

Studies On The Role Of Nitric Oxide In The Vascular Dysfunction In Experimental Renal Hypertension

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Abstract

The present study was planned to investigate the role of nitric oxide (NO) in vascular dysfunction in experimental renal hypertension. The results revealed that 6 week salt-loaded 5/6 nephrectomized rats developed significant increase in systolic (SBP), and diastolic (DBP) blood pressure, as well as left ventricular hypertrophy. Plasma creatinine and blood urea nitrogen (BUN), as well as, plasma malondialdehyde (MDA) were significantly increased. Plasma and aortic tissue nitrate were significantly decreased. Baroreflex sensitivity (BRS) and Baroreflex calculated gain (BCG) were attenuated. The relaxing effect of acetylcholine (ACh) on aortic rings was significantly reduced. Correlation studies among nephrectomized rats plus sham controls showed that both plasma creatinine and BUN exhibited significant positive correlations with each of SBP and MDA, and significant negative correlations with each of plasma and tissue nitrate as well as the relaxing effect of ACh.

L-arginine treatment of 5/6 nephrectomized rats for 6 weeks was associated with normalization of arterial blood pressure as well as lowering of BUN, plasma creatinine and MDA. Plasma and aortic tissue nitrate were significantly increased. BRS and BCG, as well as the ACh-induced vasorelaxation of the aortic rings were improved. Treatment with L-arginine also led to loss of the above mentioned correlations.

Accordingly, L-arginine may be considered as a drug of potential clinical value for safe and effective intervention in patients suffering from hypertensive renal disease. The beneficial effects of L-arginine were related to increased production and bioavailability of NO in addition to the reduction in oxidative stress.

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Introduction

A large body of experimental evidence has been accumulated demonstrating that nitric oxide released from endothelial cells is a crucial regulator of vascular tone. Deficiency of the endogenous vasodilator nitric oxide has also been implicated as potential cause of hypertension in chronic renal disease.^(1,2) The present work was planned to study the role of nitric oxide in vascular dysfunction in 5/6 nephrectomy, a model of experimentally-induced renal hypertension in rats. In addition, the effects of administration of an exogenous nitric oxide donor, L-arginine, on renal function and the possible relationships of the resulting abnormalities to possible variations in nitric oxide values were investigated.

Materials and Methods

Animals:

This work was performed on adult female Wistar rats initially weighing 160-250 g, purchased from the Research Institute of Ophthalmology (Giza). Rats were maintained in the Physiology Department Animal House under standard conditions of boarding and feeding.

Experimental protocol:

Rats were allocated into three groups:

1. **Group of sham-operated control rats**, and tap water was provided for 6 weeks *ad libitum*. (6 wk Sham-Nx group, n=24).
2. **Group of rats subjected to 5/6 nephrectomy**, and drinking water was replaced by NaCl 1% provided *ad libitum* for 6 weeks. (6 wk 5/6 Nx group, n=32).
3. **Group of rats subjected to 5/6 nephrectomy and treated with the NO donor L-arginine** 40 mg/kg/day by gavage, 6 days/week for 6 weeks, and drinking water was replaced by NaCl 1% provided *ad libitum*. (6 wk L-arginine-treated 5/6 Nx group, n=25).

The procedure for 5/6 nephrectomy was done according to the technique described previously.⁽³⁾ The technique is essentially a two-stage operation with the upper and lower poles of one kidney were excised (about 2/3), followed after one week with removal of the other kidney.

Experimental procedure: On the day of sacrifice, overnight fasted rats were weighed, and injected intraperitoneally (i.p.) with 1000 I.U. heparin sodium (Nile Co). Half an hour later, the rats were anaesthetized using pentobarbital sodium (40 mg/kg, i.p.). After passing into anesthesia, tail blood sample was collected, and the separated plasma was stored frozen at -20°C for later determination of plasma nitrate level.

Blood Pressure and Heart Rate measurements: A midline abdominal incision was made and the abdominal aorta was exposed and cannulated with polythelene catheter (0.5 mm internal diameter), which was connected through a blood pressure transducer (Omeda DTX pressure transducer) to a pressure/heart rate computer BP-1 (Columbus instruments, Ohio, U.S.A). Digital readings were taken for basal systolic (SBP) and diastolic (DBP) pressures, together with simultaneous reading of heart rate (HR).

Pressor and Depressor responses: Femoral vein was cannulated, and bolus administration of graded doses of **phenylephrine (PE)** and **sodium nitroprusside (SNP)**, (Sigma, USA), was done in order to study the responses of arterial blood pressure and heart rate to both the pressor agent PE and the depressor SNP. Phenylephrine (PE) was injected sequentially as bolus doses of 5, 10, 20 and 40 µg/kg⁽⁴⁾; following each dose, readings were taken for simultaneous responses of SBP and HR. Thereafter, the animal was left for 15 minutes to recover from the pressor agent (PE), with recording of basal SBP, DBP and HR. Then, Sodium nitroprusside (SNP) was injected sequentially as bolus doses of 0.4, 0.8, 1.2 and 1.6 µg/kg⁽⁵⁾; following each dose, readings were taken for simultaneous responses of SBP and HR. For both PE and SNP, a 10- minutes period was allowed between injections of different doses to enable blood pressure to reach a stable value before administering the next dose.

Baroreflex sensitivity (BRS) was determined by correlating delta changes in SBP (dSBP) versus delta changes in HR (dHR) in response to the 4 doses of PE (5, 10, 20 and 40 µg/kg) as well as, correlating delta changes in SBP versus delta changes in HR in response to the 4 doses of SNP (0.4, 0.8, 1.2 and 1.6 µg/kg)⁽⁶⁾. BRS was expressed numerically by correlation coefficient (r) as well as the slope (b) of regression line of the relationship between delta SBP and delta HR.

Baroreflex calculated gain (BCG) was calculated⁽⁷⁾ as the delta change in heart rate

(HR, bpm) divided by the delta change in systolic blood pressure (SBP, mmHg) obtained in response to infusion of the maximal doses of phenylephrine (40 µg/kg) and sodium nitroprusside (1.6 µg/kg) and expressed as bpm/mmHg.

After in vivo infusion of PE and SNP, the animal was left for 10-minutes period to recover. Then, blood was collected from abdominal aorta, centrifuged and the plasma was separated, frozen and stored at -20°C for later biochemical determination of plasma creatinine, blood urea nitrogen (BUN) and plasma malondialdehyde (MDA). Thereafter, the heart and the great vessels were separated for studies on the aorta as well as later determination of cardiac weight.

Aortic ring reactivity: The thoracic aorta was dissected out, excised and rapidly placed in modified Krebs' solution ⁽⁸⁾. Rings of aorta (2-4 mm in length) were suspended separately in an organ bath containing 20 ml warmed (37°C) modified Krebs' solution continually bubbled with 95% O₂ + 5% CO₂. Rings were connected to isometric force displacement transducer (UGO Basile, Comerio-Italy) for recording isometric contractions. Recording was performed on a 4 channel oscillograph (UGO Basile Quartet 9400 four channel recorder).

Aortic rings were allowed to contract in response to 20 mM KCL until reproducible evoked contractile response was obtained. After that, the contractile response of aortic rings to 1 µM PE (Sigma) was recorded. When the plateau of contraction was reached, the relaxant response to acetylcholine, 10 µM ACh (Sigma) was obtained on top of PE contraction. The amplitude of the recorded contraction and relaxation in grams were obtained from calibration curve, and were corrected for the weight of aortic rings. PE-induced contractions and ACh-induced relaxations were also calculated as percentages of KCL-induced tension. Rings were frozen at -80°C for subsequent estimation of aortic tissue nitrate level.

Determination of cardiac weights: Hearts were washed with normal saline, blotted dry by filter paper, and were further cleaned from fat and fibrous tissue. Both atria were separated together, right ventricle wall peeled evenly, and the remaining left ventricle plus the septum were all weighed in a precision balance (Metler AE163).

Biochemical analysis: Blood urea nitrogen (BUN), plasma creatinine and plasma malondialdehyde (MDA) were estimated according to the methods described by Patton

and Crouch (1977)⁽⁹⁾ Bartels et al., (1972)⁽¹⁰⁾ and Draper and Hadley (1991)⁽¹¹⁾ respectively.

Nitrate, the metabolic end-product of nitric oxide (NO), was assayed in plasma and aortic tissue according to the method described by Bories and Bories (1995)⁽¹²⁾

Statistical analysis: results were statistically analyzed according to Student's "t" test for paired and unpaired data. Correlations were determined by linear regression analysis using the Least Square Method. Statistical analysis was performed by using SPSS (version 8.0.1). A probability of P<0.05 was considered statistically significant.

Table (1): Values of blood urea nitrogen (BUN), plasma creatinine, plasma nitrate, aortic tissue nitrate and plasma malondialdehyde (MDA) levels, in the studied groups.

	<i>BUN</i> (mg/dl)	<i>Plasma Creatinine</i> (mg/dl)	<i>Plasma nitrate</i> (μ mol/l)	<i>Aortic tissue nitrate</i> (μ mol/g)	<i>Plasma MDA</i> (μ mol/l)
6 wk Sham-Nx group (24)	26.87 \pm 1.0	0.77 \pm 0.05	32.67 \pm 1.9	0.096 \pm 0.006	1.30 \pm 0.197
6 wk 5/6 Nx (32)	72.68 \pm 3.6 ^a	2.65 \pm 0.19 ^a	12.24 \pm 0.91 ^a	0.044 \pm 0.003 ^a	5.10 \pm 0.219 ^a
6 wk L-arginine-treated 5/6 Nx (25)	35.12 \pm 1.4 ^{ab}	1.38 \pm 0.04 ^{ab}	41.60 \pm 2.13 ^{ab}	0.102 \pm 0.007 ^b	2.07 \pm 0.228 ^{ab}

Data are expressed as means \pm SEM in parenthesis is the number of observations.
a: significance from 6 week sham-nephrectomized rats, calculated by Student's "t" test for unpaired data.
b: significance from 6 week 5/6 nephrectomized rats, calculated by Student's "t" test for unpaired data.

Table (2): Values of systolic (SBP), diastolic (DBP) blood pressure, absolute left ventricular weight (LV) and left ventricular weight/body weight (LV/BW) in the studied groups.

	<i>SBP</i> (mm Hg)	<i>DBP</i> (mm Hg)	<i>LV</i> (mg)	<i>LV/BW</i> (mg/g)
6 wk Sham-Nx group (24)	99.3 \pm 2.4	78.2 \pm 1.9	437.11 \pm 9.342	1.73 \pm 0.047
6 wk 5/6 Nx (32)	130.3 \pm 1.7 ^a	100.8 \pm 1.9 ^a	519.96 \pm 15.760 ^a	2.29 \pm 0.072 ^a
6 wk L-arginine-treated 5/6 Nx (25)	100.8 \pm 1.2 ^b	78.6 \pm 1.0 ^b	436.00 \pm 15.640 ^b	2.01 \pm 0.079 ^b

Data are expressed as means \pm SEM in parenthesis is the number of observations.
a: significance from 6 week sham-nephrectomized rats, calculated by Student's "t" test for unpaired data.
b: significance from 6 week 5/6 nephrectomized rats, calculated by Student's "t" test for unpaired data.

Table (3): Results of baroreflex calculated gain (bpm/mmHg) in response to maximal dose of phenylephrine (PE, 40µg/kg) and sodium nitroprusside (SNP, 1.6µg/kg) in the studied groups.

	PE	SNP
6 wk Sham-Nx group (24)	-2.22±0.16	-1.76±0.12
6 wk 5/6 Nx (32)	-1.35±0.11 ^a	-1.15±0.06 ^a
6 wk L-arginine-treated 5/6 Nx (25)	-1.92±0.08 ^b	-2.03±0.08 ^b

Data are expressed as means ±SEM in parenthesis is the number of observations
a:significance from 6 week sham-nephrectomized rats, calculated by Student's "t" test for unpaired data.
b: significance from 6 week 5/6 nephrectomized rats, calculated by Student's "t" test for unpaired data.

Table (4): Changes in aortic ring responses to potassium chloride (KCl), phenylephrine (PE) and acetylcholine (ACh) in the studied groups.

	KCl/ring wt. (g/mg)	PE/ring wt. (g/mg)	PE/KCl (%)	ACh/ring wt. (g/mg)	ACh/KCl (%)
6 wk Sham-Nx group (21)	0.027±0.002	0.061±0.005	233.5±17.89	0.017±0.002	65.3±4.63
6 wk 5/6 Nx (32)	0.020±0.002 ^a	0.040±0.003 ^a	211.9±12.86	0.005±0.001 ^a	25.8±2.16 ^a
6 wk L-arginine-treated 5/6 Nx (24)	0.029±0.002 ^b	0.060±0.003 ^b	216.0±11.31	0.017±0.001 ^b	59.2±2.95 ^b

Data are expressed as mean s± SEM in parenthesis is the number of observations.
a:significance from 6 week sham-nephrectomized rats, calculated by Student's "t" test for unpaired data.
b: significance from 6 week 5/6 nephrectomized rats, calculated by Student's "t" test for unpaired data

Table (5): Correlation coefficients (r) between plasma nitrate levels and other parameters in the groups of rats studied

Parameter	6 wk 5/6 Nx + 6 wk Sham-Nx group			6 wk L-arginine-treated 5/6 Nx + 6 wk Sham-Nx group		
	r	p		r	p	
Plasma creatinine	-0.70	<0.001	(56)	0.26	NS	(49)
BUN	-0.71	<0.001	(55)	0.27	NS	(48)
SBP	-0.66	<0.001	(56)	0.10	NS	(49)
Aortic ring responses to ACh	0.48	<0.001	(53)	-0.20	NS	(46)
BCG PE	-0.54	<0.001	(56)	0.05	NS	(49)
BCG SNP	-0.32	<0.02	(55)	-0.09	NS	(49)

In parenthesis is the number of observations.

NS: not significant.

Table (6): Correlation coefficients (r) between tissue nitrate levels and other parameters in the groups of rats studied

Parameter	6 wk 5/6 Nx + Sham-Nx group		6 wk L-arginine-treated 5/6 Nx + 6 wk Sham-Nx group	
	r	p	r	p
SBP	-0.58	<0.001 (56)	0.11	NS (49)
Aortic ring responses to ACh	0.50	<0.001 (53)	-0.03	NS (46)
BCG PE	-0.47	<0.001 (56)	-0.02	NS (49)
BCG SNP	-0.46	<0.001 (55)	-0.28	<0.05 (49)

In parenthesis is the number of observations.

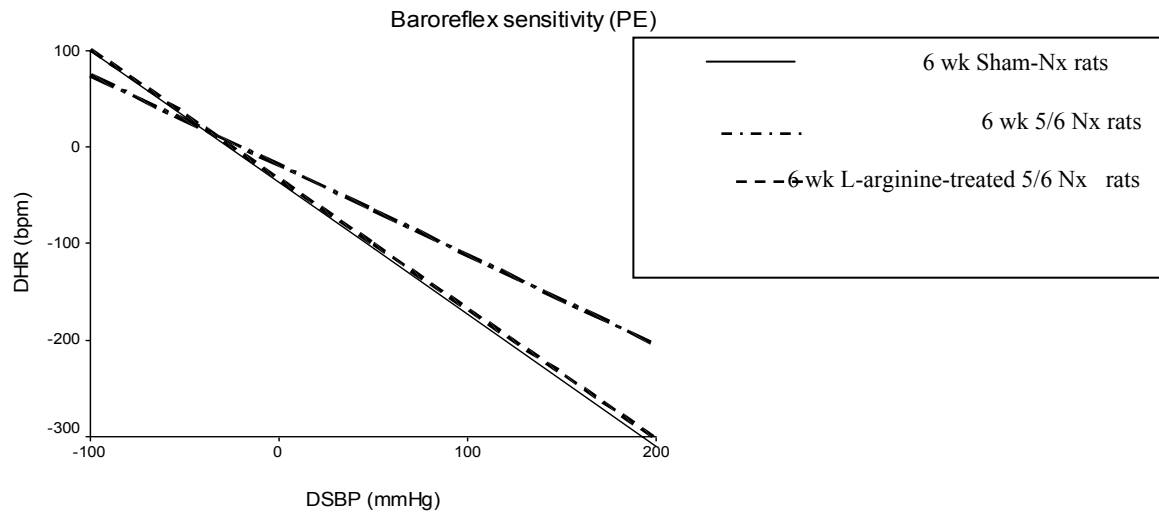
NS: not significant.

Table (7): Correlation coefficients (r) between plasma malondialdehyde (MDA) levels and other parameters in the groups of rats studied.

Parameter	6 wk 5/6 Nx + Sham-Nx group		6 wk L-arginine-treated 5/6 Nx +6 wk Sham-Nx group	
	r	p	r	p
Plasma nitrate	-0.72	<0.001 (56)	0.00	NS (49)
Tissue nitrate	-0.58	<0.001 (56)	0.29	<0.05 (49)
Aortic ring responses to ACh	-0.63	<0.001 (53)	0.15	NS (46)

In parenthesis is the number of observations.

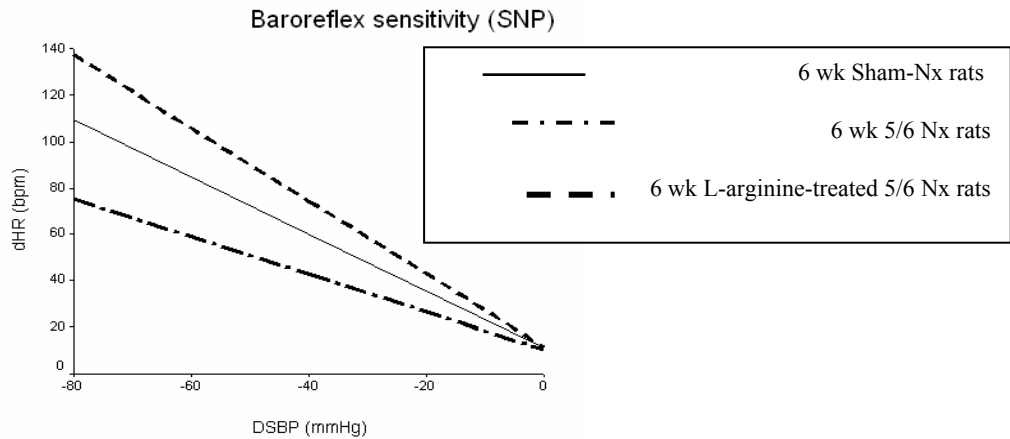
NS: not significant.



	n	Correlation coefficient (r)	Intercept (a)	Slope (b)
6 wk Sham-Nx	96	-0.7 (P<0.001)	-36.44	-1.37
6 wk 5/6 Nx	128	-0.58 (P<0.001)	-18.42	-0.93
6 wk L-arginine-treated 5/6 Nx	100	-0.76 (P<0.001)	-32.93	-1.34

n: number of observations

Figure (1): Regression lines and relationships between delta systolic blood pressure(dSBP) and delta heart rate (dHR) in response to bolus injections of Phenylephrine (PE) in the studied groups. Baroreflex sensitivity in each group is indicated by its correlation coefficient (r) and slope (b).



	n	Correlation coefficient (r)	Intercept (a)	Slope (b)
6 wk Sham-Nx	96	-0.62 (P< 0.001)	11.25	-1.23
6 wk 5/6 Nx	120	-0.69 (P< 0.001)	10.44	-0.81
6 wk L-arginine-treated 5/6 Nx	100	-0.80 (P< 0.001)	11.68	-1.57

n: number of observations

Figure (2): Regression lines and relationships between delta systolic blood pressure(dSBP) and delta heart rate (dHR) in response to bolus injections of sodium nitroprusside (SNP) in the studied groups. Baroreflex sensitivity in each group is indicated by its correlation coefficient (r) and slope (b).

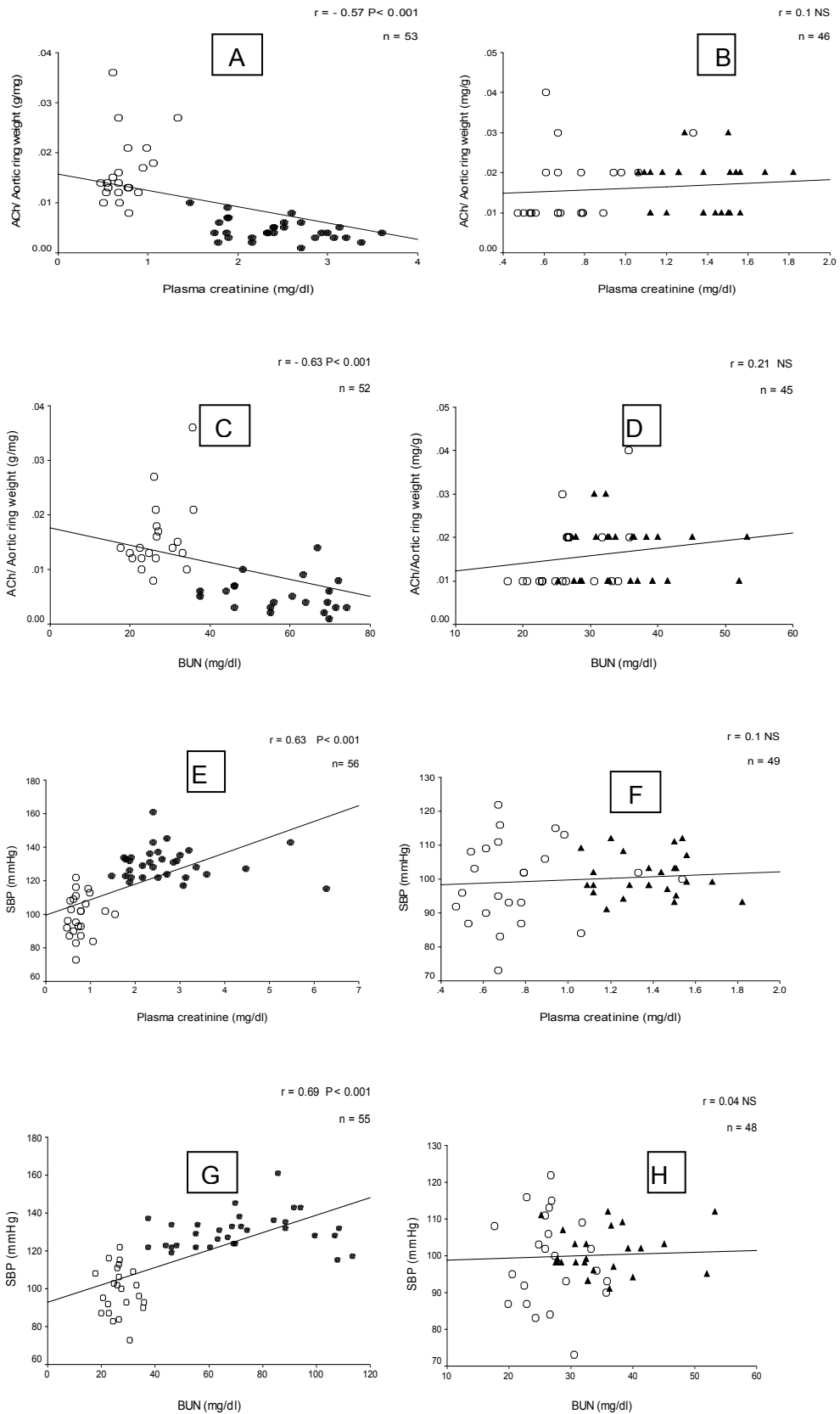


Figure (3): Graphs showing relationships between each of plasma creatinine & BUN and responses to acetylcholine/aortic ring weight (A,B,C,D) and systolic blood pressure(SBP) (E,F,G,H) among 6 wk 5/6 Nx rats (●)+6 wk Sham-Nx rats (○) and among 6 wk L-arginine-treated 5/6 Nx rats(▲)+6 wk Sham-Nx rats.

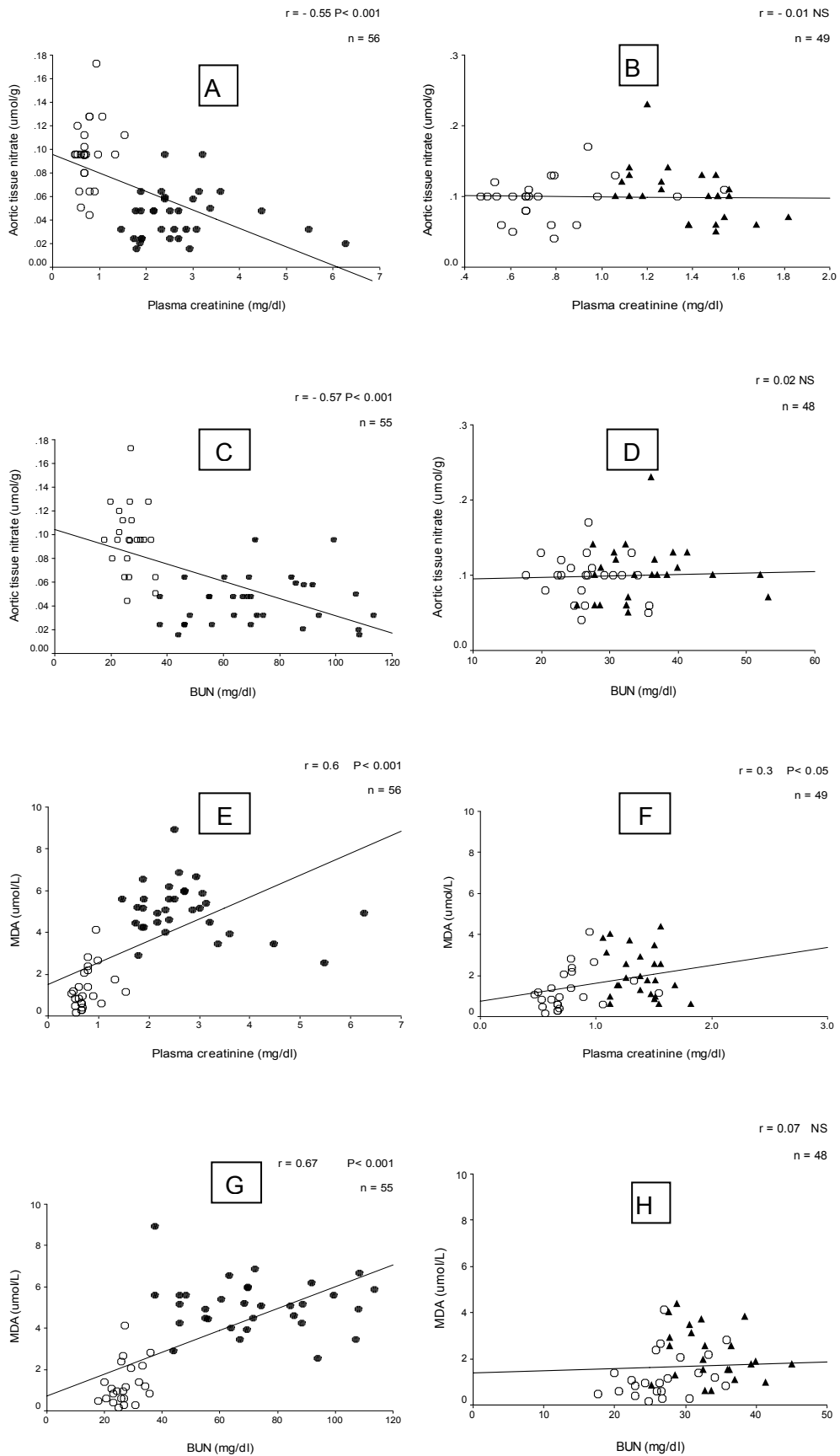


Figure (4): Graphs showing relationships between each of plasma creatinine & BUN and aortic tissue nitrate (A,B,C,D), and MDA(E,F,G,H) among 6 wk 5/6 Nx rats (●)+6 wk Sham-Nx rats (○) and among 6 wk L-arginine-treated 5/6 Nx rats (▲)+6 wk Sham-Nx rats.

Results

Responses of BUN and plasma creatinine: Table1 shows that values of BUN and plasma creatinine were significantly higher in 6 wk 5/6 Nx rats compared to 6 wk Sham-Nx rats. Treatment of 6 wk 5/6 Nx rats with L-arginine brought significant reductions in BUN and plasma creatinine compared to the untreated 6 wk 5/6 Nx rats. However, the values of these parameters were not normalized and remained significantly higher than the 6 wk Sham-Nx group.

Changes in plasma nitrate and malondialdehyde (MDA) and in aortic tissue nitrate (table1): In the group of 6 wk 5/6 Nx rats, plasma nitrate levels were significantly reduced, while plasma concentrations of MDA were higher compared to 6 wk Sham-Nx rats. 6 wk 5/6 Nx rats treated with L-arginine for 6 weeks, showed a significant increase in plasma nitrate associated with a reduction in plasma MDA compared to untreated 6 wk 5/6 Nx rats. It is to be noted that plasma nitrate and MDA levels were significantly higher than corresponding values in 6 wk Sham-Nx rats. The results of tissue nitrate levels showed significant reduction in 6 wk 5/6 Nx rats compared to 6 wk Sham-Nx controls; and treatment with L-arginine produced a significant rise in tissue nitrate values even exceeding those seen in the 6 wk Sham-Nx group.

Responses of basal blood pressure and changes in cardiac weights (table2):

6 wk 5/6 Nx rats exhibited significant rises of SBP&DBP compared to 6 wk Sham-Nx rats. Treatment of 6 wk 5/6 Nx rats with L-arginine reduced significantly the rise in SBP&DBP seen in untreated 6 wk 5/6 Nx rats. Also, 6 wk 5/6 Nx rats exhibited a significant increase of absolute weight of left ventricle (LV), and left ventricle weight /body weight (LV/BW) compared to 6 wk Sham- Nx rats. Compared to untreated 6 wk 5/6 Nx rats, the group of 6 wk L-arginine- treated 5/6 Nx showed significant decrease of absolute weights of left ventricle (LV) as well as LV/BW.

Baroreflex calculated gain (BCG) (table3): BCG became significantly less negative in response to maximal dose of PE (40 µg/kg) as well as SNP (1.6 µg/kg) in 6 wk 5/6 Nx rats when compared to 6 wk Sham-Nx rat. BCG in 6 wk L-arginine-treated 5/6 Nx rats was significantly more negative in response to maximal dose of PE and SNP compared to untreated 6 wk 5/6 Nx rats; while, it showed no significant differences when compared to 6 wk Sham-Nx rats.

Baroreceptor sensitivity:

In response to PE, in the 6 wk Sham-Nx group a negative correlation between delta SBP and delta HR was seen, while, in the 6 wk 5/6 Nx group, the same relationship but with less negative correlation coefficient (r) and less negative slope was observed. On the other hand, the group of 6 wk L-arginine-treated 5/6 Nx rats exhibited a stronger negative correlation between delta SBP and delta HR with more negative slope compared to the 6 wk 5/6 Nx rats. These values were almost similar to those observed in the 6 wk Sham-Nx controls (figures 1).

In response to SNP, the correlation between delta SBP and delta HR in the 6 wk 5/6 Nx group showed more negative correlation coefficient and less negative slope compared to their 6 wk Sham-Nx controls; while, the 6 wk L-arginine-treated 5/6 Nx group displayed the highest negative correlation coefficient and the highest negative slope compared to both 6 wk 5/6 Nx rats and 6 wk Sham-Nx controls (figure 2).

Aortic ring responses to potassium chloride (KCl), Phenylephrine (PE) and acetylcholine (ACh)(table4): Additions of KCl, PE, and ACh to rat aortas isolated from 6 wk 5/6 Nx rats evoked significant decreased responses compared to those observed with aortas from 6 wk Sham-Nx group. The ACh/KCl % ratio was significantly decreased while the PE/ KCl % ratio was not significantly different compared to 6 wk Sham-Nx rats. Treatment of 6 wk 5/6 Nx rats with L-arginine for 6 weeks, produced significant increases in the responses of the aortas to each of KCl, PE, ACh and their ACh/KCl % ratio. When compared to the data obtained with the 6 wk Sham-Nx controls, the values in all those parameters were not significantly different.

Correlation studies among the experimental groups:

1) *Correlations of plasma creatinine and BUN versus other parameters* figure (3, 4): showed significant inverse relationship between ACh-induced relaxation as well as aortic tissue nitrate and each of plasma creatinine and BUN, exhibited a positive correlation with SBP and plasma MDA levels among pooled 6 wk 5/6 Nx rats and 6 wk Sham-Nx rats. These relationships were lost by treatment with L-arginine.

2) *Correlations of plasma nitrate versus other parameters (table5)*: High significant negative correlations of plasma nitrate levels were seen with levels of plasma creatinine, BUN and SBP. Also significant negative correlations were seen between plasma nitrate and each of BCG PE and BCG SNP. In addition, a significant though positive correlation was seen with the aortic response to ACh. Non significant correlations in 6 wk L-arginine-treated 5/6 Nx rats and their 6 wk Sham-Nx controls existed between levels of plasma nitrate and plasma creatinine, BUN, SBP, BCG with both PE & SNP and aortic response to ACh.

3) *Correlations of tissue nitrate versus other parameters (table6)*: By plotting the values of tissue nitrate in 6 wk 5/6 Nx rats and their 6 wk Sham-Nx controls versus the changes in other parameters, good negative correlations were seen with SBP, and BCG with both PE and SNP. A significant positive correlation existed with the aortic response to ACh. In 6 wk L-arginine-treated 5/6 Nx rats and their 6 wk Sham-Nx controls, the values of tissue nitrate displayed insignificant relationships with SBP, BCG PE and ACh response of aortic tissue; while a negative significant relationship was observed with values of BCG SNP

4) *Correlations of plasma malondialdehyde (MDA) versus other parameters (table7)*: Among 6 wk 5/6 Nx rats and their shams, significant negative correlations were seen with the levels of plasma nitrate, tissue nitrate and the ACh response of aorta. In 6 wk L-arginine-treated 5/6 Nx rats and their 6 wk Sham-Nx controls .Significant relationship was observed between levels of MDA and tissue nitrate levels. On the other hand, the relationships with BUN, plasma nitrate, and ACh response of aorta were all weak and nonsignificant.

Discussion

The results obtained in the present work have demonstrated that salt-loaded 5/6 nephrectomy for 6 weeks in rats, produced rise of arterial blood pressure which was associated with left ventricular hypertrophy. Renal function in the 5/6 nephrectomized rats was impaired. In the present study, the decrease in plasma and aortic tissue nitrate was related to the compromised renal function as indicated by the significant negative correlations established between the high values of plasma creatinine and BUN, and low levels of plasma and aortic tissue nitrate. Also, plasma and aortic tissue nitrate levels exhibited strong significant negative correlations with the high levels of systolic

blood pressure. These data indicate that nitric oxide deficiency may be involved in the deterioration of renal function encountered in this model. Also, these results suggest the possibility of contribution of nitric oxide lack in the development of hypertension in 5/6 nephrectomized rats. This view is favored by other investigators, Rocznik et al. (1999)⁽¹³⁾ demonstrated that 5/6 nephrectomy in rats inhibits expression of nNOS mRNA and protein in the renal cortex and inner medulla. Inhibition of inner medullary nNOS expression was shown to induce salt-sensitive hypertension in rats ⁽¹⁴⁾. The reduced nitric oxide production observed in the present study may be ascribed to the decreased expression of constitutive and inducible isoforms of NOS in the kidney and vasculature ⁽¹⁵⁾. Another explanation of the decreased plasma and tissue nitric oxide reported in the present study, may be due to excessive oxidative stress and lipid peroxidation as indicated by the associated rise of malondialdehyde values in the 5/6 nephrectomized rats. This explanation is favored by the negative correlation between malondialdehyde values and levels of plasma and tissue nitrate demonstrated in 5/6 nephrectomized rats. Reduction in nitric oxide levels during kidney failure has been related to the reaction of nitric oxide with superoxide anions to yield peroxynitrite which possesses the biological activity responsible for renal damage ⁽¹⁶⁾.

The baroreflex calculated gain for phenylephrine as well as for sodium nitroprusside were decreased in 5/6 nephrectomized rats. This impaired baroreflex function affecting both vagal and sympathetic limbs may explain the decrease in bradycardic and tachycardic responses to PE and SNP respectively. Oxidant stress encountered in the present study may play a role in the attenuated baroreflex activity. This assumption is supported by Wang et al., 1988 ⁽¹⁷⁾ who reported that free radicals and lipid peroxides may inhibit cyclooxygenase to reduce PGI₂ formation which exerts an excitatory influence on baroreceptor activity ⁽¹⁸⁾. In addition, free radicals and oxidant stress have been shown to alter membrane excitability ⁽¹⁹⁾ and blunt baroreceptor responsiveness; this may functionally contribute to the impaired baroreflex response ⁽²⁰⁾. The attenuated baroreflex sensitivity and baroreflex calculated gain seen in this study may be involved in the development of hypertension.

The present data clearly demonstrated reduction of the relaxing effect of acetylcholine in the nephrectomized rats; such impairment was associated with and negatively correlated with impairment in kidney function as shown by the levels of plasma creatinine and BUN. In addition, this impaired relaxing effect of acetylcholine

could be related to the reduction in nitrate levels measured in aortic tissue and plasma in the nephrectomized group. This view is favored by the demonstration in this study of strong correlations with levels of tissue and plasma nitrate. This aortic tissue response was negatively correlated with the levels of malondialdehyde in plasma. In view of these data, the compromised relaxing effect of acetylcholine on vascular smooth muscle, together with the decrease in vascular nitric oxide provide evidence for endothelial dysfunction in 5/6 nephrectomized rats. The proposed mechanism for endothelial dysfunction in nephrectomized rats is oxidative stress as evidenced by the increased values of malondialdehyde in the 5/6 nephrectomized rats. Oxidative stress leads to diminished nitric oxide ⁽²¹⁾ and correlates with the degree of impairment of endothelium-dependent vasodilatation ⁽²²⁾.

The present study revealed that L-arginine treatment of 5/6 nephrectomized rats for 6 weeks produced pronounced decrease in arterial blood pressure. These data indicate that L-arginine treatment was effective in preventing the development of hypertension induced by salt-loaded 5/6 nephrectomy. This study agrees with those of others who showed that L-arginine attenuated the hypertension in salt-sensitive rats ⁽²³⁾ and in rats treated with L-NAME⁽²⁴⁾, and extends previous findings from this laboratory by Ragwa-Taher et al., (2007)⁽²⁵⁾ that L-arginine treatment abolished hypertension induced by L-NAME for 2 weeks. It is conceivable that reduction of blood pressure in uremic rats supplemented with L-arginine seen in this study was due in part to the enhanced renal nitric oxide formation. This compensatory mechanism is probably related to the renal haemodynamic and the natriuretic effects of nitric oxide ⁽²⁶⁾. Accordingly, it may be proposed that treatment with L-arginine, which acts as the substrate for NOS enhances nitric oxide bioavailability and reduces oxidative stress, and consequently improves vasorelaxation. Previous studies of other workers have shown that L-arginine-treatment in uremic rats develops less glomerular injury than untreated uremic rats ⁽²⁷⁾, and attenuates glomerular capillary pressure in rats with reduced renal mass ⁽²⁸⁾.

The finding in the present study that L-arginine treatment in 5/6 nephrectomized rats improved the net pressor response to phenylephrine, may be attributed to prevention of the downregulation of α_1 -adrenoceptors. ⁽²⁹⁾ L-arginine treatment of 5/6 nephrectomized rats improved the impaired baroreceptor sensitivity in its vagal limb and sympathetic limb, suggesting a possible local role of nitric oxide

synthesis in regulating baroreceptor sensitivity.⁽³⁰⁾ The presence in the current study of strong correlation between plasma and tissue nitrate levels and baroreceptor sensitivity to both phenylephrine and sodium nitroprusside in 5/6 nephrectomized rats, and the disappearance of these relationships following the raised plasma and tissue nitrate values in L-arginine-treated 5/6 nephrectomized rats strongly suggest the role of plasma and tissue nitric oxide in the baroreceptor function.

In the present study, L-arginine treatment increased the relaxing effect of acetylcholine on aortic tissue and abated its relationships that existed in untreated 5/6 nephrectomized rats with plasma and tissue nitrate. This provides also another supportive evidence for the role of nitric oxide in the vasorelaxant responses of blood vessels. The decreased levels of plasma malondialdehyde in the L-arginine-treated rats, and the presence of a negative correlation between the high levels of plasma malondialdehyde and the response of aorta to acetylcholine in 5/6 nephrectomized rats, both provide evidence for the role of oxidative stress in the impaired vasorelaxing responses of blood vessels in renal failure.

From the data presented in the current study, we may conclude that hypertension in rats with reduced renal mass is associated with inappropriate nitric oxide levels that could in part be attributed to the accumulation of uremic nitrogenous waste products as creatinine and BUN, and its inactivation by oxidative stress which is markedly increased in uremic animals. In this respect the effects of L-arginine are mediated through enhancing nitric oxide bioavailability and reducing oxidative stress as documented in the present study. Based on these results, L-arginine may be considered as a putative candidate for safe and effective clinical intervention in patients suffering from chronic renal failure.

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