determined after VE and nNOS inhibition.

Results. Acute VE decreased ΔPsf and ΔSNGFR while increasing the turning point, indicating decreased sensitivity of tubuloglomerular feedback response. After administration of L-NAME or 7-NI, ΔPsf was maximally sensitized and the turning point and ΔSNGFR were restored. GFR decreased after VE and nNOS inhibition compared to that after VE alone.

Conclusion. These results suggest that a functioning nitric oxide system, especially through the nNOS, is important in mediating normal renal responses and that increased production of and/or sensitivity to nitric oxide during sustained VE plays an important role in the adaptive mechanism of the tubuloglomerular feedback.

Salt and water overload is a condition that can occur in human beings and animals. The body therefore has powerful mechanisms to excrete excess fluid volumes and salt that would otherwise endanger the life of the organism. In the mammalian kidney there is accurate control of tubular reabsorption, with appropriate adjustment of the glomerular filtration rate (GFR). One of the most important factors in this control process is the tubuloglomerular feedback mechanism located in the juxtaglomerular apparatus. This mechanism operates by sensing the tubular load to the distal tubule via the Na+/K+2 Cl− cotransport at the macula densa site, sending signals to the rest of the apparatus to adjust the tonus of the glomerular arterioles, which regulates GFR [1, 2]. The sensing step in this control mechanism, the macula densa cells, is located in the distal tubule at the point of contact with the glomerulus and the glomerular arterioles.

From earlier studies it is known that the sensitivity of the tubuloglomerular feedback mechanism can be reset by several different factors. Many hormones and local factors such as prostaglandins, angiotensin II, thromboxane, and others can reset the tubuloglomerular feedback sensitivity by altering its response curve [3, 4]. Soon after the tubuloglomerular feedback operation had been characterized in detail, it became clear that changes in the body fluid volume were associated with changes in the sensitivity of the tubuloglomerular feedback mechanism. Under conditions of volume expansion a resetting of tubuloglomerular feedback to a low sensitivity was found [5]. This indicated that a high tubular flow was needed to activate the tubuloglomerular feedback. This allows a greater fluid flow to pass the macula densa cells without contraction of the glomerular arterioles, leading to reduced glomerular filtration.

Nitric oxide is involved in many functions and conditions throughout the body, including vasodilatation, cell growth, inflammation, neurotransmission, hormone release, and renal sodium handling etc. Nitric oxide is formed together with L-citrulline from L-arginine and oxygen by the enzyme nitric oxide synthase (NOS). There are three known isoforms of NOS, the neuronal isoform (nNOS), the inducible isoform (iNOS), and the endothelial isoform (eNOS). The nNOS and the eNOS isoforms are constitutively expressed and Ca2+-dependent,
while iNOS is considered to be inducible and Ca$^{2+}$-independent. However, it has been reported that iNOS is also constitutively expressed in the kidney [6, 7]. In the kidney the macula densa cells have been found to contain a high density of nNOS [8–10]. It has also been found that the nitric oxide system has an important function in the tubuloglomerular feedback mechanism [11, 12]. It has been reported that nitric oxide produced in the juxtaglomerular apparatus can influence glomerular capillary pressure and the tubuloglomerular feedback mechanism [11, 12]. Acute blockade of nitric oxide with Nω-Nitro-L-arginine (L-NNA) resets tubuloglomerular feedback sensitivity to a high level, implying that nitric oxide is produced continuously in the juxtaglomerular apparatus to reduce the tubuloglomerular feedback sensitivity. Most of this effect of nitric oxide seems to be attributed to nitric oxide produced by nNOS located in the macula densa cells, since 7-nitro indazole (7-NI), a relatively selective nNOS inhibitor, reduces tubuloglomerular feedback sensitivity to the same extent as L-NNA when administered into the tubular lumen [13].

Since volume expansion (VE) and increased nitric oxide production result in a decrease in tubuloglomerular feedback sensitivity, the present investigation was undertaken to determine to what extent nitric oxide is involved in the effect of tubuloglomerular feedback sensitivity resetting that occurs upon extracellular VE. To study this question, we measured the tubuloglomerular feedback response in rats with acute saline VE after general NOS inhibition and specific inhibition of nNOS. This response was elucidated both with a stop-flow technique and by measurement of the single nephron glomerular filtration rate (SNGFR). We also measured GFR after VE and inhibition of nNOS. We found that the tubuloglomerular feedback sensitivity, which was reset to a low level after VE, could be restored to a normal or even supranormal level, as observed both from the stop-flow pressure and by SNGFR measurements by blockade of the NOS system. These results indicate that the resetting of the tubuloglomerular feedback mechanism to a low sensitivity that occurs during VE takes place by increased production of nitric oxide through activation of the NOS system.

**METHODS**

The experiments were performed on male Sprague-Dawley rats (Møllegaard, Copenhagen, Denmark) weighing 220 to 340 g. The rats were anesthetized with an intraperitoneal injection of Inactin® (thiobutabarbitual sodium, 120 mg/kg) Sigma Chemical Co., St. Louis, MO, USA).

**Surgery**

The animals were placed on a servo-controlled heating pad to maintain the body temperature at 37.5°C. A tracheostomy was performed to allow spontaneous breathing. The carotid artery and jugular vein were cannulated for blood pressure measurements and infusion of maintenance fluid (0.9% NaCl, 5 mL/hour * kg), respectively. The bladder was catheterized for urine collection. For experiments involving micropuncture, the left kidney was exposed through a subcostal flank incision. The kidney was dissected free from surrounding tissue, placed in a plastic cup, and fixed with a 3% agar solution. The ureter of the left kidney was cannulated to allow free urine flow. The kidney was covered with oil to prevent drying. A 5% VE was achieved after surgery by increasing the infusion rate to 50 mL/hour * kg saline for 60 to 90 minutes and then adjusting it to match the urine flow. When the urine flow was stabilized, the tubuloglomerular feedback characteristics were evaluated.

**Experimental protocols**

The rats were divided into four groups, treated in different ways as described and submitted to stop-flow and single nephron GFR (SNGFR) measurements.

**Normovolemic controls (NC).** After the surgical preparations, saline was infused continuously at 5 mL/hour * kg and after 90 minutes of equilibration stop-flow measurements were made or SNGFR was determined (N = 5 nephrons/3 rats).

**Volume expansion controls (VEC).** After surgery, saline was infused continuously at 50 mL/hour * kg and after 90 minutes of equilibration stop-flow measurements were made or SNGFR was determined (N = 9 nephrons studied/5 rats studied).

**Volume expansion, intratubular NOS inhibition (VELNAME).** After surgery, saline was infused continuously at 50 mL/hour * kg and after 90 minutes of equilibration stop-flow measurements were made or SNGFR was made (N = 8 nephrons/5 rats) with addition of the nonspecific NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (10$^{-3}$ mol/L) to the artificial ultrafiltrate.

**Volume expansion, nNOS inhibition (VEN7-NI).** After surgery, saline was infused continuously at 50 mL/hour * kg. After 90 minutes of equilibration, a single intraperitoneal dose of 25 mg/kg of the specific nNOS inhibitor 7-NI (Sigma Chemical Co.), dissolved in heated (80°C) peanut oil (Sigma Chemical Co.), was administered, and a minimum of 15 minutes was allowed before either stop-flow measurements were performed or SNGFR was determined (N = 7 nephrons/4 rats).

**Stop-flow pressure measurements**

Tubuloglomerular feedback characteristics were determined by the stop-flow technique. Randomly chosen proximal tubular segments on the kidney surface were punctured with a sharpened glass pipette [outer diameter
maximal stop-flow response, and PSF/ΔP1 rate eliciting half-maximal loglomerular feedback reactivity and the tubular SNGFR measurements or activity [14]. It should be mentioned that PSF is not always a perfect measure of tubuloglomerular feedback sensitivity responded. Between these two pipettes a solid wax block was placed with a third pipette (OD 7 to 9 μm). The pressure upstream to the block, the proximal tubular stop-flow pressure (PSF), was determined at different perfusion rates in the loop of Henle with artificial ultrafiltrate. The maximal change in stop flow pressure (ΔPSF) was used to indicate tubuloglomerular feedback reactivity and the tubular flow rate eliciting half-maximal ΔPSF, the turning point (TP), served to indicate tubuloglomerular feedback sensitivity. For plotting the response curve in Figure 1, normalized data were fitted to the following equation by means of a nonlinear least-squares curve-fitting program:

\[ PSF = PSF_{\text{min}} + \Delta PSF/1 + e^{w(PR-TP)} \]

where PSF is the stop-flow pressure, ΔPSF is the average maximal stop-flow response, and PSF_{\text{min}} is the average minimum stop-flow pressure when the distal delivery of fluid is increased. TP is the turning point, PR is the end proximal perfusion rate and w is the factor determining the width of the perfusion interval during which the PSF responded. It should be mentioned that PSF is not always a perfect measure of tubuloglomerular feedback sensitivity or activity [14].

**SNGFR measurements**

For measuring SNGFR, [3H] methoxy-inulin (NEN, Boston, MA, USA) was given intravenously as a bolus dose of 25 μCi, followed by a continuous intravenous infusion of 50 μCi/hr. SNGFR characteristics were determined by micropuncture techniques. Randomly chosen tubular segments on the kidney surface were punctured with a sharpened glass pipette, filled with artificial ultrafiltrate (140 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L CaCl₂, 1 mmol/L MgCl₂, 4 mmol/L NaHCO₃, 7 mmol/L urea, and 2 g/L Lissamine green, pH 7.4) and connected to a micropump (Hampel, Frankfurt, Germany). Between the oil block was perfused. In some experiments either L-NAME was added to the perfusion fluid or 7-NI was given intraperitoneally. In the experiments where L-NAME was used, the inhibitor was used to perfuse the nephron at a rate of 10 nL/min between SNGFR investigations and also in the periods between tubuloglomerular feedback determinations. A change in perfusion rate from 10 to 40 nL/min always gave the same result as an increase from 0 to 40 nL/min. The volumes of the collected samples were
measured in constant-bore capillaries. Clearance calculations were made from standard formulas.

GFR measurements

Measurements of the urine flow rate, Na\(^+\) and K\(^+\) excretion, and GFR were commenced after 1 hour of continuous saline infusion at 5 mL/hour * kg. Urine from both kidneys was sampled through a catheter placed in the bladder. After completion of surgery, infusion of \[^{3}H\] methoxy-inulin in normal saline into the jugular vein was commenced. In whole kidney clearance measurements a continuous dosage of 5 \(\mu\)Ci/hour was given. After an equilibration period of 60 minutes, a 20-minute urine collection period was begun, and this was followed by a bolus intraperitoneal dose of 7-NI (25 mg/kg). Fifteen minutes after the 7-NI administration a second 20-minute urine collection period was begun. The urine volume was determined by weight. At the midpoint of the collection periods, blood samples were taken. These samples were centrifuged and aliquots of plasma were analyzed in a multipurpose scintillation counter (Beckman LS 6500) (Beckman Industries, Inc., Fullerton, CA, USA), together with aliquots of urine. Inulin clearance was then calculated as a measure of the GFR. The concentrations of sodium and potassium were assayed with a flame photometer (FLM 3) (Radiometer, Copenhagen, Denmark).

Statistics

The results are presented as mean ± SEM. Differences between the groups were tested for significance with Student’s test for paired or unpaired observations. When multiple groups were compared, one-way analysis of variance (ANOVA) was employed. The Bonferroni test for pairwise 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. Statistical significance was defined as \(P < 0.05\).

RESULTS

The mean arterial pressures in the animals used in the different groups were constant throughout the experiments (N\(_C\), 118 ± 2.6 mm Hg; VE\(_C\), 123 ± 2.9 mm Hg; VE\(_L\)-NAME, 124 ± 3.7 mm Hg; and VE\(_7\)-NI, 129 ± 6.1 mm Hg) and did not differ after induction of 5% extracellular VE or addition of L-NAME or 7-NI. The hematocrit was 47.4% ± 0.3% during normovolemia and fell to 44.2% ± 0.4% after initiation of VE; thereafter it remained constant.

Stop-flow pressure measurements

The influence of intratubular infusion of L-NAME or intraperitoneal administration of 7-NI on the characteristics of the tubuloglomerular feedback is shown in Table 1 and in Figures 1 and 2. Throughout the stop-flow pressure measurements, blood pressure (P\(_a\)) remained stable. Although P\(_a\) had a tendency to be higher in the VE group treated with 7-NI (VE\(_7\)-NI), this was not statistically significant. In the N\(_C\) group, proximal tubular pressure (P\(_T\)) was 13.2 mmHg. Acute VE caused an elevation of P\(_T\) in all of the three VE groups. Despite this increase in P\(_T\), acute VE did not seem to affect PSF. In all of the four groups PSF was similar, averaging between 42 and 45 mm Hg. Measurements of PSF at different perfusion rates of the loop of Henle showed that VE greatly affected the tubuloglomerular feedback. Acute VE led to a significant decrease in the reactivity of tubuloglomerular feedback response, as seen by the changes in maximal stop-flow pressure response (ΔPSF) from 9.9 mm Hg in the N\(_C\) group to 2.7 mm Hg in the VE\(_C\) group (from 23.5% to 6.0%). The flow rate eliciting a half-maximal PSF response (i.e., TP) increased significantly from 20.3 to 32.9 nL/min following VE, showing a great attenuation in the sensitivity of the tubuloglomerular feedback. When the loop of Henle was perfused with L-NAME, ΔPSF increased fourfold in the VE rats to 34.5%, compared to the VE\(_C\) group. At the same time TP was reset back to the control level found in the N\(_C\) group, from 32.9 to 20.5 nL/min, which was seen as a leftward shift in the response curve in Figure 1. When the animals were treated with 7-NI, there was a more than fivefold increase in ΔPSF (from 6.0% to 38.8%). The magnitude of the tubuloglomerular feedback response was even greater than that found in the N\(_C\) group and similar to the response observed previously with 7-NI treatment of control animals [12]. As seen with L-NAME, inhibition of nNOS with 7-NI also restored the sensitivity of the tubuloglomerular feedback to that noted in the N\(_C\) animals (from 32.9 to 22.1 nL/min).

SNGFR measurements

The results of the SNGFR measurements are summarized in Figure 2. During this series of late proximal micropuncture collections, the loop perfusion rate increased from 0 to 40 nL/min, resulting in maximal activation of the tubuloglomerular feedback mechanism. In the N\(_C\) animals, the maximal decrease in SNGFR that occurred

### Table 1. Tubuloglomerular feedback characteristics in normovolemic control rats (N\(_C\)) and in rats submitted to volume expansion (VE) and N\(_\alpha\)-nitro-L-arginine methyl ester (L-NAME) or 7-nitro indazole (7-NI)

<table>
<thead>
<tr>
<th></th>
<th>N(_C)</th>
<th>VE(_C)</th>
<th>VE(_L)-NAME</th>
<th>VE(_7)-NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_a) mm Hg</td>
<td>118 ± 2.6</td>
<td>119 ± 2.9</td>
<td>121 ± 3.3</td>
<td>127 ± 3.7</td>
</tr>
<tr>
<td>(P_T) mm Hg</td>
<td>13.2 ± 0.3(^a)</td>
<td>21.0 ± 0.9(^b)</td>
<td>21.0 ± 0.9 (^b)</td>
<td>18.7 ± 1.2(^b)</td>
</tr>
<tr>
<td>(P_{\text{EG}}) mm Hg</td>
<td>42.2 ± 0.8</td>
<td>45.2 ± 0.8</td>
<td>42.5 ± 0.6</td>
<td>45.6 ± 1.8</td>
</tr>
<tr>
<td>TP nL/min</td>
<td>20.3 ± 0.8(^a)</td>
<td>32.9 ± 2.6</td>
<td>20.5 ± 1.2(^b)</td>
<td>22.1 ± 1.7(^b)</td>
</tr>
<tr>
<td>ΔPSF mm Hg</td>
<td>9.9 ± 0.3(^a)</td>
<td>2.7 ± 0.6</td>
<td>14.7 ± 1.3(^a)</td>
<td>17.7 ± 2.3(^a)</td>
</tr>
<tr>
<td>%ΔPSF</td>
<td>23.5 ± 0.7(^a)</td>
<td>6.0 ± 1.3</td>
<td>34.5 ± 3.0(^a)</td>
<td>38.8 ± 5.1(^a)</td>
</tr>
<tr>
<td>N/M</td>
<td>7/5</td>
<td>7/5</td>
<td>7/5</td>
<td>6/5</td>
</tr>
</tbody>
</table>

Abbreviations are: \(P_a\), blood pressure; \(P_T\), proximal tubular pressure; PSF, proximal tubular stop-flow pressure; TP, turning point; M, rats; N, nephrons. Values are given as mean ± SE.

\(^a\)P < 0.05 vs. VE\(_C\); \(^b\)P < 0.05 vs. N\(_C\).
when the perfusion rate was increased to 40 nL/min was 7.8 ± 1.4 nL/min (38.3% ± 3.4% per 100 g body weight). In the VEC group no such reduction was observed when the perfusion rate was increased (0.85 ± 0.2 nL/min; 4.9% ± 0.9% per 100 g body weight), indicating diminished tubuloglomerular feedback activity. Addition of L-NAME to the tubular perfusion fluid had a great effect on the SNGFR and restored the response to the same level as was observed in the NC group (7.8 ± 1.1 nL/min; 39.2% ± 3.5% per 100 g body weight). The VE7-NI group also showed an increase in TGF activity, with a decrease in SNGFR by 9.3 ± 2.4 nL/min (37.3% ± 7.8%), when the loop perfusion rate was increased, and the response was thus restored to the normovolemic level.

**GFR measurements**

Mean arterial pressure was not significantly altered after 7-NI administration (Table 2). Acute nNOS inhibition with 7-NI after volume expansion decreased GFR. Salt excretion values are also given in Table 2. The elevated Na\(^+\) and osmolar excretions were increased after administration of 7-NI, while the K\(^+\) excretion was unaltered following this nNOS inhibition.

**DISCUSSION**

The main finding in the present study is that inhibition of nNOS, with the specific inhibitor 7-NI, sensitizes the
greatly attenuated tubuloglomerular feedback response to a higher than normal reactivity and decreases the GFR after 5% extracellular VE. Thus, our results indicate that macula densa–derived nitric oxide is a mandatory participant in the tubuloglomerular feedback resetting that occurs in response to extracellular VE.

Experiments employing different techniques and approaches have shown the existence of the basic tubuloglomerular feedback loop in the juxtaglomerular apparatus [15]. Resetting of the tubuloglomerular feedback to a lower level during VE, as was observed in this study, is an important mechanism, since the attenuation of the tubuloglomerular feedback response allows a greater distal delivery of fluid before a tubuloglomerular feedback–induced reduction of the GFR takes place [5, 16, 17]. This can be seen in Figure 1A as a rightward shift of the tubuloglomerular feedback response curve. This adaptation of the tubuloglomerular feedback response plays a part in facilitating the return of the extracellular fluid volume to a normovolemic level. If no resetting of the tubuloglomerular feedback occurred, the tubuloglomerular feedback–induced vasoconstriction of the afferent arteriole would reduce the GFR and thereby prevent an adjustment of the extracellular fluid volume.

The mechanisms underlying the resetting process are complex and derive from many different sources, and the resulting sensitivity of the tubuloglomerular feedback will be influenced by several factors. However, it appears as if some factors are of greater importance than others. It seems that angiotensin II, nerve stimulation, and nitric oxide all have powerful influence on the setting of the sensitivity of the tubuloglomerular feedback mechanism [9, 18, 19].

It has been reported that angiotensin II can importantly sensitize the tubuloglomerular feedback mechanism in a situation with volume expansion [18]. The authors of that study demonstrated that after a large volume expansion the tubuloglomerular feedback sensitivity was reduced, as also was the plasma concentration of angiotensin II. Subsequently, the tubuloglomerular feedback sensitivity could be increased by infusion of angiotensin II. With large doses of angiotensin II they were able to maximally sensitize the tubuloglomerular feedback sensitivity, but when they restored the angiotensin II concentration to the same level as they found in euvolemic, they observed an increase in reactivity (ΔPf) from 6% to 12%. Earlier studies at our laboratory in the control state showed a shift in turning point from 18 to 14 nL/min and an increase in the stop-flow pressure response from 20% to 36%, while the corresponding changes in the present study during 7-NI administration were from 20 to 22 nL/min and 23.5% to 38.5%. The shift in turning point was less pronounced in the present study, but the change in reactivity was the same, indicating almost maximal tubuloglomerular feedback sensitivity.

In the resetting during VE one important causative factor of a reduction of tubuloglomerular feedback sensitivity is obviously a decrease in the angiotensin II concentration. Furthermore, VE causes reduced sympathetic activation and reduced nerve stimulation to the renal nerves, which could also lower the tubuloglomerular feedback sensitivity [20]. In addition to these nervous or general hormonal factors, local factors may also play an important role. Prior studies at our laboratory have addressed the question whether the resetting of the tubuloglomerular feedback response following VE depends on extrarenal or local intrarenal factors [21]. By reducing the colloid osmotic pressure in the peritubular capillary network, the tubuloglomerular feedback sensitivity could be reset to a lower level [21], indicating that altered activity of the tubuloglomerular feedback system during VE might be achieved via locally derived factors. Studies by Morsing and Persson [22] indicate that bradykinin is responsible for the resetting of the tubuloglomerular feedback sensitivity during VE. Aprotinin, a blocker of bradykinin formation could also inhibit the reduction in tubuloglomerular feedback sensitivity caused by VE [22]. The same authors also found that administration of bradykinin to euvolemic animals could reset the tubuloglomerular feedback sensitivity to a low level. Thus, one possible explanation for the tubuloglomerular feedback resetting during VE could be that VE leads to a local release of bradykinin within the kidney, which in turn could increase prostaglandin (prostacyclin) release in the renal interstitium [23]. Concerning the mechanism of the resetting, it may be speculated that bradykinin could release intracellular calcium in the macula densa cells as in other renal epithelial cells [24, 25], and that this increase in calcium in the macula densa cells could then stimulate the formation of nitric oxide from calcium-calmodulin–dependent nNOS and in this way counteract the afferent arteriolar constriction. Nitric oxide could vasodilate the renal vasculature and thereby increase the interstitial hydrostatic pressure and reduce interstitial oncotic pressure. Such interstitial pressure changes are known to be associated with a reduced tubuloglomerular feedback sensitivity [26].

Earlier studies have indicated that nNOS is greatly expressed in the macula densa cells [8–10]. It seems that this NOS has an important function in tonically influencing the tubuloglomerular feedback mechanism to reduce its sensitivity and reactivity [27]. Studies of the tubuloglomerular feedback mechanism through Psf measurements indicated that tubular inhibition of NOS with a general NOS inhibitor shifts the tubuloglomerular feedback response to a low TP, indicating a high sensitivity and a large reactivity [27]. Hence, nitric oxide production continuously desensitizes the tubuloglomerular feedback mechanism. Furthermore, it has been shown that this effect is mediated mainly by the action of nNOS and not by...
other NOS isoforms [12, 13]. Thus, an increased production of nitric oxide from nNOS would lead to a shift of the tubuloglomerular feedback sensitivity to a higher TP and reduced reactivity. It has recently been demonstrated that an increased sodium chloride concentration at the macula densa site in isolated perfused thick ascending limbs with attached glomeruli increases nitric oxide release [28]. It would therefore be expected that during VE, when the distal delivery of fluid is increased, the sodium chloride concentration at the macula densa would be raised. This would then lead to an increased rate of production of nitric oxide by the macula densa cell.

In the present study we found that extracellular VE reduced the reactivity and sensitivity of the tubuloglomerular feedback response, as seen both in the PSF and SNGFR measurements. General NOS inhibition achieved through L-NAME or nNOS inhibition with 7-NI restored the tubuloglomerular feedback sensitivity to the control level, while the tubuloglomerular feedback reactivity was maximal. Thus, these findings indicate that in a situation with a low angiotensin II concentration and low sympathetic tone, as in volume expansion, nitric oxide essentially controls the full range of tubuloglomerular feedback sensitivity. Nitric oxide blockade by tubular administration of L-NAME returns the reactivity in a matter of seconds to the maximal achievable, and the turning point to the control level. It is also interesting to note that 7-NI gives rise to a significant decrease in total kidney GFR that can be explained on the basis of the increased tubuloglomerular feedback sensitivity during VE on administration of the drug.

Many investigators have studied the effect of different salt diets on the nNOS expression in the macula densa cells. In these cells there seems to be an inverse relationship between a salt diet and long-term nNOS expression [29–31]. Even though NOS expression is increased after low salt treatment, the nitric oxide produced by macula densa nNOS is not elevated [32, 33]. This is probably due to limited delivery and/or uptake of L-arginine in this situation [32, 34]. It has been shown that both short-term saline infusion and chronic dietary NaCl loading increase the nitric oxide production in the kidney [35, 36]. Wilcox, Deng, and Welch [33] found that after 8 to 10 days of a high salt diet the excretion of NO2 and NO3 was increased. This increase seemed to be mediated through the nNOS derived nitric oxide [33]. This is in line with our finding that the resetting of the tubuloglomerular feedback response after VE was restored in the nNOS-inhibited rats, as seen both in the PSF and the SNGFR measurements.

In the experiments in which whole kidney GFR was measured after extracellular VE, we noted that GFR was attenuated by NOS inhibition. In an earlier study [13], we found a decrease in GFR after acute nNOS inhibition. It is very likely that tubuloglomerular feedback activation is one important factor underlying the reduction in total kidney GFR seen after administration of 7-NI. By contrast, we also found increases in sodium and osmolar excretion after systemic nNOS inhibition during VE, while the potassium excretion was unaffected. The effect of nitric oxide on sodium excretion has been greatly studied, but the results are conflicting. In vitro there is evidence that nitric oxide has direct tubular actions leading to inhibition of sodium reabsorption in the proximal tubules and collecting duct [7, 37–39]. However, in vivo the results are more variable. It has been found that nitric is important in mediating pressure natriuresis [40, 41], but it has also been reported that NOS inhibition gives rise to a natriuretic response [41, 42]. It has been suggested that this natriuretic response following NOS inhibition and VE may be related to the renal nerves [43].

CONCLUSION

During VE the tubuloglomerular feedback response is greatly attenuated. This resetting allows for increased water and solute excretion, so that the extracellular fluid volume can be normalized. Part of this resetting depends on a reduced sympathetic tone and a reduced production rate of angiotensin II. During VE the sensitivity of the tubuloglomerular feedback is under the control of the nitric oxide production rate. An inhibition of the nitric oxide production by the macula densa cell nNOS leads to maximal sensitization the tubuloglomerular feedback reactivity and normalization of the turning point. Thus, the present study also suggests that a functional nitric oxide system is important in mediating normal renal responses and that an increased production of or increased sensitivity to nitric oxide during volume expansion plays an important role in the adaptive mechanisms.

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