

Dysfunctional Renal Nitric Oxide Synthase as a Determinant of Salt-Sensitive Hypertension: Mechanisms of Renal Artery Endothelial Dysfunction and Role of Endothelin for Vascular Hypertrophy and Glomerulosclerosis

MATTHIAS BARTON,* INGRID VOS,[‡] SIDNEY SHAW,[†] PETER BOER,[‡] LIVIUS V. D'USCIO,* HERMANN-JOSEF GRÖNE,[§] TON J. RABELINK,[‡] THOMAS LATTMANN,* PIERRE MOREAU,* and THOMAS F. LÜSCHER*

*Cardiology, University Hospital Zürich and Cardiovascular Research, Institute of Physiology, University of Zürich, [†]Department of Clinical Research, University Hospital Bern, Switzerland; [‡]Nephrology and Hypertension, University Hospital Utrecht, The Netherlands; and [§]Department of Pathology, Philipps-University Marburg, Germany.

Abstract. This study investigated the role of renal nitric oxide synthase (NOS), endothelin, and possible mechanisms of renovascular dysfunction in salt-sensitive hypertension. Salt-sensitive (DS) and salt-resistant (DR) Dahl rats were treated for 8 wk with high salt diet (4% NaCl) alone or in combination with the ET_A receptor antagonist LU135252 (60 mg/kg per d). Salt loading markedly increased NOS activity (pmol citrulline/mg protein per min) in renal cortex and medulla in DR but not in DS rats by 270 and 246%, respectively. Hypertension in DS rats was associated with renal artery hypertrophy, increased vascular and renal endothelin-1 (ET-1) protein content, and glomerulosclerosis. In the renal artery but not in the aorta of hypertensive DS rats, endothelium-dependent relaxation to acetylcholine was unchanged; however, endothelial dysfunction

due to enhanced prostanoid-mediated, endothelium-dependent contractions and attenuation of basal nitric oxide release was present. Treatment with LU135252 reduced hypertension in part, but completely prevented activation of tissue ET-1 without affecting ET-3 levels. This was associated with a slight increase of renal NOS activity, normalization of endothelial dysfunction and renal artery hypertrophy, and marked attenuation of glomerulosclerosis. Thus, DS rats fail to increase NOS activity in response to salt loading. This abnormality may predispose to activation of the tissue ET-1 system, abnormal renal vasoconstriction, and renal injury. Chronic ET_A receptor blockade normalized salt-induced changes in the renal artery and reduced glomerular injury, suggesting therapeutic potential for ET antagonists in salt-sensitive forms of hypertension.

The pathomechanisms of salt-sensitive hypertension, an independent determinant of cardiovascular risk (1), are unknown. Changes in expression and/or activity of endothelium-derived factors such as nitric oxide (NO) (2), vasoconstrictor prostanoids (3), and endothelin-1 (ET-1) (4) have been implicated in the pathogenesis of arterial hypertension. In the Dahl rat, an animal model of salt-sensitive hypertension (5), impaired renal vasodilatory capacity (6) and NO-mediated endothelial dysfunction

in conduit arteries have been reported (7–9). However, the exact mechanisms underlying the impaired vasodilatory capacity are not known.

Animal studies using infusions or oral treatment with NO synthase (NOS) inhibitors have suggested that NO plays an important role in maintaining renal function and structure. Indeed, short-term treatment with NOS inhibitors increases BP and inhibits sodium excretion (10), whereas a more prolonged inhibition also causes fibrinoid deposition in both the vasculature and renal parenchyma (11). Genetic studies (12,13) as well as indirect functional evidence suggested a role of the L-arginine/NO pathway for hypertension also in salt-sensitive Dahl (DS) rats, because both oral L-arginine treatment (14) and intramedullary L-arginine infusion (15) normalize BP and improve renal hemodynamics (16). High salt diet increases protein expression of Nos2 in the kidney of Sprague Dawley rats without affecting BP (17), suggesting a potential compensatory mechanism to counteract the increase in volume load and pressure. Consistent with this notion, Sprague Dawley rats treated with the Nos2 inhibitor aminoguanidine develop hypertension, which can be reversed by L-arginine treatment (18). These data, together with the observation that dietary sodium

Received March 18, 1999. Accepted October 2, 1999.

This work was presented at the 30th Annual Meeting of the American Society of Nephrology, San Antonio, TX, November 2–5, 1997, and at the 52nd Annual Fall Conference and Scientific Session of the American Heart Association Council for High Blood Pressure Research, Philadelphia, PA, September 15–18, 1998. Parts of this work have been published previously (*J Am Soc Nephrol* 8: 295A, 1997, *Hypertension* 32: 623, 1998, and *Science* 281: 1962, 1998).

Correspondence to Dr. Thomas F. Lüscher, University Hospital, Rämistrasse 100, CH-8091 Zürich, Switzerland. Phone: +41 1 255 21 21 or 21 77; Fax: +41 1 255 42 51; E-mail: 101527.3476@compuserve.com

1046-6673/1105-0835

Journal of the American Society of Nephrology

Copyright © 2000 by the American Society of Nephrology

aggravates hypertension and renal injury during chronic NOS inhibition (19), suggest that the effects of sodium on BP may be mediated through interaction with NO synthesis. Of note, ET-1, a potent vasoconstrictor and mitogen synthesized by the vasculature and the kidney which interacts with the L-arginine/NO pathway (11) (20,21), has recently been implicated in the pathogenesis of salt-induced Dahl hypertension (9,22). Although the ET-3 gene locus has been linked to hypertension in Dahl rats (23), it is unknown whether changes in ET-3 expression occur in hypertensive animals.

The aims of this study were: (1) to characterize potential mechanisms of endothelial function in the renal artery in normotensive and hypertensive Dahl rats; and (2) to determine whether and to what extent the L-arginine/NO pathway and/or the endothelin system play a role for renovascular functional and structural changes.

Materials and Methods

Animals, BP, and Body Weight

Male salt-sensitive (DS; $n = 7$ to 10 per group) and salt-resistant (DR; $n = 6$ to 8 per group) Dahl rats (13 wk of age; Charles River WIGA, Sulzfeld, Germany) were randomly assigned to control diet (standard chow), high salt diet (NaCl 4%; Harlan Teklad, Madison, WI), or high salt diet in combination with LU135252, an orally active, ET_A receptor-selective endothelin antagonist. LU135252 was a gift from Knoll AG (Ludwigshafen, Germany) and administered by chow (60 mg/d + NaCl 4%) for 8 wk. Body weight and food intake were continuously monitored, and systolic BP was measured at the beginning, after 4 wk, and at the end of treatment as described (9,24). Study design and experimental protocols were approved by the institutional animal care committee.

Arterial and Renal Tissue Preparations

Rats were anesthetized (thiopental, 50 mg/kg body wt, intraperitoneally) and sacrificed. The left and right main renal artery and the thoracic aorta were isolated, removed, and placed into cold (4°C) Krebs Ringer bicarbonate solution (in mmol/L): 118.6 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25.1 NaHCO₃, 0.026 ethylenediaminetetra-acetic acid (EDTA) calcium disodium, and 11.1 glucose. Arteries were dissected in cold Krebs solution under a microscope (Wild-Heerbrugg, Switzerland), cleaned from perivascular tissue, and rinsed with a cannula to remove residual blood cells. Two rings of each renal artery (length: DS 2.37 ± 0.03 mm, $n = 67$ rings; DR 2.43 ± 0.03 mm, $n = 51$ rings) were used for organ chamber experiments. For endothelium-dependent contractions, aortic rings were used for comparison (9,25). Remaining renal artery tissue was snap-frozen in liquid nitrogen and stored at -80°C . The right kidney was cut in half, and one half was fixed in phosphate-buffered saline-buffered paraformaldehyde (4%) at 4°C, processed for histologic analysis, and embedded in paraffin. The other half and the left kidney were decapsulated, sliced horizontally, separated between outer medulla and inner cortex, immediately snap-frozen in liquid nitrogen, and kept at -80°C until assayed.

Renal Artery Endothelial Function and Structure

Renal artery rings were suspended to fine tungsten holders (diameter, 100 μm) in organ chambers containing 25 ml of Krebs-bicarbonate solution (37°C, pH 7.4, 95% O₂ and 5% CO₂) and equilibrated for 1 h. Resting tension was gradually increased, and rings were

repeatedly exposed to 100 mmol/L KCl until the optimal tension for generation of force during isometric contraction was reached (DS group: 1.48 ± 0.01 g, $n = 90$ rings; DR group: 1.46 ± 0.01 g, $n = 72$ rings, NS). After equilibration for 30 min, rings were exposed to cumulative concentrations of ET-1 (10^{-11} – 3×10^{-7} mol/L) or norepinephrine (10^{-10} – 3×10^{-5} mol/L). In quiescent renal artery and aortic rings, basal NO release was assessed by contractions to NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-5} – 3×10^{-4} mol/L). In rings pretreated with L-NAME (3×10^{-4} mol/L, 30 min of preincubation), acetylcholine (10^{-8} – 3×10^{-4} mol/L) in the presence or absence of the thromboxane A₂/prostaglandin H₂ receptor antagonist SQ 30741 (10^{-7} mol/L, 30 min of preincubation) or cyclooxygenase inhibitor indomethacin (10^{-5} mol/L, 30 min of preincubation) was used to study endothelium-dependent contractions. Other rings were precontracted with norepinephrine (1×10^{-8} – 2×10^{-7} mol/L) until a stable plateau was reached (approximately 70% of contraction induced by 100 mmol/L KCl); precontraction was not different between groups (data not shown). Relaxations to acetylcholine (10^{-10} – 3×10^{-5} mol/L)—with or without indomethacin (10^{-5} mol/L, 30 min of preincubation)—and to sodium nitroprusside (10^{-10} – 3×10^{-5} mol/L) were then performed. In some preparations, the endothelium was removed and its absence was confirmed by the lack of relaxation in response to acetylcholine (3×10^{-6} mol/L). For the assessment of vascular hypertrophy, rings were blotted dry after the experiments and weighed, and arterial surface area (mm²) was calculated by microscopic planimetry as described previously (9). Arterial diameter of renal artery rings was calculated using the following equations: Given that the circumference (c) of the artery equals the length of the transversely cut, opened arterial strip, the formula ($c = 2\pi r$) was used ($\pi = 3.1415$, $r =$ vessel radius). Renal artery diameter ($2r = c/\pi$) was calculated by use of formulas for diameter ($d = 2r$) and radius ($r = c/2\pi$) of a cylinder for each individual ring, and values were averaged. Mean diameter of renal arteries was 444 ± 14 μm (DS rats, $n = 67$ rings) and 428 ± 11 μm (DR rats, $n = 51$ rings), with no statistical difference between treatment groups (Table 1).

Determination of NOS Activity in Renal Tissue

NOS activity was determined as the formation of L-[2,3,4,5-³H]-citrulline from L-[2,3,4,5-³H]-arginine of tissue homogenates from renal cortex and medulla. After excision, kidneys were dissected into cortex and medulla, frozen in liquid nitrogen, and stored at -80°C until assay. For determination, tissue samples were homogenized using an Ultra-Turrax in 1.5 ml of buffer (pH 7.4) containing Tris (50 mmol/L), sucrose (320 mmol/L), EDTA (1 mmol/L), dithiothreitol (1 mmol/L), aprotinin (2 mg/L), and phenylmethylsulfonyl fluoride (100 mg/L). The homogenates were used for measuring protein concentration using the Bradford method and NOS activity. All measurements were performed in duplicate. Total (Ca²⁺-dependent plus Ca²⁺-independent) activity was measured by incubating 50 μl of homogenate during 30 min at 37°C with 50 μl of KH₂PO₄ buffer (50 mmol/L, pH 7.2) containing (final concentrations) dithiothreitol (1 mmol/L), NADPH (0.5 mmol/L), tetrahydrobiopterin (0.3 mmol/L), calmodulin (300 U/ml), CaCl₂ (1 mmol/L), L-citrulline (1 mmol/L), L-arginine (0.01 mmol/L), and L-[2,3,4,5-³H]-arginine (3.7 KBq). Ca²⁺-independent activity was measured by incubation in the presence of EDTA (10 mmol/L) instead of CaCl₂, and nonspecific activity was determined by incubation in the presence of L-NAME (100 mmol/L) instead of NADPH. Lower concentrations of EDTA and L-NAME were found to give incomplete inhibition of Ca²⁺-dependent and total NOS activity, respectively. Incubations were stopped by placing the

Table 1. Physiology, renal artery measurements, and renal artery ET-1 content^a

Group	Salt-Sensitive			Salt-Resistant		
	DS Control	DS + Salt	DS + Salt + LU	DS Control	DS + Salt	DS + Salt + LU
Body weight, before (g)	464 ± 11	428 ± 16	430 ± 13	407 ± 11	406 ± 14	424 ± 15
Body weight, after (g)	503 ± 16 ^b	416 ± 16	474 ± 9 ^b	442 ± 15	419 ± 15	445 ± 14
Systolic blood pressure (mmHg)	154 ± 2	190 ± 4 ^c	168 ± 3 ^d	145 ± 3	147 ± 2	150 ± 3
Renal artery diameter (μm)	417 ± 27	448 ± 20	459 ± 26	389 ± 19	431 ± 18	456 ± 17
Renal artery hypertrophy (mg/mm ²)	0.169 ± 0.009	0.232 ± 0.010 ^c	0.179 ± 0.011 ^d	0.185 ± 0.012	0.161 ± 0.012	0.166 ± 0.006
Renal artery ET-1 content (pg/g)	165 ± 33	484 ± 57 ^c	124 ± 11 ^d	136 ± 28	174 ± 49	163 ± 46

^a Effects of high-sodium diet with or without ET_A receptor antagonist LU135252 (LU) on body weight before and at the end of treatment, systolic blood pressure, diameter, vascular hypertrophy, and ET-1 protein content of the renal artery at the end of 8 wk of treatment. Data are means ± SEM. *n* = 7 to 9 in salt-sensitive rats, *n* = 6 to 8 in salt-resistant rats. ET-1, endothelin-1; DS, Dahl salt-sensitive rat; LU, LU135252; DR, Dahl salt-resistant rat.

^b *P* < 0.05 versus beginning of treatment.

^c *P* < 0.05 versus control.

^d *P* < 0.05 versus salt.

incubation mixtures in ice, followed by addition of 1 ml of ice-cold Hepes buffer (20 mmol/L, pH 5.5). The mixtures were loaded onto 1-ml Dowex 50X8-200 (Na⁺ form) columns, followed by washing with 1 ml of stop buffer. Citrulline was eluted with 2 ml of stop buffer, collected into scintillation vials, and counted in a beta counter in the presence of 15 ml of scintillation fluid. By addition of L-[¹⁴C]-citrulline before or after the incubation step, the recovery of this procedure was found to be >95%. After correction for nonspecific counts, total and Ca²⁺-independent NOS activity was calculated from the percent conversion of [³H]-arginine into [³H]-citrulline in the presence of CaCl₂ or EDTA, respectively, and expressed as picomoles per milligram of protein per minute. Ca²⁺-dependent activity was calculated by subtraction of Ca²⁺-independent activity from total activity. In additional experiments, we compared NOS enzyme activity in membrane fractions from 20,000 × *g* supernatants with the activity measured in crude tissue homogenates.

Morphologic Analysis of Glomerular Injury

Renal injury was assessed as described previously (26). Briefly, paraffin-embedded sections of whole kidneys (5 to 7 μm) stained with periodic acid-Schiff reagent were viewed by light microscopy at a magnification of ×40 using a Zeiss microscope. One hundred glomeruli per slide were evaluated. Morphologic evaluation of glomerular injury was performed by two of the authors (I.V., H.J.G.) blinded to the groups, using semiquantitative scoring methods. Lesions were graded by glomerulosclerosis (grade 0 to 4, *i.e.*, 1 to 25, 26 to 50, 51 to 76, and 76 to 100% sclerosis), mesangiosclerosis, mesangiolytic, and mesangioproliferation (grade 1 to 3), ischemia, and thrombosis. The glomerular injury score was calculated by summarizing the products of severity score (index) and the percentage of glomeruli or arteries displaying the same degree of severity. The total injury index was defined as total amount of morphologic changes.

Quantification of Tissue Endothelin Protein Content

Determination of tissue ET-1 and ET-3 protein content was performed in a blinded manner. Frozen renal artery tissue was pulverized and homogenized using a polytron for 60 s in ice-cold chloroform:methanol (2:1 dilution) containing 1 mmol/L *N*-ethylmaleimide and 0.1% trifluoroacetic acid. Frozen tissue from renal cortex and medulla (300 mg) was homogenized without pulverization. ET-1 and ET-3 protein was extracted from tissue homogenates as described (24). RIA measurements and reversed-phase HPLC was used for ET-1 and ET-3 protein content determination according to previously published protocols (24,27), and vascular and renal ET-1 and ET-3 tissue content was related to tissue weight (pg/g) (24).

Materials

Acetylcholine chloride, calmodulin, CaCl₂, dichlorodiphenyltrichloroethane, Dowex resin AG 50W-X4, EDTA, ET-3, indomethacin (dissolved in 5 mmol/L sodium carbonate), L-arginine, L-NAME, NADPH, norepinephrine bitartrate salt, phenylmethylsulfonyl fluoride, potassium chloride, sucrose, sodium nitroprusside dihydrate, and Tris salt were purchased from Sigma Chemical Co. (St. Louis, MO). ET-1 was from Calbiochem/Novabiochem AG (Läufingen, Switzerland). Rabbit antibodies against synthetic ET-1 and ET-3 were from Peninsula Laboratories (San Carlos, CA). Radiolabeled ¹²⁵I-ET-1, ¹²⁵I-ET-3, and L-[2,3,4,5-³H]-arginine was purchased from Amersham (Amersham, Buckinghamshire, United Kingdom). SQ 30741 was a gift of Bristol-Myers Squibb (Princeton, NJ), and pentobarbital was from Abbott Laboratories (Chicago, IL).

Statistical Analyses

Data are given as mean \pm SEM, and n equals the number of animals used. Relaxations are expressed as percent contraction to norepinephrine, and contractions are given as percent contraction to potassium chloride. For multiple comparisons, results were analyzed using ANOVA followed by Bonferroni correction. For comparison between two values, the unpaired t test or the nonparametric Mann-Whitney test were used when appropriate. The Pearson correlation coefficient was calculated by linear regression analysis. A P value <0.05 was considered significant.

Results

BP and Body Weight

Systolic BP increased in DS ($P < 0.05$) but not in DR rats on high salt diet after 4 wk and increased further after 8 wk (Figure 1). Hypertensive DS rats had significantly lower body weight than salt-loaded DR rats ($P < 0.05$ versus control) (Table 1). ET_A receptor antagonist LU135252 treatment only in part reduced the BP increase ($P < 0.05$) (Figure 1) but largely prevented the inadequate weight gain ($P < 0.05$) (Table 1). No significant effect on BP or body weight was observed in DR rats.

Endothelium-Dependent Relaxations to Acetylcholine

Despite hypertension, relaxations to acetylcholine were unaffected in DS rats (NS) (Figure 2); however, concomitant treatment with LU135252 slightly enhanced relaxations in DS rats ($P < 0.05$ versus salt). Surprisingly, relaxations were reduced in salt-treated DR rats after chronic treatment with LU135252 ($P < 0.05$) (Figure 2A, right panel), which could be prevented by cyclooxygenase inhibition with indomethacin *in vitro* ($P < 0.05$) (Table 2).

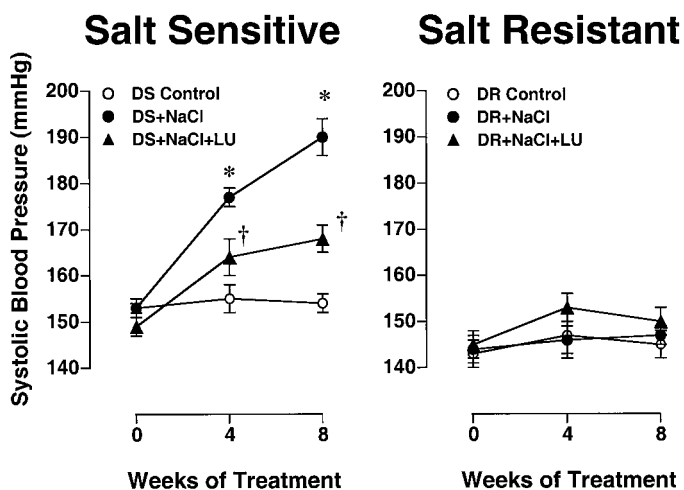


Figure 1. BP in Dahl salt-sensitive (DS) and salt-resistant (DR) rats. (A) In DS but not DR rats on a high salt diet, systolic BP increased after 4 and 8 wk compared with DS control rats ($P < 0.05$). Concomitant LU135252 in part reduced the increase in pressure in DS rats ($P < 0.05$), and had no effect in DR rats. Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt.

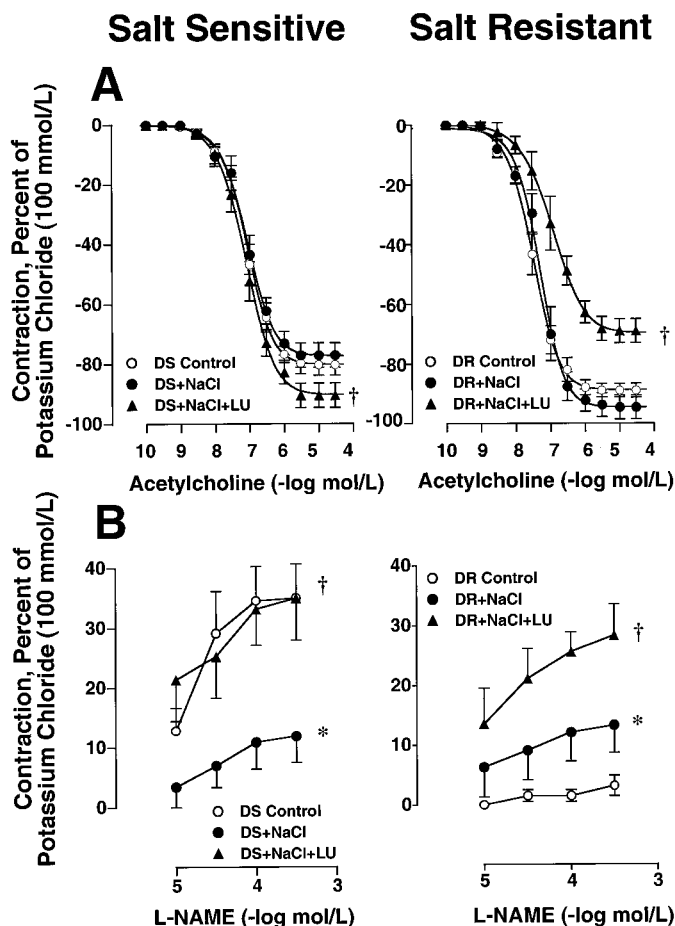


Figure 2. Endothelium-dependent relaxations to acetylcholine and endothelium-dependent contractions to nitric oxide (NO) synthase inhibitor N^G -nitro-L-arginine methyl ester (L-NAME) in renal arteries. (A) High sodium diet alone had no effect on relaxations to acetylcholine in DS or DR animals. However, in animals concomitantly treated with LU135252, relaxations were slightly but significantly increased (left panel), whereas they were reduced in DR rats (right panel) ($P < 0.05$). (B). High salt diet attenuated the contraction to L-NAME in DS rats, whereas salt treatment increased contractions in DR rats. Concomitant LU135252 treatment prevented the attenuation of basal NO release in DS rats and further enhanced contractions to L-NAME in DR rats. Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt.

Endothelium-Dependent Contractions to NOS Inhibitor L-NAME

NOS inhibitor L-NAME caused concentration-dependent contractions in quiescent renal artery and aortic rings. Treatment with high salt diet attenuated contractile responses to L-NAME in DS rats, while contractions increased in DR rats (Figure 2B) ($P < 0.05$ versus low salt). In DS rats, LU135252 treatment normalized the attenuated response ($P < 0.05$ versus salt). In comparison, maximal contractions to L-NAME (300 μ mol/L) in the aorta of DS control rats were about one-third in magnitude compared with the renal artery ($10 \pm 2\%$ of KCl) ($P < 0.01$ versus renal artery) and unaffected by high-salt diet or LU135252 (Table 2).

Table 2. Vascular physiology in isolated renal artery and aorta^a

Group	Salt-Sensitive			Salt-Resistant		
	DS Control	DS + Salt	DS + Salt + LU	DS Control	DS + Salt	DS + Salt + LU
Renal artery						
contractions						
potassium chloride (mN/mm)	3.69 ± 0.27	3.89 ± 0.19	4.12 ± 0.31	3.69 ± 0.31	4.22 ± 0.32	3.96 ± 0.42
acetylcholine + SQ30741	2 ± 2	11 ± 5 ^b	1 ± 1 ^c	4 ± 1	16 ± 6 ^b	3 ± 3 ^c
relaxations						
acetylcholine	80 ± 4	77 ± 4	91 ± 2 ^c	89 ± 2	95 ± 4	69 ± 3 ^c
acetylcholine + indomethacin	91 ± 2	98 ± 3 ^d	99 ± 1 ^d	92 ± 2	100 ± 1	96 ± 2 ^d
sodium nitroprusside	107 ± 2	105 ± 1	104 ± 1	107 ± 1	105 ± 1	103 ± 1
Aorta						
contractions						
potassium chloride (mN/mm)	7.48 ± 0.05	7.3 ± 0.04	7.3 ± 0.08	7.48 ± 0.04	7.4 ± 0.4	7.38 ± 0.02
acetylcholine	3 ± 2	4 ± 2	1 ± 1	1 ± 1	4 ± 1	1 ± 1
acetylcholine + SQ30741	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0 ^c
L-NAME (0.3 mmol/L)	10 ± 2	13 ± 4	10 ± 3	0 ± 0	9 ± 4 ^b	0 ± 0 ^c

^a Effects of high-sodium diet with or without ET_A receptor antagonist LU135252 (LU) on force (mN/mm) induced by potassium chloride, contractions to acetylcholine (0.3 mmol/L, in the presence and absence of thromboxane receptor antagonist SQ30741 (0.1 μmol/L), in renal artery and aorta. Maximal responses of relaxations to acetylcholine (30 μmol/L, with or without indomethacin 10 μmol/L) and to sodium nitroprusside (30 μmol/L) of renal arteries are also given. Contractions are normalized to contractions to KCl (100 mmol/L), and relaxations are expressed as percent relaxation of precontraction to norepinephrine. Data are means ± SEM. *n* = 7 to 9 per group in salt-sensitive rats, *n* = 6 to 8 per group in salt-resistant rats. L-NAME, N^G-nitro-L-arginine methyl ester. Other abbreviations as in Table 1.

^b *P* < 0.05 versus control.

^c *P* < 0.05 versus salt.

^d *P* < 0.05 versus acetylcholine alone.

Endothelium-Dependent Contractions to Acetylcholine

The magnitude of responses was similar in renal arteries from DS and DR control rats (NS) (Figure 3). High salt diet markedly potentiated contractions in DS rats (approximately threefold, from 25 ± 3 to 78 ± 4%, *P* < 0.05 versus control) and, to a lesser extent, in DR rats (approximately 1.6-fold) (Figure 3). Contractions were correlated with vascular ET-1 protein content in DS rats only (*r* = 0.7, *P* < 0.01). LU135252 treatment completely normalized the increased contractions (Figure 3) (*P* < 0.05 versus high salt). Responses to acetylcholine were markedly inhibited by pretreatment with the thromboxane receptor antagonist SQ 30741 (10⁻⁷ mol/L, *P* < 0.05) (Table 2) or indomethacin (10⁻⁵ mol/L) in all groups (data not shown). In the aorta of DS rats, contractions to acetylcholine were only about 1/20 in magnitude compared with the renal artery (DS control rats 3 ± 2% and DS rats 4 ± 2% on a high salt diet, NS) and unaffected by concomitant LU135252 treatment (Table 2).

Endothelium-Independent Responses

Potassium chloride-induced contractions were comparable between groups (NS) (Table 2). Contractions to ET-1 but not those to norepinephrine were attenuated in salt-loaded DS rats; however, this was prevented by concomitant LU135252 treatment (Figure 4, A and B). LU135252 normalized the response to ET-1 (*P* < 0.05 versus salt) (Figure 5, right panel). Endothelium-independent relaxation to sodium nitroprusside was unaffected by the treatments (NS) (Table 2).

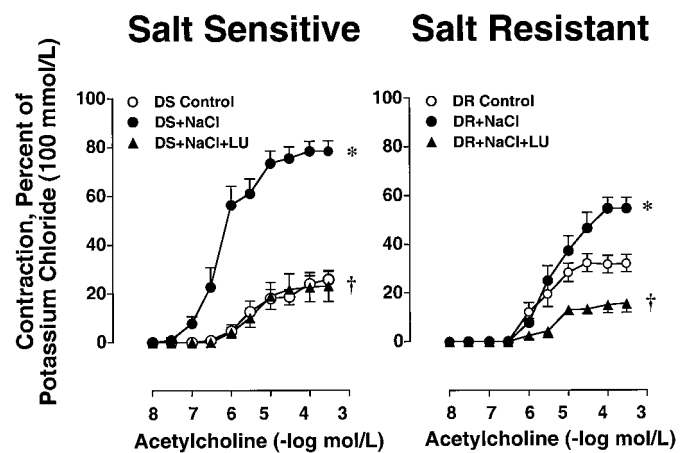


Figure 3. Endothelium-dependent, prostanoid-mediated contractions to acetylcholine in renal arteries. In renal arteries of control animals, contractions to acetylcholine (in the presence of L-NAME, 0.3 mmol/L) were similar between strains. High salt diet markedly enhanced contractions in DS and, to a lesser extent, in DR rats (*P* < 0.05); the effect of high salt diet was completely prevented by LU135252 in both strains. Data are mean ± SEM. **P* < 0.05 versus control; †*P* < 0.05 versus salt.

Renal Artery Structure

Vascular hypertrophy of the renal artery (expressed as ratio of tissue weight in mg per mm² vascular surface area) was observed after salt loading in DS rats only. The ratio increased

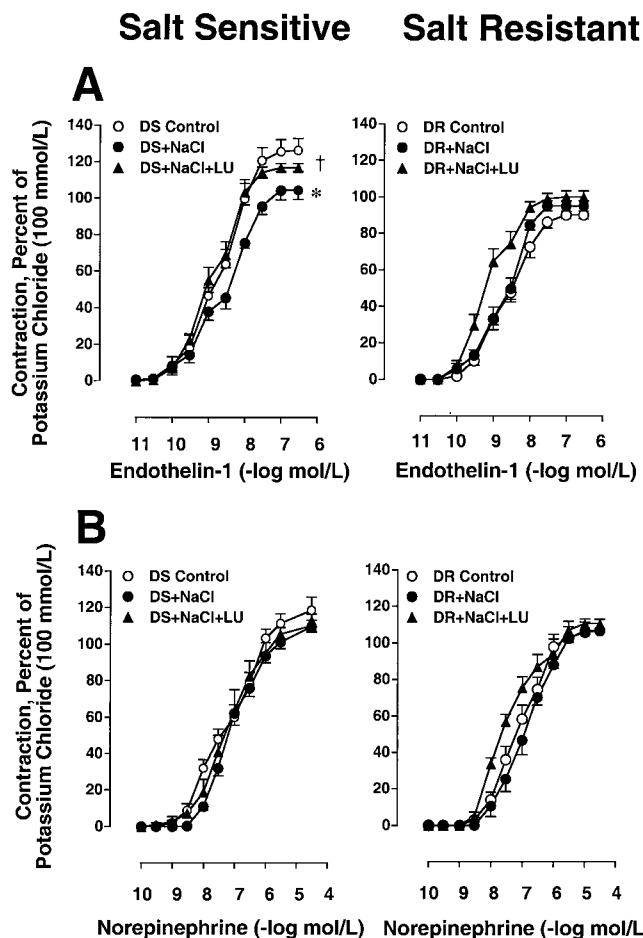


Figure 4. Contractions to endothelin-1 (ET-1) and norepinephrine in isolated renal arteries. (A) In control animals, the response to ET-1 was greater in DS than DR rats ($P < 0.05$). High salt diet reduced contractions in DS but not DR rats. LU135252 treatment restored this attenuation in DS rats, whereas contractions were slightly enhanced in DR rats. (B) Contractions to norepinephrine were unaffected by either treatment in DS or DR rats. Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt.

from 0.17 ± 0.01 to 0.23 ± 0.01 mg/mm², and was completely normalized by concomitant LU135252 treatment (0.18 ± 0.01 mg/mm², $P < 0.001$ versus salt). High salt diet with or without LU135252 had no effect on vascular structure in DR rats (NS) (Table 1).

Renal NOS Activity

Total NOS activity is given as the L-NAME-inhibitable portion of L-citrulline formation. L-NAME concentrations < 100 mmol/L showed incomplete inhibition of total NOS activity. Total activity was comparable between untreated DS and DR rats and higher in the medulla than in the cortex (Figure 5). Total NOS activity was of the same order of magnitude as reported previously in Dahl rats, Wistar Kyoto rats, and spontaneously hypertensive rats (8,28). The majority of renal NOS activity was calcium-independent (Table 3). Salt loading markedly increased total NOS activity (about threefold) in DR rats ($P < 0.05$). In contrast, DS rats failed to

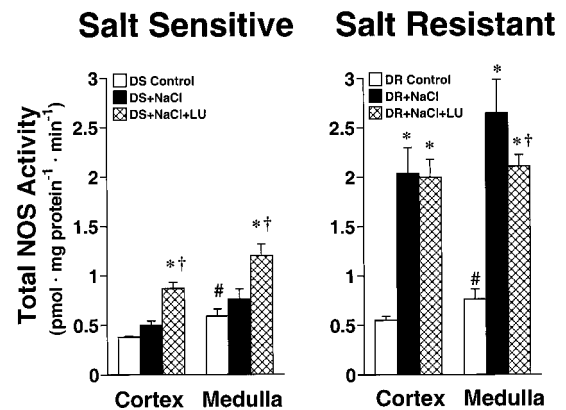


Figure 5. Renal nitric oxide synthase (NOS) activity. In DR rats (right panel), high salt diet induced a threefold increase in total NOS activity in renal cortex and medulla. No increase in response to salt loading was observed in DS rats (left panel). However, concomitant treatment with LU135252 slightly increased renal NOS activity in kidneys of DS rats only ($P < 0.05$, left panel). Note that NOS activity was higher in the medulla than in the cortex in both strains. Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt; # $P < 0.05$ versus cortex.

increase total NOS activity after salt loading (Figure 5). Interestingly, LU135252 treatment was associated with an increase of total NOS activity in both cortex ($74 \pm 10\%$) and medulla ($58 \pm 8\%$) in DS rats only ($P < 0.05$) (Figure 5). Using membrane fractions instead of crude homogenates resulted in no net difference with regard to enzyme activity and had no effect on the ratio of calcium-dependent and -independent conversion (data not shown).

Glomerular Injury

Compared with DS control animals, the number of structurally normal glomeruli (grade 0 renal injury) in hypertensive DS rats was reduced to $49 \pm 2\%$ ($P < 0.05$). Hypertension in DS rats was associated with pronounced glomerular damage with thickening of Bowman's capsule, adhesion formation, mesangial proliferation, and hypertrophy of the preglomerular arteries and arterioles (Figure 6, middle panels). Concomitant LU135252 treatment increased the number of healthy glomeruli from 49 ± 2 to $79 \pm 4\%$ ($P < 0.05$ versus high salt) (Figure 7) and markedly reduced structural glomerular changes ($P < 0.05$) (Figure 6, right panels and Figure 7). In salt-treated DR rats, renal morphology was essentially identical to untreated DR rats, which showed no signs of renal damage (authors' unpublished observation and reference 26).

Endothelin Protein Content in Renal Artery and Kidney

In hypertensive DS rats, renal artery ET-1 protein content was increased from 165 ± 33 to 484 ± 57 pg/g tissue ($P < 0.05$). This increase was prevented by chronic LU135252 treatment (124 ± 11 pg/g tissue, $P < 0.05$ versus salt). No changes occurred in DR rats (Table 1). LU135252 also prevented the increase in ET-1 protein content in renal cortex and medulla of DS rats only (Figure 8) ($P < 0.05$). Renal ET-3 protein content, which was predominantly expressed in the medulla of

Table 3. Nitric oxide synthase activity in kidney homogenates^a

Group	Salt-Sensitive			Salt-Resistant		
	DS Control	DS + Salt	DS + Salt + LU	DR Control	DS + Salt	DS + Salt + LU
Cortex						
Ca ²⁺ -dependent NOS activity	0.02 ± 0.01	0.02 ± 0.01	0.16 ± 0.05 ^{b,c}	0.04 ± 0.01	0.56 ± 0.12 ^{b,d}	0.50 ± 0.11 ^d
Ca ²⁺ -independent NOS activity	0.36 ± 0.02	0.49 ± 0.04 ^b	0.72 ± 0.04 ^{b,c}	0.5 ± 0.04 ^d	1.48 ± 0.18 ^{b,d}	1.51 ± 0.11 ^d
Medulla						
Ca ²⁺ -dependent NOS activity	0.08 ± 0.03	0.19 ± 0.03 ^b	0.43 ± 0.05 ^{b,c}	0.12 ± 0.03	1.1 ± 0.13 ^{b,d}	0.77 ± 0.09 ^{b,c,d}
Ca ²⁺ -independent NOS activity	0.51 ± 0.07	0.61 ± 0.08	0.86 ± 0.06 ^{b,c}	0.65 ± 0.09	1.58 ± 0.2 ^{b,d}	1.34 ± 0.09 ^{b,d}

^a Effect of high-salt diet and ET_A antagonist LU135252 (LU) on Ca²⁺-dependent and -independent NOS activity (pmol citrulline/mg protein per min) in renal cortex and medulla of DS and DR rats. Values are given as L-citrulline production inhibitable by L-NAME (100 mmol/L); total NOS activity (Ca²⁺-dependent + Ca²⁺-independent) is depicted in Figure 5. Note that a large portion of renal NOS activity was Ca²⁺-independent. Data are mean ± SEM. NOS, nitric oxide synthase. Other abbreviations as in Table 1.

^b *P* < 0.05 versus control.

^c *P* < 0.05 versus salt.

^d *P* < 0.05 DR versus DS.

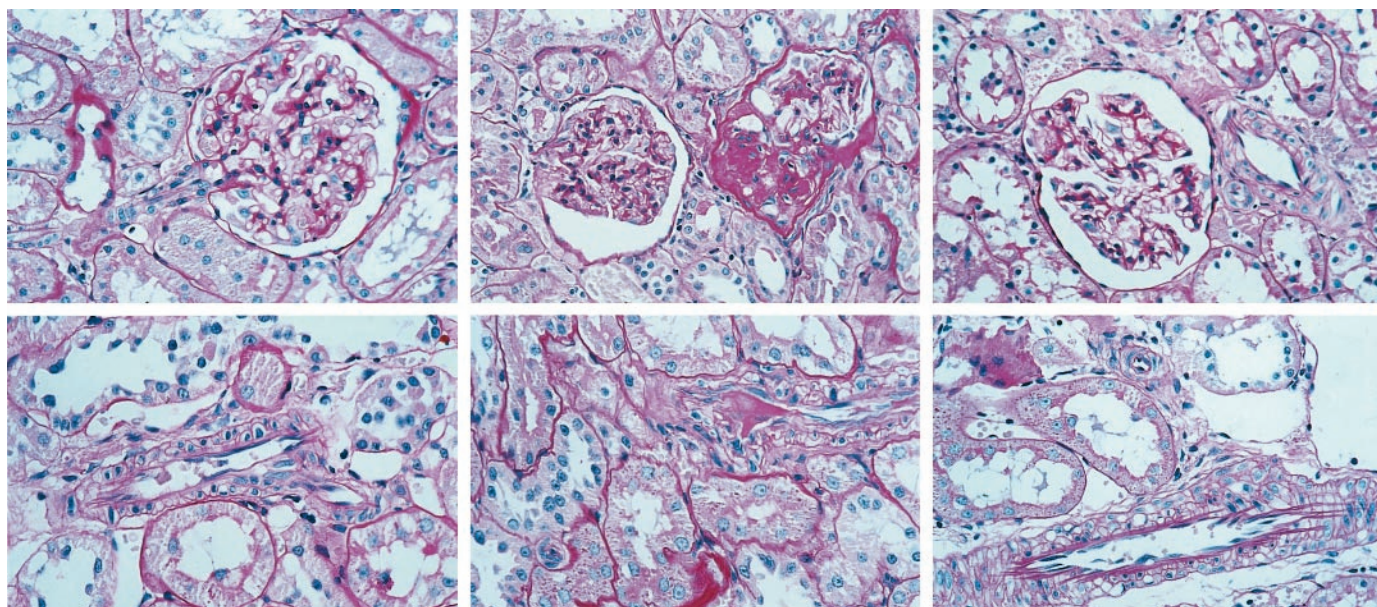


Figure 6. Morphology of glomerular injury in kidneys from DS rats. (Left) Paraffin sections from a DS control rat kidney showing a normal glomerulus and preglomerular artery (top panel) and normal intrarenal artery (bottom panel). (Middle) Glomerulosclerosis (top panel) and marked intrarenal artery hypertrophy (below) in a salt-loaded, hypertensive DS rat. (Right) Glomerular injury (top panel) and vascular hypertrophy (bottom panel) were largely prevented by ET_A antagonist LU135252. Magnification, ×100 (oil immersion), paraffin, periodic acid-Schiff (PAS) stain.

both DS and DR rats, was unaffected by high salt diet (NS) (Figure 8B).

Discussion

In this study, we have presented several novel findings. First, this study provides direct evidence for a functional defect of NOS in the kidney in salt-sensitive Dahl rats, and shows that a substantial portion of enzyme activity is calcium-independent. Second, we have demonstrated that independent of endothelium-dependent relaxation, endothelial dysfunction and vascular hypertrophy occur in the renal artery, which likely contribute to the impaired vasodilatory capacity in hyperten-

sive animals. Third, hypertension was shown to be associated with selective activation of the tissue ET-1 system (but not the ET-3 system) in the vasculature and renal parenchyma. Fourth, the ET system appears to contribute significantly to functional and structural abnormalities, because chronic ETA receptor blockade largely normalized these changes, while only partially inhibiting the salt-induced increase in BP.

Salt-Induced Hypertension: A Failure to Increase Renal NOS Activity

The key finding of our study was the observation that DS rats failed to increase renal NOS activity in response to salt

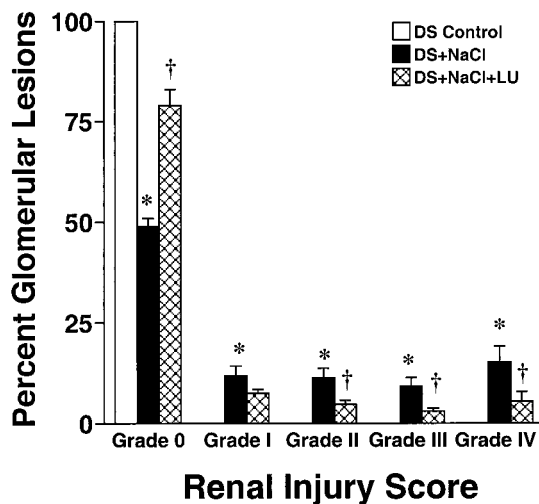


Figure 7. Morphologic assessment of glomerular injury in DS rats. Salt-induced hypertension markedly reduced the number of healthy glomeruli (grade 0 renal injury) and was associated with renal injury (grades 1 to 4, black bars), which was inhibited by chronic ET_A receptor blockade. No morphologic changes were observed in DS control rats or DR rats (not shown). Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt.

loading. In contrast, and consistent with increased renal NOS2 protein expression in salt-loaded Sprague Dawley rats (17), renal NOS activity in DR rats increased markedly after high salt diet treatment. Neither in Sprague Dawley rats (17) nor in DR rats in the present study did salt loading affect BP. The observed failure in DS rats to adequately increase NOS activity could be linked to a genetically determined defect of NOS or its regulation. In this context, it is of interest that the gene of calcium-independent *Nos2* cosegregates with BP in DS rats (12,13). A functional defect of NOS is also suggested by studies demonstrating that treatment with the NOS substrate L-arginine abolishes hypertension and inhibits renal injury in this model (14,15,29).

An unexpected and novel finding in contrast to previous studies (8,28) was that in all groups more than half of renal NOS activity was calcium-independent. It is important to note that in our experiments L-NAME concentrations < 100 mmol/L did not completely block NOS activity in renal tissue. The concentration used was 100-fold higher compared to previously published protocols (8,28), and may in part explain the difference between calcium-dependent and -independent NOS activity compared with these previous studies. Unlike other calcium-independent NOS enzymes, expression of *Nos2* is constitutive in Dahl and Wistar-Kyoto rat kidneys (30,31), and can be stimulated by salt loading (17). Also, sodium-mediated effects in humans may involve the L-arginine/NO pathway, as L-arginine-induced increases in sodium excretion and inhibition of fractional sodium reabsorption are dependent on salt loading (32). NOS2 regulates BP under normal conditions (18), and chronic inhibition of NOS2 is associated with reduced urinary nitrate excretion and proteinuria (33), indeed suggesting a role for NOS2-derived NO in renal function. Thus,

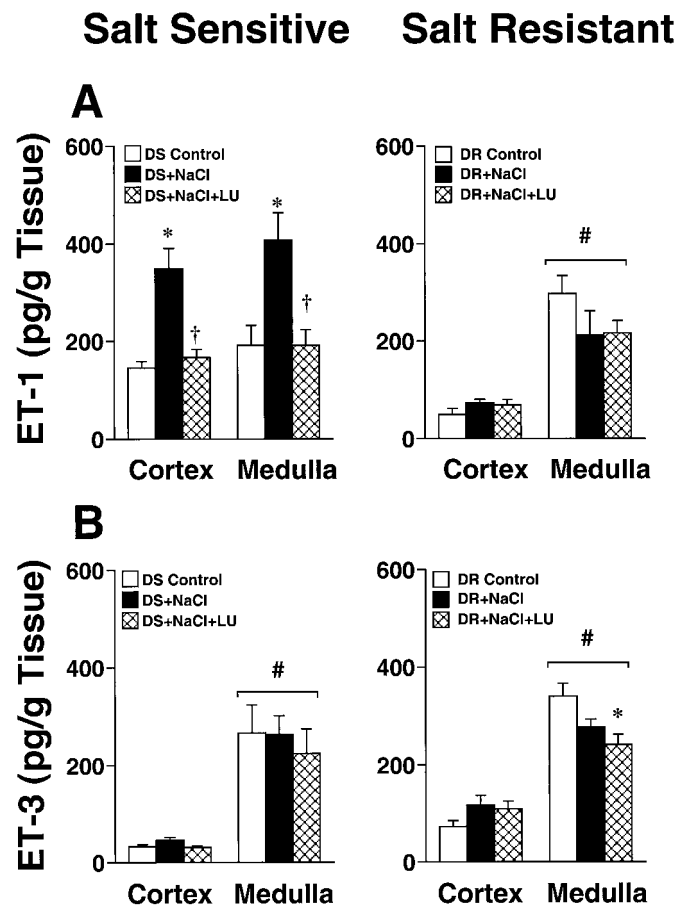


Figure 8. ET-1 and ET-3 protein content in renal cortex and medulla. (A) In control DR animals, ET-1 protein content in the medulla was higher than in the cortex (right panel, $P < 0.05$). No difference was observed in DS rats (left panel). High salt diet increased ET-1 content in DS rats only, and this increase was prevented by chronic ET_A receptor blockade. (B) In all groups of rats, ET-3 protein content in the medulla was higher than in the cortex ($P < 0.05$) and not significantly affected by treatments. Data are mean \pm SEM. * $P < 0.05$ versus control; † $P < 0.05$ versus salt; # $P < 0.05$ versus cortex.

compensatory upregulation of *Nos2* expression and/or NOS activity in the kidney (as observed in DR rats in the present study) may represent a novel mechanism regulating the increased volume load and BP. Two distinct forms of *Nos2* mRNA—macrophage *Nos2* and vascular smooth muscle cell *Nos2*—have been identified in the rat kidney (30). In addition, evidence from a very recent study indicates that *Nos2* mRNA is present in both rat cortex and medulla and that the enzyme is functionally active (34). Also, *Nos2* is present in vascular smooth muscle of intrarenal vessels (30), and reduced nitrate production by *Nos2* has been demonstrated in vascular smooth muscle cell of DS rats (13).

Future studies will determine whether the increase in calcium-independent NOS activity observed in DR rats is derived from NOS2. In this context, it is interesting to note that the paradigm of calcium dependency of NOS isoenzymes has recently been modified by studies showing that *Nos2* activity—although completely calmodulin-independent—can be

partly inhibited by removal of calcium (35). On the other hand, calcium-independent actions of Nos3 have recently been reported (36,37). Although we did not investigate gene expression of NOS isoenzymes, our study unequivocally demonstrates that salt sensitivity appears to involve a failure to increase renal NOS activity in response to salt loading, and that a substantial portion of the activity is calcium-independent. These findings are in agreement with the previously mentioned molecular data and a recent preliminary report (38).

Renal Artery Endothelial Dysfunction: Role of NO and Vasoconstrictor Prostanoids

Impaired renal vasodilatory capacity after salt loading has been described in Dahl hypertension (6). Here, we provide evidence that “selective” endothelial dysfunction may be one of the mechanisms interfering with renal artery vasodilation. Unexpectedly, and in contrast to aorta (9,25) and mesenteric artery (39), endothelium-dependent relaxations in the renal artery were unaffected by hypertension. However, endothelial dysfunction due to abnormal increased basal NO release and prostanoid-mediated contractions was present, which could result in inappropriate vasoconstriction. To our knowledge, this is the first study reporting the release of endothelium-derived vasoconstrictor prostanoids as part of endothelial dysfunction in Dahl hypertension. These mechanisms are likely to contribute to impaired renal vasodilatory capacity (6). Furthermore, our data suggest that endothelium-dependent relaxation *per se* should not be used as the sole indicator to indicate “preserved” endothelial function.

Interestingly, our study also shows that endothelin plays an important role in these functional changes. Although chronic ET_A receptor blockade only partly reduced hypertension in DS rats, the treatment completely prevented the attenuation of basal NO release and the enhanced vasoconstrictor prostanoid release. Renal NOS activity was also slightly increased, suggesting that ET_A receptor blockade can interfere with the L-arginine/NO pathway *in vivo*, in line with recent *in vitro* observations in mesangial (40) and vascular (41) cells in culture and normotensive animals *in vivo* (20). Both ET-1 (through ET_A receptor-mediated mechanisms) (42,43) and NO deficiency (44) have been implicated in vasoconstrictor prostanoid formation *in vitro*. As chronic treatment with LU135252 completely normalized prostanoid-mediated vasoconstriction and increased tissue ET-1 levels, increased vascular ET-1 concentrations may well contribute to endothelial vasoconstrictor prostanoid release *in vivo*. As observed previously in other vascular beds (9,39), contractions to exogenous ET-1 but not those to norepinephrine were reduced in the renal artery, and this was likely caused by agonist-induced receptor downregulation due to increased vascular ET-1 content. Again, treatment with LU135252 prevented both the increase of ET-1 protein and the attenuation of contractile responses.

Endothelin: Mediator of Renal Injury and Endothelial Dysfunction

In this study, we have demonstrated that both renal and vascular ET-1 protein levels, but not renal ET-3 protein, mark-

edly increase in DS rats after salt loading. This was associated with renal artery hypertrophy and glomerulosclerosis in hypertensive DS rats, confirming previous reports (26,45). Although genetic analyses have linked the ET-3 gene locus (23) but not the ET-1 gene locus (46) to hypertension in DS rats, and despite altered renal ET-3 expression in other forms of renal injury (47), we found no evidence that ET-3 expression is modulated in hypertensive DS rats. Thus, ET-1 but not ET-3 appeared to be the likely mediator of salt-mediated injury. Consistent with this hypothesis, the structural and particularly the functional changes associated with hypertension were largely normalized by concomitant treatment with an orally active ET_A antagonist, which had only moderate effects on the salt-induced BP increase. It is noteworthy that no activation of the ET system was observed in DR rats in which NOS activity (in contrast to DS rats) almost tripled. We speculate that the failure to adequately increase NOS activity may facilitate activation of the ET system in hypertensive DS rats, which is regulated by NO (16,48). This “intrinsic” NO deficiency in DS rats, in turn, may promote the detrimental effect of ET-1 on renal structure and function. Indeed, chronic NO deficiency induced pharmacologically by L-NAME treatment is associated with hypertension (2) and with pronounced changes in renal morphology and function, which can be attenuated by ET receptor antagonism (11,49). One of the limitations of our study is that we did not use another antihypertensive agent to examine the effect of BP lowering. However, it is unlikely that normalization of the observed changes was due to the antihypertensive effects of LU135252, which lowered BP only in part. In line with this notion, a previous study demonstrated that antihypertensive therapy with the diuretic indapamide actually normalized BP, but did not provide effective renal protection (8). Unfortunately, these investigators did not examine vascular hypertrophy. Schiffrin and colleagues have suggested that the effects of ET-1 on vascular structure may be, at least in part, pressure-independent (50), which is in keeping with the present study and our observations in atherosclerotic mice (20).

We have reported previously that chronic ET_A receptor blockade normalizes elevated tissue levels of ET-1 protein in hypertension and atherosclerosis (9,20,24). We have confirmed these findings in the present study, in which ET-1 tissue levels in LU135252-treated animals were comparable to those seen in untreated DS control rats. The underlying mechanism is presently unknown. It is possible, however, that increased endothelial NO activity, which regulates ET-1 production (51) and was observed after LU135252 treatment, was involved. The effects of LU135252 may also involve ET_B receptor-mediated mechanisms such as NO formation, because ET_B blockade worsens renovascular function and injury in deoxycorticosterone acetate-salt hypertension (52). Other possible mechanisms include ET_A receptor-coupled autocrine regulation of ET-1 synthesis at the transcriptional (53) or protein (54,55) level.

Clinical Implications

Salt sensitivity in patients with essential hypertension is associated with increased cardiovascular risk (1) and is partic-

ularly common among African-Americans. These patients are often resistant to conventional antihypertensive therapy (56) and commonly develop hypertensive renal injury. Two recent studies in this population demonstrated both endothelial dysfunction (57) and increased renal vasoconstriction in response to salt loading (58), consistent with our observations in experimental salt-sensitive hypertension. The ET system appears to be activated in these patients because plasma levels of ET-1 are extraordinarily high (59). Therefore, it is reasonable to speculate that selective ET_A receptor antagonism will provide additional therapeutic benefit for the treatment of salt-sensitive forms of essential hypertension.

In conclusion, we have demonstrated: (1) a failure to increase NOS activity; (2) enhanced renal artery vasoconstriction due to selective endothelial dysfunction; and (3) a role for ET-1 as mediator of functional and structural changes that act as novel mechanisms in the pathogenesis of experimental salt-sensitive hypertension. Clinical studies will show whether ET_A receptor blockade has the therapeutic potential to inhibit end-organ damage in patients with salt-sensitive hypertension.

Note Added in Proof: While this manuscript was under revision, a study was published reporting that chronic inhibition of Nos2 by use of several isoform-specific enzyme inhibitors resulted in salt-induced hypertension without affecting endothelial function in resistance arteries in formerly salt-resistant DR rats (60).

Acknowledgments

This work was supported by the Swiss National Foundation (Grant 32-51069.97 to Dr. Lüscher and Grant 32-49648.96 to Dr. Shaw), the Deutsche Forschungsgemeinschaft (Grant Ba 1543/1-1 to Dr. Barton and Grant Gr 728/5-1 to Dr. Gröne), the ADUMED Foundation (Dr. Barton), the Royal Dutch Academy of Sciences (to Dr. Rabelink), and the Intermedia Foundation (Bern, Switzerland) (Dr. d'Uscio). The authors are much indebted to Jane Boden for her invaluable help with the radioimmunoassays.

References

- Morimoto A, Uzu T, Fujii T, Nishimura M, Kuroda S, Nakamura S, Inenaga T, Kimura G: Sodium sensitivity and cardiovascular events in patients with essential hypertension. *Lancet* 350: 1734–1737, 1997
- Arnal JF, Warin L, Michel JB: Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. *J Clin Invest* 90: 647–652, 1992
- Lüscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 8: 344–348, 1986
- Yokokawa K, Tahara H, Kohno M, Murakawa K, Yasunari K, Nakagawa K, Hamada T, Otani S, Yanagisawa M, Takeda T: Hypertension associated with endothelin-secreting malignant hemangioendothelioma. *Ann Intern Med* 114: 213–215, 1991
- Dahl LK, Heine M, Tassinari L: Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. *Nature* 194: 480–482, 1962
- Simchon S, Manger WM, Carlin RD, Peeters LL, Rodriguez J, Batista D, Brown T, Merchant NB, Jan KM, Chien S: Salt-induced hypertension in Dahl salt-sensitive rats: Hemodynamics and renal responses. *Hypertension* 13: 612–621, 1989
- Lüscher TF, Vanhoutte PM, Raij L: Antihypertensive treatment normalizes decreased endothelium-dependent relaxations in rats with salt-induced hypertension. *Hypertension* 9[Suppl III]: 193–197, 1987
- Hayakawa H, Coffee K, Raij L: Endothelial dysfunction and cardiorenal injury in experimental salt-sensitive hypertension: Effects of antihypertensive therapy. *Circulation* 96: 2407–2413, 1997
- Barton M, d'Uscio L, Shaw S, Meyer P, Moreau P, Lüscher TF: ET_A receptor blockade prevents increased tissue endothelin-1, vascular hypertrophy and endothelial dysfunction in salt-sensitive hypertension. *Hypertension* 31: 499–504, 1998
- Mattson DL, Lu S, Nakanishi K, Papanek PE, Cowley AW Jr: Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol* 266: H1918–H1926, 1994
- Chatziantoniou C, Boffa JJ, Ardaillou R, Dussaule JC: Nitric oxide inhibition induces early activation of type I collagen gene in renal resistance vessels and glomeruli in transgenic mice: Role of endothelin. *J Clin Invest* 101: 2780–2789, 1998
- Deng AY, Rapp JR: Locus for the inducible, but not a constitutive, nitric oxide synthase cosegregates with blood pressure in the Dahl salt-sensitive rat. *J Clin Invest* 95: 2170–2177, 1995
- Chen PY, Gladish RD, Sanders PW: Vascular smooth muscle nitric oxide synthase anomalies in Dahl/Rapp salt-sensitive rats. *Hypertension* 31: 918–924, 1998
- Chen PY, Sanders PW: L-Arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J Clin Invest* 88: 1559–1567, 1991
- Miyata N, Zou AP, Mattson DL, Cowley AW: Renal medullary interstitial infusion of L-arginine prevents hypertension in Dahl salt-sensitive rats. *Am J Physiol* 275: R1667–R1673, 1998
- He H, Kimura S, Fujisawa Y, Tomohiro A, Kiyomoto K, Aki Y, Abe Y: Dietary L-arginine supplementation normalizes regional blood flow in Dahl-Iwai salt-sensitive rats. *Am J Hypertens* 10: 89S–93S, 1997
- Mattson DL, Higgins DJ: Influence of dietary sodium intake on renal medullary nitric oxide synthase. *Hypertension* 27: 688–692, 1996
- Mattson DL, Maeda CY, Bachmann TD, Cowley AW: Inducible nitric oxide synthase and blood pressure. *Hypertension* 31: 15–20, 1998
- Fujihara CK, Michellazzo SM, de Nucci G, Zatz R: Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. *Am J Physiol* 266: F697–F705, 1994
- Barton M, Haudenschild CC, d'Uscio LV, Shaw S, Münter K, Lüscher TF: Endothelin ET_A receptor blockade restores NO-mediated endothelial function and inhibits atherosclerosis in apoE-deficient mice. *Proc Natl Acad Sci USA* 95: 14367–14372, 1998
- Hirahashi J, Nakaki T, Hishikawa K, Marumo T, Hayashi M, Saruta T: Endothelin ET_A receptor antagonist reverses the inhibitory effect of platelet-derived growth factor on cytokine-induced nitric oxide production. *Eur J Pharmacol* 365: 119–123, 1999
- Goligorsky MS, Iijima K, Morgan M, Yanagisawa M, Masaki T, Lin L, Nasjletti A, Kaskel F, Frazer M, Badr KF: Role of endothelin in the development of Dahl hypertension. *J Cardiovasc Pharmacol* 17[Suppl 7]: S484–S491, 1991
- Cicila GT, Rapp JP, Bloch KD, Kurtz TW, Pravenec M, Kren V, Hong CC, Quertermous T, Ng SC: Cosegregation of the endothelin-3 locus with blood pressure and relative heart weight in inbred Dahl rats. *J Hypertens* 12: 643–651, 1994
- Barton M, Shaw S, d'Uscio LV, Moreau P, Lüscher TF: Angio-

- tensin II increases vascular and renal endothelin-1 and functional endothelin converting enzyme activity in vivo: Role of ETA-receptors for endothelin regulation. *Biochem Biophys Res Commun* 238: 861–865, 1997
25. Lüscher TF, Raij L, Vanhoutte PM: Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* 9: 157–163, 1987
 26. Sterzel RB, Luft FC, Gao Y, Schnermann J, Briggs JP, Ganten D, Waldherr R, Schnabel E, Kriz W: Renal disease and the development of hypertension in salt-sensitive Dahl rats. *Kidney Int* 33: 1119–1129, 1988
 27. Kaasjager KAH, Shaw S, Koomans HA, Rabelink TJ: Role of endothelin receptor subtypes in the systemic and renal responses to endothelin-1 in humans. *J Am Soc Nephrol* 8: 32–39, 1997
 28. Hayakawa H, Raij L: Nitric oxide synthase activity and renal injury in genetic hypertension. *Hypertension* 31: 266–270, 1998
 29. Chen PY, St. John PL, Kirk KA, Abrahamson DR, Sanders PW: Hypertensive nephrosclerosis in the Dahl/Rapp rat: Initial sites of injury and effect of dietary L-arginine supplementation. *Lab Invest* 68: 174–184, 1993
 30. Mohaupt MG, Elzie JL, Ahn KY, Clapp WL, Wilcox CS, Kone BC: Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. *Kidney Int* 46: 653–665, 1994
 31. Ikeda Y, Saito K, Kim JI, Yokoyama M: Nitric oxide synthase isoform activities in kidney of Dahl salt-sensitive rats. *Hypertension* 26: 1030–1034, 1995
 32. Barri YM, Wilcox CS: Salt intake determines the renal response to L-arginine infusion in normal human subjects. *Kidney Int* 53: 1299–1304, 1998
 33. Waz WR, van Liew JB, Feld LG: Nitric oxide-inhibitory effect of aminoguanidine on renal function in rats. *Kidney Blood Press Res* 20: 211–217, 1997
 34. Wu F, Park F, Cowley AW, Mattson DL: Quantification of nitric oxide synthase activity in microdissected segments of the rat kidney. *Am J Physiol* 276: F874–F881, 1999
 35. Venema RC, Sayegh HS, Kent JD, Harrison DG: Identification, characterization, and comparison of the calmodulin-binding domains of the endothelial and inducible nitric oxide synthases. *J Biol Chem* 271: 6435–6440, 1996
 36. Fleming I, Bauersachs J, Schafer A, Scholz D, Aldershvile J, Busse R: Isometric contraction induces the Ca²⁺-independent activation of the endothelial nitric oxide synthase. *Proc Natl Acad Sci USA* 96: 1123–1128, 1999
 37. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM: Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605, 1999
 38. Rudd MA, Toolan G, Hope SK, Daumerie GJ, Trolliet MR, Loscalzo J: Salt-dependent increase in blood pressure in mice lacking inducible nitric oxide synthase [Abstract]. *Circulation* 98: I-4, 1998
 39. d'Uscio LV, Barton M, Shaw S, Moreau P, Lüscher TF: Structure and function of small arteries in salt-induced hypertension. *Hypertension* 30: 905–911, 1997
 40. Beck K-F, Mohaupt MG, Sterzel BR, Peters S, Fees H: Endothelin-1 inhibits cytokine-stimulated transcription of inducible nitric oxide synthase in glomerular mesangial cells. *Kidney Int* 48: 1893–1899, 1995
 41. Ikeda U, Yamamoto K, Maeda Y, Shimpo M, Kanbe T, Shimada K: Endothelin-1 inhibits nitric oxide synthesis in vascular smooth muscle cells. *Hypertension* 29: 65–69, 1997
 42. de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, Vane JR: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 85: 9797–9800, 1988
 43. Takimoto M, Oda K, Sasaki Y, Okada T: Endothelin-A receptor mediated prostanoid secretion via autocrine and deoxyribonucleic acid synthesis via paracrine signaling in human bronchial epithelial cells. *Endocrinology* 137: 4542–4550, 1996
 44. Pomposiello S, Yang XP, Liu YH, Surakanti M, Rhaleb NE, Sevilla M, Carretero OA: Autacoids mediate coronary vasoconstriction induced by nitric oxide synthesis inhibition. *J Cardiovasc Pharmacol* 30: 599–606, 1997
 45. Wu X, Scholey JW, Sonnenberg H: Renal vascular morphology in male Dahl rats on high-salt diet: Effect of potassium. *J Am Soc Nephrol* 7: 338–344, 1996
 46. Deng AY, Dene H, Pravenec M, Rapp JP: Genetic mapping of two new blood pressure quantitative trait loci in the rat by genotyping endothelin system genes. *J Clin Invest* 93: 2701–2709, 1994
 47. Firth JD, Schricker K, Ratcliffe PJ, Kurtz A: Expression of endothelins 1 and 3 in the rat kidney. *Am J Physiol* 269: F522–F528, 1995
 48. Sventek P, Li JS, Grove K, Deschepper CF, Schiffrin EL: Vascular structure and expression of endothelin-1 gene in L-NAME-treated spontaneously hypertensive rats. *Hypertension* 27: 49–55, 1996
 49. Verhagen AM, Rabelink TJ, Braam B, Ogenorth TJ, Grone HJ, Koomans HA, Joles JA: Endothelin A receptor blockade alleviates hypertension and renal lesions associated with chronic nitric oxide synthase inhibition. *J Am Soc Nephrol* 9: 755–762, 1998
 50. Schiffrin EL, Lariviere R, Li JS, Sventek P, Touyz RM: Enhanced expression of endothelin-1 gene may cause blood pressure-independent vascular hypertrophy. *J Cardiovasc Pharmacol* 26: S5–S8, 1995
 51. Boulanger C, Luscher TF: Release of endothelin from the porcine aorta: Inhibition by endothelium-derived nitric oxide. *J Clin Invest* 85: 587–590, 1990
 52. Matsumura Y, Hashimoto N, Taira S, Kuro T, Kitano R, Ohkita M, Ogenorth TJ, Takaoka M: Different contributions of endothelin-A and endothelin-B receptors in the pathogenesis of deoxycorticosterone acetate-salt-induced hypertension in rats. *Hypertension* 33: 759–765, 1999
 53. Fujisaki H, Ito H, Hirata Y, Tanaka M, Hata W, Lin M, Adachi S, Akimoto H, Marumo F, Hiroe M: Natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking endothelin-1 gene expression. *J Clin Invest* 96: 1059–1065, 1995
 54. Hahn AW, Resink TJ, Scott-Burden T, Powell J, Dohi Y, Buhler FR: Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: A novel autocrine function. *Cell Regul* 1: 649–659, 1990
 55. Alberts GF, Peifley KA, Johns A, Kleha JF, Winkles JA: Constitutive endothelin-1 overexpression promotes smooth muscle cell proliferation via an external autocrine loop. *J Biol Chem* 269: 10112–10118, 1994
 56. Sullivan JM: Salt sensitivity: Definition, conception, methodology, and long-term issues. *Hypertension* 17[Suppl 1]: I61–I68, 1991
 57. Cardillo C, Kilcoyne CM, Cannon RO, Panza JA: Attenuation of

- cyclic nucleotide-mediated smooth muscle relaxation in blacks as a cause of racial differences in vasodilator function. *Circulation* 99: 90–95, 1999
58. Schmidlin O, Forman A, Tanaka M, Sebastian A, Morris RC: NaCl-induced renal vasoconstriction in salt-sensitive African-Americans: Antipressor and hemodynamic effects of potassium bicarbonate. *Hypertension* 33: 633–639, 1999
59. Ergul S, Parish DC, Puett D, Ergul A: Racial differences in plasma endothelin-1 concentrations in individuals with essential hypertension. *Hypertension* 28: 652–655, 1996
60. Rudd MA, Trolliet M, Hope S, Scribner AW, Daumerie G, Toolan G, Cloutier T, Loscalzo J: Salt-induced hypertension in Dahl salt-resistant and salt-sensitive rats with NOS II inhibition. *Am J Physiol* 277: H732–H739, 1999