Increased Blood Pressure in Rats after Long-Term Inhibition of the Neuronal Isoform of Nitric Oxide Synthase

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Abstract

In the kidney, nitric oxide synthase (NOS) of the neuronal isoform (nNOS) is predominantly located in the macula densa cells. Unspecific chronic NOS inhibition in rats leads to elevated blood pressure (P_A), associated with increased renal vascular resistance. This study was designed to examine the effect of chronic selective inhibition of nNOS with 7-nitro indazole (7-NI) on P_A, GFR, and the tubuloglomerular feedback (TGF) system. P_A was repeatedly measured by a noninvasive tail-cuff technique for 4 wk in rats treated orally with 7-NI, and in control rats. After treatment, the animals were anesthetized and renal excretion rates, GFR, and TGF activity were determined. After 1 wk of 7-NI treatment P_A was increased from 129±4 to 143±2 mmHg. GFR (1.85±0.1 vs. 1.97±0.2 ml/min in controls) was unchanged, but micropuncture studies revealed a more sensitive TGF than in controls. After 4 wk of 7-NI treatment P_A was 152±4 mmHg, but no change in GFR (1.90±0.5 ml/min) or TGF sensitivity was detected. Acute administration of 7-NI to nontreated rats did not affect P_A, but decreased GFR (1.49±0.1 ml/min) and increased TGF sensitivity. In conclusion, chronic nNOS inhibition leads to increased P_A. Our results suggest that the elevated P_A could be caused by an initially increased TGF sensitivity, leading to decreased GFR and an increased body fluid volume. (*J. Clin. Invest.* 1997. 99:2212–2218.) Key words: hypertension • 7-nitro indazole • glomerular filtration rate • tubuloglomerular feedback • renal hemodynamics

Introduction

A new experimental model for studying systemic hypertension in rats has been developed in recent years. Chronic administration of unselective inhibitors (L-arginine analogues) of nitric oxide (NO)[1] synthase (NOS) has been reported by several laboratories to cause a sustained and dose-dependent elevation of arterial blood pressure (P_A) and an increase in renal vascular resistance (1–8). Chronic NOS blockade by various forms of L-arginine analogues results in an increase in glomerular filtration pressure (P_{GC}), and in the resistance of both afferent and efferent arterioles, and a decrease in the glomerular capillary ultrafiltration coefficient (8). The hypertension leads to mild to substantial glomerular damage and proteinuria, depending on the duration of treatment with the NOS inhibitor and the dose used (3). Although convincing evidence for a strong renal influence in this type of hypertension has been demonstrated, the use of unselective NOS inhibitors is combined with a wide range of unselective pressor effects on peripheral vascular resistance (9, 10), as well as unknown effects on the central nervous system (11, 12).

The tubuloglomerular feedback (TGF) mechanism is an important regulator of GFR (13). It is mediated by the macula densa (MD) cells and determines the tonus of the afferent arteriole. The MD cells have been shown to contain NOS of the neuronal isoform (nNOS; 14–16), and NO produced from these cells counterbalances the TGF-mediated constriction of the afferent arteriole (15–17). Local administration of L-arginine analogues leads to a potent increase in TGF reactivity and sensitivity. We showed recently that selective inhibition of the neuronal NOS-isoform with 7-nitro indazole (7-NI) was as potent as unselective NOS inhibition in enhancing TGF responsiveness, which emphasizes the obligatory role of MD-produced NO in the TGF-mediated regulation of P_{GC} (18). Such strong activation of TGF after NOS inhibition could explain the decrease in GFR found after systemic (19, 20) and intrarenal NOS inhibition (21).

This study was designed to investigate the chronic effects of selective inhibition of neuronal NOS on the blood pressure and renal hemodynamics in male Sprague-Dawley rats. We hypothesized that long-term (4 wk) 7-NI treatment would activate TGF, leading to decreased GFR and thereby elevated blood pressure. But because the results were partly contradictory to our expectations, additional experiments were conducted to investigate the effects of 7-NI after 1 wk of chronic inhibition and in the acute state. Blood pressure was continuously measured, using the noninvasive tail-cuff technique. All experiments on renal hemodynamics were performed on anesthetized rats. Both whole kidney clearance measurements and micropuncture experiments were carried out to evaluate renal filtration on the whole kidney GFR and single nephron GFR (SNGFR) basis. To assess TGF activation, the difference between proximal and distal SNGFR was determined, and the

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1. *Abbreviations used in this paper:* eNOS, endothelial NOS; iNOS, inducible NOS; L-NAME, N^{ω}-nitro-L-arginine methyl ester; MD, macula densa; 7-NI, 7-nitro indazole; nNOS, neuronal NOS; NO, nitric oxide; NOS, nitric oxide synthase; P_A, arterial blood pressure; P_{GC}, glomerular capillary pressure; P_{SF}, proximal tubular stop-flow pressure; P_T, proximal tubular free-flow pressure; SNGFR, single nephron GFR; TGF, tubuloglomerular feedback; TP, turning point.

changes in stop-flow pressure (P_{SF}) to various loop of Henle perfusion rates were measured.

A separate series of experiments was carried out to determine the selectivity of 7-NI as an nNOS inhibitor. Using the isolated perfused juxtamedullary nephron preparation, the effects of carbachol-mediated vasodilation on afferent arterioles were investigated in rats treated for 3 wk with vehicle, 7-NI, and N^{ω}-nitro-L-arginine methyl ester (L-NAME), respectively. Also, measurements of NO_2^-/NO_3^- excretion were made to get an estimate of total NO production in these rats.

Methods

Series I. Male Sprague-Dawley rats (Møllegaard, Copenhagen, Denmark) were divided into four groups. The first group, control ($n = 8$), was given vehicle (tap water) for 4 wk. The second group (7-NI_a; $n = 8$) was treated in the same way as the control group up to the time of an experiment. After surgery they received 7-NI (Research Biochemicals Inc., Natick, MA) dissolved in heated (80°C) peanut oil (Sigma Chemical Co., St. Louis, MO), in a single intraperitoneally administered dose of 25 mg/kg body wt. The third (7-NI_{1w}; $n = 9$) and fourth (7-NI_{4w}; $n = 7$) groups were given 7-NI in their drinking water (2.5 mg/d) for 1 and 4 wk, respectively. In the control and 7-NI_{4w} groups a tail-cuff blood pressure measuring system (Kent Scientific Co., Lichfield, United Kingdom) was used to measure systolic blood pressure in a noninvasive way. This was done two to three times a week for 5 wk. The rats were trained to become accustomed to the blood pressure measurement procedure for 1 wk before the experimental series was begun. All animals were given free access to standard rat chow and tap or 7-NI water until the day before an experiment. They were fasted overnight but had free access to a sugar solution (5% glucose in tap water).

Surgical preparation. On the day of the experiment, the rat was anesthetized by an intraperitoneal injection of thiopenthal sodium (Trapanal, 120 mg/kg body wt), which was supplemented if necessary during the experiment. The rats were placed on a servo-regulated heating pad with a rectal probe to maintain their body temperature at 37.5°C. The trachea was catheterized to allow spontaneous breathing. Catheters were inserted into the carotid artery and the jugular vein for arterial blood pressure measurements and infusion of maintenance fluid (0.9% NaCl; 10 ml/h per kg body wt), respectively. The bladder was cannulated for urine release. The rat was allowed to recover for 30 min after which a subcostal flank incision was made and the left kidney was exposed. The kidney was dissected free from surrounding tissue, placed in a Lucite cup, and fixed with a 3% agar–agar solution. The kidney surface was covered with mineral oil to prevent drying. After an equilibration period of at least 45 min, clearance and micropuncture measurements were started.

Whole kidney clearance measurements. The urine flow rate, excretion of sodium and potassium, and GFR were determined ~ 75 min after completion of surgery. Urine from both kidneys was sampled through a catheter placed in the bladder. 30 min after completion of surgery, infusion of [^{3}H]inulin in normal saline into the jugular vein was commenced. In whole kidney clearance measurements an initial bolus of 5 μCi was followed by a continuous dosage of 5 μCi/h. After an equilibration period of 45 min, two 20-min urine collection periods were begun. The urine volume was determined by weight. Before and after each collection period, blood samples were taken. These samples were centrifuged and aliquots of plasma were analyzed in a multipurpose scintillation counter (model LS 6500; Beckman Instruments, Inc., Fullerton, CA), together with aliquots of urine. Inulin clearance was then calculated as a measure of GFR. The concentrations of sodium and potassium were assayed with a flame photometer (model FLM 3; Radiometer, Copenhagen, Denmark).

Measurements of SNGFR. For measurements of SNGFR, [^{3}H]inulin (80 μCi/h) in normal saline was infused into the jugular vein. Proximal and early distal tubular segments were identified with a perfusion pipette (o.d. 7–9 μm) with which dye (Lissamine green) was injected intratubularly. For distal collections a glass pipette (o.d. 6–8 μm) filled with black-stained mineral oil was used. An oil drop was injected downstream from the puncture site and all fluid proximal to the pipette was collected. The proximal collection was done in a similar way but using a pipette with an o.d. of 8–10 μm in a segment as proximal as possible. Before the collection was begun, a hole was made in a late proximal segment to avoid a stop-flow situation. Timed samples (3–5 min) of tubular fluid were taken and analyzed for ^{3}H activity for calculations of SNGFR. The presented results are mean values of measurements in one to four nephrons per kidney. Because the difference between distal and proximal collections is an estimate of the activity of the TGF system, only nephrons from which both distal and proximal collections had been made were used. The initial distal collection was performed with fluid through the MD area and hence with TGF activated. The subsequent proximal collection would be done with TGF inactivated, because all fluid was collected proximal to the oil drop in the proximal tubule. The larger the difference between proximally and distally measured SNGFR, the stronger the activation of TGF.

Stop-flow pressure measurements. TGF characteristics were determined by the stop-flow technique. Under a stereo microscope, randomly chosen proximal tubular segments on the kidney surface were punctured with a sharpened glass pipette (o.d. 3–5 μm) filled with 1 M NaCl solution stained with Lissamine green. The pipette was connected to a servo-nulling pressure system (WP Instruments, New Haven, CT) to determine the proximal tubular free-flow pressure (P_T). By injections of the stained fluid, the tubular distribution on the kidney surface was determined. In nephrons where more than three proximal segments were identified, a second pipette (o.d. 7–9 μm) was inserted in the last accessible segment of the proximal tubule. This pipette was filled with an artificial ultrafiltrate (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $NaHCO_3$, 7 mM urea, 2 g/liter Lissamine green, pH 7.4), and connected to a microperfusion pump (Hampel Inc., Frankfurt, Germany). Between these two pipettes a solid wax block was placed, with a third pipette (o.d. 7–9 μm).

The pressure upstream to the block, the P_{SF}, was measured at different perfusion rates (0–40 nl/min) in the loop of Henle. The flow was increased or decreased in steps of 2.5–5 nl/min and the maximal feedback response ΔP_{SF} was determined as the decrease in P_{SF} at the 40 nl/min perfusion rate, compared with P_{SF} at zero perfusion. The tubular flow rate at which 50% of the maximal pressure response was obtained, called the turning point (TP), was determined; by definition TP is a measure of the TGF sensitivity.

Series II. Series II experiments were conducted in 29 rats (290–370 g). The animals were distributed into three groups: control animals ($n = 10$) received tap water for 3 wk; 7-NI animals ($n = 9$) were treated with 7-NI (10 mg/kg per day dissolved in tap water) over 3 wk before the experiments; L-NAME animals ($n = 10$) received a similar treatment with L-NAME (6 mg/kg per day dissolved in tap water; Sigma Chemical Co.).

Isolated blood-perfused juxtamedullary nephron preparation. The acute experiments were conducted as described originally by Casellas and Navar (22) and later by Casellas and Moore (23). Briefly, the animals were anesthetized with pentobarbital sodium (60 mg/kg Mebumal; NordVacc). The right kidney was removed and the left kidney was acutely denervated with phenol solution applied around the renal artery. A double-barrel cannula was introduced into the left renal artery via the aorta and pressure-controlled perfusion of the left kidney was started with Krebs-Ringer-bicarbonate solution containing 5 mM Hepes buffer, 4% dialyzed BSA (BSA fraction V; Sigma Chemical Co.) and a mixture of amino acids (L-methionine 0.33, L-isoleucine 0.3, L-alanine 2.0, glycine 2.3, L-arginine 0.5, L-proline 0.9, L-aspartic acid 0.2, L-glutamine 0.5, L-serine 1.0 mM; Sigma Chemical Co.). This perfusate was pressurized and equilibrated with a 95% O_2/5% CO_2 gas mixture. The perfused kidney was removed, longitudinally sectioned, and the papilla was reflected upwards. The veins

were cut open and the pelvic mucosa and connective tissue were removed. All major arteries supplying the rest of the kidney were ligated except for a few afferent arterioles or a small arcuate artery with its branches in the observed area. The preparation was then transferred to a fixed stage Zeiss Axioskop microscope equipped with a Zeiss LD Achroplan 32×/0.40 long-working distance objective (Carl Zeiss, Inc., Thornwood, NY). The images of the afferent arteriole were displayed on a video monitor connected to an MTI CCD 72 camera (DAGE-MTI Inc., Michigan City, IN) and to a personal computer. The images were annotated, stored, and diameter measurements were taken using the software Image-1 (Universal Imaging Co., West Chester, PA). The surface of the preparation was superfused with warmed (37°C) Krebs-Ringer-bicarbonate solution containing 1% BSA at a rate of ~ 3 ml/min. During measurements, freshly separated and washed homologous red blood cells were added to the perfusate. At 100 mmHg perfusion pressure, as measured at the level of the renal artery, basal vascular diameter of a selected afferent arteriole (~ 150 μm upstream from the glomerulus) was measured and recorded. After this, carbachol (10^{-5} M; Sigma Chemical Co.) was added to the perfusing blood. After a 15-min period the diameter was measured again.

NO_2^- /NO_3^- excretion measurements. The rats from the series II experiments were placed in metabolic cages during 4 h after 3 wk of treatment with vehicle, 7-NI, and L-NAME. Urine samples were collected for NO_2^-/NO_3^- concentration determination. The NO_2^-/NO_3^- analysis was conducted using an assay kit (Cayman Chemical Company, Ann Arbor, MI) that measures total nitrate/nitrite concentration in a two-step process. The first step was the conversion of nitrate to nitrite using nitrate reductase. The second step was the addition of the Griess reagents which convert nitrite into a deep purple azo compound. Photometric measurement of the absorbance due to this azo chromophore determines NO_2^- concentration.

Statistical analyses. All values are given as mean±SE. Values from tail-cuff measurements of blood pressure were tested with analysis of variance for repeated measures, followed by the Bonferroni test for pair-wise multiple comparisons. The one-sample Kolmogorov-Smirnov test for normal distribution was used for all other parameters. Normally distributed parameters were tested for significance with Student's paired or unpaired *t* test and others with the Mann-Whitney U test. $P < 0.05$ was accepted for significance.

Results

Noninvasive blood pressure measurements

Noninvasive blood pressure measurements with the tail-cuff technique were performed during a period of 5 wk in the control and 7-NI_{4w} groups (Fig. 1). During the week before treatment (−1–0), the rats were trained to become accustomed to the blood pressure measurements. P_A decreased in both groups during this week, indicating lowered stress levels in the rats as they became accustomed to the technique. At the beginning of treatment/sham treatment (week 0), the control animals had a P_A of 134±3 mmHg, that continued to decline throughout the 4 wk and ended at 125±3 mmHg. In 7-NI_{4w} rats the initial P_A was 129±5 mmHg and after only 1 wk of treatment this increased to 143±6 mmHg. After 4 wk the value was 152±4 mmHg. 90 min after induction of anesthesia, P_A was 118±4 mmHg in the control animals and 138±3 mmHg in rats treated chronically with 7-NI as measured from a catheter in the carotid artery.

Series I

Whole kidney clearance measurements. The effects of 7-NI on whole kidney function in the controls, in the rats treated with a single dose of 7-NI, and in those treated for 1 and 4 wk are summarized in Fig. 2. All values are presented as absolute values because the body weight (286±9 g), and, more importantly, the kidney weight (control = 1.23±0.1 g; 7-NI_a = 1.24±0.1 g; 7-NI_{1w} = 1.22±0.1 g; and 7-NI_{4w} = 1.25±0.1 g) were similar between groups. There was no difference in GFR between the control, 7-NI_{1w}, and 7-NI_{4w} groups (1.97±0.2, 1.85±0.1, and 1.90±0.5 ml/min, respectively), whereas 7-NI_a animals had a significantly lower GFR level (1.49±0.1 ml/min). There were only relatively small differences in urine production between the groups, although 7-NI_{4w} showed a tendency to a somewhat increased urinary output, which might indicate a mild pressure diuresis. Further, the group differences in sodium and potassium excretion rates were small. No difference in hematocrit was found between the groups.

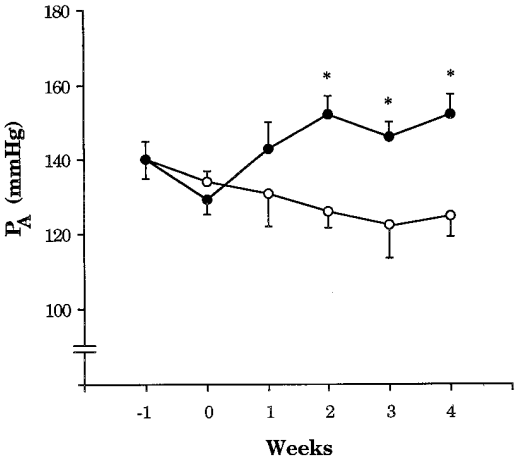

Figure 1. Blood pressure (P_A) measured by noninvasive tail-cuff technique during a 5-wk period in control rats (○) and in rats treated with 7-NI (●). Note that recording of P_A was begun 1 wk before the treatment was initiated to make the rats accustomed to the measuring equipment and handling. *$P < 0.05$ vs. vehicle-treated rats.

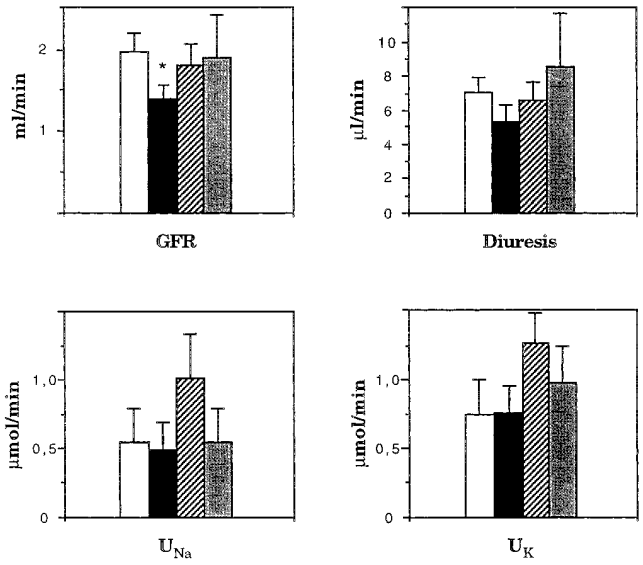

Figure 2. Whole kidney glomerular filtration rate and excretion rates of urine, sodium, and potassium in control rats (□), and in rats treated acutely (7-NI_a, ■) and for 1 and 4 wk (7-NI_{1w}, ▨; 7-NI_{4w}, ▩) with 7-NI. *$P < 0.05$ vs. control.

Table I. SNGFR Measured in Proximal and Distal Tubules

	Proximal SNGFR	Distal SNGFR	ΔProximal-Distal SNGFR	ΔProximal-Distal SNGFR
	nl/min	*nl/min*	*nl/min*	%
Control (n = 8)	32.7±4.5	23.4±3.9	9.2±4.4	28.1±9.2
7-NI_a (n = 5)	36.0±4.6	15.3±3.0	20.7±4.4*	57.5±11.5*
7-NI_{1w} (n = 5)	35.2±2.9	19.3±4.2	15.9±6.9	45.2±17.9
7-NI_{4w} (n = 6)	39.3±4.7	26.4±6.9	12.9±6.2	32.8±12.3

Values are given as mean±SE. 7-NI_a, after acute treatment; 7-NI_{1w}, after 1 wk of treatment; 7-NI_{4w}, after 4 wk of treatment; n, rats. *$P < 0.05$ vs. control.

Table II. Tubuloglomerular Feedback Characteristics in Control Rats and in Rats after Acute 7-NI Treatment (7-NI_a), and after 1 and 4 wk of 7-NI Treatment

	Control	7-NI_a	7-NI_{1w}	7-NI_{4w}
P_A (mmHg)	118±4.0	122±1.6	130±2.1*	138±2.8*
P_T (mmHg)	14.7± 0.3	13.2±0.6	14.2±0.4	14.1±0.3
P_{SF} (mmHg)	41.3±2.0	40.3±2.1	40.3±1.2	43.2±1.7
ΔP_{SF} (mmHg)	10.0±1.3	15.4±1.1*	13.7±1.1*	11.1±1.2
ΔP_{SF} (%)	25.4±4.0	39.3±4.2*	33.4±2.8	26.1±3.0
TP (nl/min)	21.0±0.9	15.5±1.6*	17.9±0.5*	20.5±0.9
m/n	6/10	8/8	7/19	7/11

Values are given as mean±SE. m, rats; n, nephrons. *$P < 0.05$ vs. control.

Single nephron GFR measurements. The results from the proximal and distal collections of tubular fluid are presented in Table I. The proximal SNGFR was similar between the groups, whereas the distal SNGFR in the 7-NI_a group was clearly lower than in the other groups. The proximal–distal ΔSNGFR in that group was 20.7±4.4 nl/min, compared with 9.2±4.4 nl/min in the controls. Indications of a more active TGF, although less pronounced and not significant, were also seen in 7-NI_{1w}, where ΔSNGFR was 15.9±6.9 nl/min. 7-NI_{4w} did not differ (ΔSNGFR = 12.9±6.2 nl/min) from the control group.

Stop-flow pressure measurements. Stop-flow pressure measurements were performed to characterize the sensitivity and the reactivity of the TGF mechanism (Table II; Fig. 3). P_T did not differ between the groups and P_{SF} showed no difference between the controls and the 7-NI_a, 7-NI_{1w}, and 7-NI_{4w} animals (41.3±2.0, 40.3±2.1, 40.3±1.2, and 43.2±1.7 mmHg, respectively). Elicitation of the maximal TGF response (i.e., an increase in the late proximal flow rate from 0 to 40 nl/min) in the controls caused a decrease in P_{SF} by 10.0±1.3 mmHg, which was not different from that found in 7-NI_{4w} (by 11.1±1.2 mmHg). But in more than 50% of the studied nephrons the initial transient drop in P_{SF} was more or less doubled as a response to maximal TGF activation. This transient response then faded away, and the sustained P_{SF} did not differ from that in the controls. In the 7-NI_{1w} group, P_{SF} fell from 40.3 to 26.6 mmHg (ΔP_{SF} 13.7±1.1 mmHg), which is a significantly greater reduction than seen in the controls. The strongest TGF response was elicited in 7-NI_a, where P_{SF} fell from 40.3 to 24.9 mmHg (ΔP_{SF} 15.4±1.1 mmHg) after maximal TGF activation. No transients were seen in these two groups. The TGF sensitivity was determined by measurements of TP. Small changes

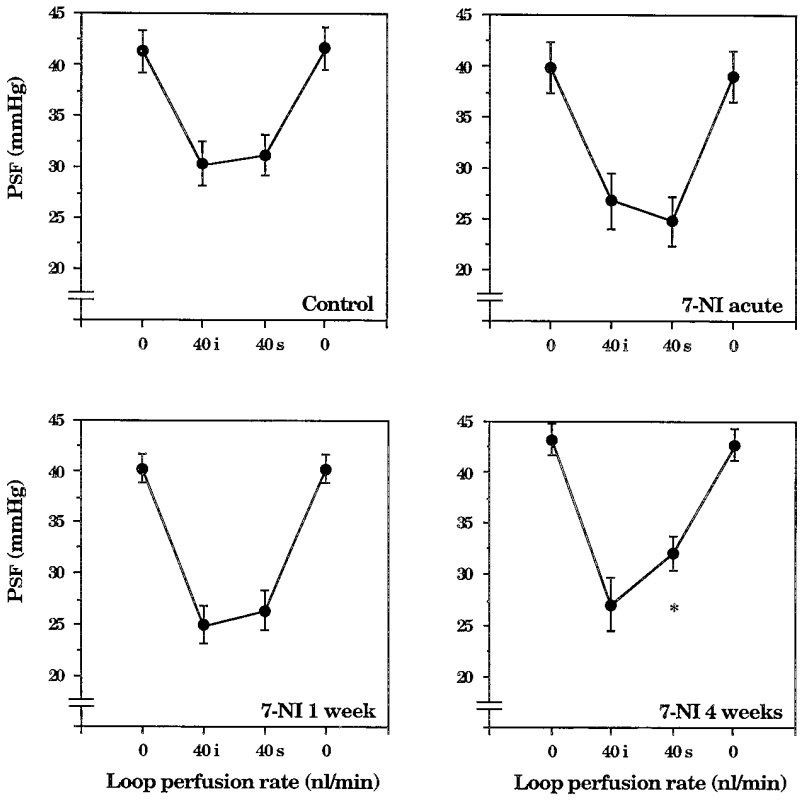

Figure 3. Tubuloglomerular feedback response in control rats and in rats treated acutely, and for 1 and 4 wk with 7-NI. Curves represent the proximal tubular stop-flow pressure (P_{SF}) at 0 and 40 nl/min of loop of Henle perfusion. 40i stands for the initial response and was defined as the maximal change in P_{SF} that occurred during the first minute of 40 nl/min perfusion. 40s is the sustained response after a stabilization time of 3–5 min. Note the strong initial but transient response to an increased loop of Henle perfusion rate in 7-NI_{4w} animals. *$P < 0.05$ vs. P_{SF} at 40i.

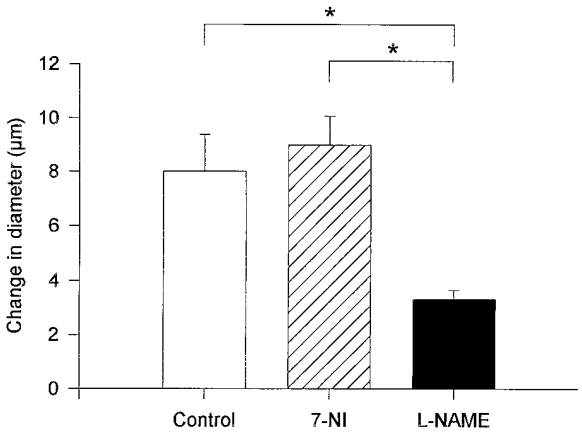

Figure 4. Increases in afferent arteriolar diameter after acute administration of 10^{-5} M carbachol to the perfusate in rats treated for 3 wk with vehicle ($n = 5$; □), 7-NI ($n = 4$; ▨), and L-NAME ($n = 4$; ■), respectively. *$P < 0.05$ vs. L-NAME.

in this parameter may be very important for the regulation of GFR. TP in control animals was 21.0±0.9 nl/min, which was not different from that in 7-NI_{4w} (20.5±0.9 nl/min). 7-NI_{1w} rats showed a significantly lower TP (17.9±0.5 nl/min) and the lowest TP was found in 7-NI_a, where it was 15.5±1.6 nl/min. Interestingly, acute administration of 7-NI (25 mg/kg body wt) to 7-NI_{1w} and 7-NI_{4w} rats did not affect either ΔP_{SF} or TP, indicating that 1 or 4 wk of treatment with 7-NI was sufficient to inhibit MD nNOS.

Series II

Isolated blood-perfused juxtamedullary nephron preparation. The results from the series II experiments are shown in Fig 4. These experiments were conducted after 3 wk of treatment with 7-NI, L-NAME, or vehicle. The perfusion pressure was kept at 100 mmHg throughout the entire experiment. Acute administration of carbachol (10^{-5} M) elicited an increase in the diameter of the afferent arteriole by 8.0±1.4 μm (50.2±9.0%) in the control group and by 9.0±1.1 μm (50.1±6.3%) in 7-NI rats. In the L-NAME–treated group the afferent arterioles were dilated by only 3.3±0.3 μm (16.0±1.6%). This latter value was found significantly different from both the control and the 7-NI group.

NO_2^-/NO_3^- excretion measurements. Measurements of urinary NO_2^-/NO_3^- showed that vehicle-treated animals had an urinary excretion rate of 3.26±0.26 pmol/min, whereas 7-NI animals had a significantly higher excretion rate of 5.31±0.35 pmol/min. L-NAME–treated rats had a corresponding value of 2.69±0.18 pmol/min that is significantly less than 7-NI–treated rats but it was not different from the control group. Thus, it seems likely that NOS other than that of neuronal origin is unaffected by 7-NI treatment.

Discussion

Long-term treatment of rats with L-arginine analogues such as L-NAME and N^{ω}-nitro-L-arginine has developed into a new model of experimental hypertension (1–8). This type of hypertension has been shown to be associated with dramatic changes in total vascular resistance and also in renal hemodynamics. Baylis et al. (3) demonstrated that chronic administration of an L-arginine analogue for 2 mo led to sustained and stable systemic hypertension which was combined with renal vasoconstriction. Using micropuncture, Ribeiro et al. (8) found that this model of hypertension was associated with an increased P_{GC}, increased afferent and efferent resistance, and lowered glomerular capillary ultrafiltration coefficient. These results imply that renal NO production is essential for the control of body fluid homeostasis and hence the blood pressure. On the other hand, a conceivable reason for the elevated blood pressure could be that systemic administration of L-arginine analogues affects peripheral resistance vessels, leading to a general vasoconstriction (9, 10). The resultant increase in blood pressure could then bring about the renal vasoconstriction as a secondary effect.

Renal NOS is present in at least three different isoforms (24). Endothelial NOS (eNOS) is located in the cytoplasm of endothelial cells in renal vessels. The nNOS has been found mainly in MD cells of the juxtaglomerular apparatus (14–16) although some investigators have demonstrated the existence of nNOS in the inner medulla (25). The third type of NOS, the inducible NOS (iNOS), has been found in a wide range of renal structures. Glomerular, proximal tubular, distal convoluted, and mesangial cells have all been shown to contain iNOS (26, 27). Administration of L-arginine analogues blocks NOS of all three isoforms, and the recent development of more specific inhibitors was therefore greatly appreciated. 7-NI has been shown to selectively inhibit nNOS without systemic effects (28, 29) and we recently observed that 7-NI was as potent as L-arginine analogues in enhancing TGF responsiveness but without effects on blood pressure in acute experiments (18). The present studies were performed to examine the effect of chronic 7-NI treatment on blood pressure in Sprague-Dawley rats. Measurements of whole kidney excretion rates as well as micropuncture studies on TGF activity were undertaken to test the hypothesis that 7-NI selectively inhibits nNOS in the MD cells, thereby increasing the TGF activity. The subsequent decrease in GFR should then lead to diminished renal excretion of water and electrolytes, which would increase the blood pressure.

From earlier studies on the development of arterial hypertension in spontaneously hypertensive rats (from both the Okamoto spontaneously hypertensive rat strain, and the Milan hypertensive strain), we know that the sensitivity of TGF in these animals is initially very high. The increased TGF activity in these rats is associated with a reduction of total kidney GFR and SNGFR. Consequently, they retain water and solutes and their blood pressure rises (30, 31). In this study, acute 7-NI treatment induced no change in blood pressure but potently enhanced the TGF responsiveness, as indicated both by decreased TP and increased maximal response. Our data also indicate that this increased TGF sensitivity leads to an activation of the TGF system as suggested by a significant increase in the difference between proximal and distal SNGFR compared with that in control animals. It is very likely that this TGF activation is one important factor underlying the reduction in total kidney GFR seen in the 7-NI_a animals. After 1 wk of treatment, TGF sensitivity was still increased, although the increase in maximal response and the reduction in TP were not as pronounced as in 7-NI_a animals. The difference between proximally and distally measured SNGFR in 7-NI_{1w} animals was not found to be significantly different from that in the controls, but

still was somewhat larger. The blood pressure after 1 wk of 7-NI treatment was higher, however, than in the controls. After 4 wk of this treatment the blood pressure was further increased, but the TGF sensitivity was completely normalized and there was no difference in TGF activation compared with that in control animals. Neither whole kidney GFR nor the excretion rates of sodium and potassium were different. The urine production was somewhat higher than in the other groups, but this was most likely an effect secondary to the increased blood pressure. A single dose of 7-NI acutely administered to both 7-NI_{1w} and 7-NI_{4w} animals did not alter the TGF sensitivity, indicating that the inhibition of neuronal NOS was complete during dietary addition of the drug. Thus there seems to be a parallel between the development of hypertension in spontaneously hypertensive rats and that resulting from chronic administration of 7-NI. It is therefore particularly interesting to note that we recently found an impaired effect of NOS inhibition in spontaneously hypertensive rats and Milan hypertensive rats as compared with their normotensive control strains (32). Taken together, these results indicate that disturbed NO synthesis might be an important factor in the development of arterial hypertension.

Interestingly, in more than 50% of the studied nephrons of 7-NI_{4w} rats, increased loop of Henle perfusion resulted in an initial transient drop in P_{SF} to almost the same magnitude as the sustained level in acutely treated nephrons. This pressure drop lasted for $\sim$ 1 min and P_{SF} then gradually recovered to a sustained and stable level which was not different from the sustained level in control nephrons. As stated earlier, acute administration of 7-NI to 7-NI_{4w} animals did not increase the TGF response, so it is very likely that the MD-NOS system was totally blocked after 4 wk of 7-NI treatment. This might indicate that chronic neuronal NOS inhibition, which in the kidney predominately affects MD-NOS, exposes the involvement of some of the other NOS isoforms. We have shown recently that under normal conditions iNOS is not involved in TGF regulation (18), but a feasible influence by this isoform cannot be excluded in the hypertensive state. Another possibility is that MD-NOS inhibition unmasks the influence of eNOS in the afferent arteriole and that NO synthesis by this enzyme is stimulated by shear stress (33–35), induced by the TGF-mediated decrease in arteriolar diameter or by some other stimuli. It is also conceivable that chronic MD-NOS inhibition unveils a hitherto unknown vasodilator that is not as fast as NO but capable of taking over the role as a modulator of the afferent tonus. The existence of a second vasodilator involved in TGF modulation was proposed recently by Vallon and Thomson (36). Furthermore, the results of another recent study of chronic NOS inhibition by Bouriquet and Casellas (4) might support the idea of a second vasodilation system in renal arterioles. They studied the autoregulatory response in rats subjected to chronic NOS inhibition by measurements of the arteriolar diameter in the isolated juxtamedullary nephron preparation and found a diminished arteriolar response to increases in renal perfusion pressure. In fact, vessels of these rats were dilated by supranormal perfusion pressures, and the dilation was most pronounced in the juxtaglomerular afferent arterioles. They used an unspecific NOS inhibitor and this dilation therefore cannot be explained by upgraded NO synthesis by any of the currently known isoforms of NOS. Combined with the fact that the impaired contractility did not affect the ability of the vessels to generate their basal tonus and that this occurred in the absence of detectable changes in the structure of the vessel walls (4), their findings might point to a possible involvement of a second dilator system in renal vessels.

Whether the effects of chronic unspecific NOS inhibition on P_A are a result of the previously reported changes in renal hemodynamics or are due to unspecific effects on the peripheral resistance vessels has not yet been determined. The results of the present study demonstrate that chronic inhibition of neuronal NOS can, by itself, cause a sustained increase in P_A, although 7-NI has no systemic effects in acute experiments (18, 29). These findings indicate that the TGF mechanism plays a crucial role in the regulation of renal filtration and hence of P_A. But our results do not allow us to exclude some other ways in which disturbed neuronal NO synthesis might affect P_A. The blood pressure of chronically 7-NI–treated animals could, for example, be affected by altered renin secretion. The effect of NO on renin release is still controversial and will not be discussed here. Another possible explanation for the hypertension observed after chronic NO inhibition that cannot be excluded is that both unselective NOS inhibition and selective neuronal NOS inhibition might have direct effects on the central nervous system. It has been found that injections of very low doses of an NOS inhibitor into the lateral cerebral ventricle of the rat cause a rise in blood pressure (11). Systemic infusion of the same dose had no effect. Furthermore, intracerebroventricularly administered NO donors have been shown to cause a decrease in blood pressure in rats (12). These observations imply that NOS activity in the cardiovascular regulatory center of the brain might be involved in blood pressure regulation. Sander et al. (37) demonstrated that sympathectomy markedly attenuated the hypertensive effect of chronic L-NAME treatment on rats. In their study, sympathectomy did not alter eNOS-dependent vasodilation to acetylcholine and bradykinin, indicating that the hypertensive effect of L-NAME was related more to inhibition of nNOS than to eNOS. Consequently, the effects of 7-NI on the central nervous system and on presynaptic sympathetic ganglia might substantially contribute to the presently described hypertension.

One critical point in this study is the question about the specificity of 7-NI as an inhibitor of nNOS. In acute experiments 7-NI has been shown to exert its effect on nNOS without changes in blood pressure of the treated animals (18, 32), which indicates that this substance does not interfere with eNOS. Series II experiments of this study were performed to investigate a plausible inhibitory effect on eNOS or iNOS by the long-term 7-NI treatment. Using the isolated perfused juxtamedullary nephron preparation, the vasodilatory response of the vessels upon carbachol administration was compared between vehicle, 7-NI– and L-NAME–treated rats. The extent of vasodilation after administration of carbachol in 7-NI–treated rats was similar to that found in vehicle-treated rats. The vasodilatory response of the vessels of L-NAME–treated rats was markedly blunted upon acute carbachol administration, indicating that 7-NI does not interfere with isoforms of NOS other than the expected neuronal isoform.

Furthermore, measurements of urinary excretion rates of NO_2^-/NO_3^- give an estimate of the total NO production of the body. Most bodily produced NO is a product of the eNOS, and long-term L-NAME treatment is known to decrease the excretion rates of NO_2^-/NO_3^- (38). In our study, urinary NO_2^-/NO_3^- levels were significantly decreased in L-NAME–treated animals compared with 7-NI animals, also indicating that NO

production from eNOS and iNOS is undisturbed by 7-NI treatment.

To summarize, the present findings demonstrate that acute selective inhibition of neuronal NOS results in increased TGF sensitivity as indicated both by increased maximal TGF responsiveness and by reduction of the tubular flow rate required to activate the TGF response. Measurements of SNGFR after acute administration of 7-NI showed that the TGF system was strongly activated to reduce total kidney GFR. After 1 wk of treatment TGF sensitivity was still somewhat increased, but the strong effect on GFR had faded, and the blood pressure was now increased. After 4 wk of 7-NI treatment the blood pressure was even more elevated, but the TGF activity was normalized. These results might imply that the change in TGF sensitivity induced by the 7-NI treatment activates the TGF mechanism to reduce GFR which might lead to volume retention. The increased extracellular volume could elevate the blood pressure. Thereby a new steady state would be reached, where a normalization of TGF occurs at the expense of an increased blood pressure. A sequence of events that is similar to that seen during development of arterial hypertension on spontaneously hypertensive rats of the spontaneously hypertensive rats and Milan hypertensive rat strains. However, because this study was performed using systemic administration of 7-NI, we cannot exclude that both the elevated blood pressure and our findings on renal hemodynamics are consequences of direct effects of 7-NI on the central nervous system and/or presynaptic sympathetic ganglia.

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