

Renal Nerves Participation in the Effects of Nitric Oxide and ET_A/ET_B Receptor Inhibition in Spontaneously Hypertensive Rats

R. GIRCHEV, P. MARKOVA

Department of Physiology, Medical University, Sofia, Bulgaria

Received November 28, 2005

Accepted January 17, 2006

On-line available February 23, 2006

Summary

The influence of renal nerves on the effects of concurrent NO synthase inhibition (10 mg kg⁻¹ b.w. i.v. L-NAME) and ET_A/ET_B receptor inhibition (10 mg kg⁻¹ b.w. i.v. bosentan) on renal excretory function and blood pressure in conscious spontaneously hypertensive rats (SHR) was investigated. L-NAME increased blood pressure, urine flow rate, fractional excretion of sodium, chloride and phosphate in both normotensive Wistar rats and SHR with intact renal nerves ($p < 0.01$). GFR or RBF did not change in any of the groups investigated. The effects of L-NAME on renal excretory function were markedly reduced by bosentan and the values returned to control level in the normotensive rats, while in SHR the values were reduced by bosentan, but they remained significantly elevated as compared to control level ($p < 0.05$). The hypertensive response induced by L-NAME in SHR is partially due to activation of endogenous endothelins, but it does not depend on renal nerves. Chronic bilateral renal denervation abolished the effect of L-NAME on sodium and chloride excretion in normotensive rats, whereas it did not alter this effect in SHR. The participation of endogenous endothelins in changes of renal excretory function following NO synthase inhibition is diminished in SHR as compared to Wistar rats.

Key words

Bilateral renal denervation • L-NAME • Bosentan • SHR • Renal excretory function

Introduction

Renal sympathetic nerve activity, nitric oxide (NO) and endogenous endothelins (ET) play an important role in the regulation of blood pressure, renal hemodynamics and renal excretory function. It is well known that renal sympathetic nerve activity is elevated in spontaneously hypertensive rats (SHR) compared to normotensive rats and that increased renal sympathetic

nerve activity contributes to the development of hypertension (Grisk and Rettig 2004). It was shown that chronic renal denervation delays the development of L-NAME-induced hypertension (Matsuoka *et al.* 1994), whereas acute NO synthesis blockade increases renal sympathetic nerve activity (Sakuma *et al.* 1992). It was demonstrated that activation of α_2 -adrenergic receptors in the thick ascending limb of Henle's loop, stimulated the production of NO, which in turn increased urinary

sodium excretion by a direct tubular effect (Plato and Garvin 2001). Alpha₂-adrenoceptors also determined the response to NO synthase (NOS) inhibition in the proximal tubule (Thomson and Vallon 1995).

On the other hand, it was observed that ET partially mediate the pressure action of acute NO blockade in anesthetized rats (Richard *et al.* 1995). ET-1 acutely inhibits NaCl reabsorption in the thick ascending limb of Henle's loop by activating the ET_B receptor, stimulating endothelial NO synthase and thereby increasing NO production (Herrera and Garvin 2004). It is well known that both ET and NO are implicated in oxidative stress (Leclercq *et al.* 2002, Li *et al.* 2003). The existence of cross-talk between ET and NO in renal tubular cells was suggested (Plato *et al.* 2000). It has also been found that ET-1 is an inhibitory modulator of renal noradrenergic neurotransmission (Matsuo *et al.* 1997). We have recently shown that renal nerves influence the effects of endogenous ET on renal excretory function in conscious normotensive as well as hypertensive rats (Girchev *et al.* 2004a).

However, there is little information about a possible interaction between NO and ET-1 in the regulation of renal excretion function. At present, our understanding of the influence of renal sympathetic nerves on the action of ET and NO on renal function and blood pressure remains incomplete. More precisely, it is still unknown whether renal nerves affect the concomitant action of ET and NO on renal excretory function in spontaneous hypertension.

Since it is well known that renal sympathetic nerves are important for the regulation of renal hemodynamics and excretory function, we focused our attention on the participation of renal nerves in the regulation of hemodynamic and renal effects of NO and ET. Therefore, the aim of the present study was to determine the contribution of renal nerves to the regulation of renal excretory function during concurrent NO synthase and ET_A/ET_B receptor inhibition in SHR.

Methods

The experiments were carried out on conscious, male, 11- to 12-week-old spontaneously hypertensive rats (SHR) and normotensive Wistar rats. The animals were kept in individual cages and were housed under standard conditions: temperature 22 °C, light/dark cycle 12/12 hours, free access to tap water and normal rat chow. The study was performed in accordance with institutional care

and use of laboratory animal guidelines, Medical University, Sofia, and is in conformity with the European Convention on Animal Protection.

For surgical preparation the rats were anesthetized with pentobarbital sodium (Narcoren, Merial) 35 mg kg⁻¹ b.w. given intraperitoneally. Bilateral renal denervation was performed in about half of the animals prior to vascular and bladder catheterization. After a retroperitoneal flank incision the kidneys were denervated by stripping all visible nerves from renal arteries and by coating the renal arteries and veins with a 10 % solution of phenol in absolute alcohol. Sham operation was performed exposing the kidneys through a retroperitoneal flank incision. One week was allowed for the rats to recover. The effectiveness of the denervation was assessed by noradrenaline concentration in renal cortical tissue homogenate on the day of sacrifice by high-performance liquid chromatography using a modified procedure of Lozanov *et al.* (2004).

During the general anesthesia (Narcoren 35 mg kg⁻¹ b.w. i.v.) through a small suprapubic incision a modified polyethylene catheter was placed in the urinary bladder eliminating its dead space. For urine collection the catheter was tunneled and exteriorized on the flank. Urine was collected in a small plastic tube secured in a plastic loop sutured to the skin during the surgery. After a small skin incision the femoral artery and vein were isolated. A catheter (PE-50) was placed into the femoral vein for L-NAME (N^o-nitro-L-arginine methyl ester, Sigma) or L-NAME and bosentan administration as well as for inulin and paraaminohippuric acid (PAH) infusion. An indwelling catheter (PE-10, connected to PE-50) was inserted into the femoral artery for blood pressure measurement. The catheters were tunneled to the back of the neck and exteriorized. Thereafter, the animals were returned into their individual cages. To avoid clotting the femoral artery and vein catheters were flushed with 20 IU ml⁻¹ heparin in 0.9 % sterile saline. Rats were allowed for 24 h to recover from the surgical intervention and the experiments were performed on conscious, freely moving animals. During the experiments, the femoral artery and vein catheters were freely connected to a pressure transducer and an infusion pump.

Control experiments

Blood pressure was measured and urine was collected over a time period of 100 min for two clearance periods in sham-operated SHR with intact renal nerves (n=18) and renal denervated SHR (n=10), as well as in

sham-operated (n=14) and renal denervated (n=12) normotensive Wistar rats.

NO synthase inhibition

Renal excretory function, blood pressure and heart rate (HR) were investigated in the sham-operated SHR with intact renal nerves (n=11) and in the renal denervated SHR (n=10) as well as in the sham-operated (n=14) and the renal denervated (n=10) normotensive Wistar rats. The animals were studied during a 40-min control clearance period, a 20-min equilibration, and a 40-min experimental clearance period. An intravenous bolus injection of 10 mg kg⁻¹ b.w. L-NAME (Baylis *et al.* 1997) was performed after the end of the control period. Twenty minutes later, urine collection and blood pressure registration were started again. The administration of 10 mg kg⁻¹ b. w. L-NAME as a single i.v. injection produced a rapid and pronounced increase in systolic, mean and diastolic arterial pressure sustained over the 60-min period of observation. The increase in blood pressure was almost maximal 5 min after L-NAME administration.

NO synthase and non-selective ET_A/ET_B receptor inhibition

Renal excretory function, blood pressure and HR were investigated in the sham-operated SHR with intact renal nerves (n=9) and in the renal denervated SHR (n=10) as well as in the sham-operated (n=13) and the renal denervated (n=10) normotensive Wistar rats. Ten min after 10 mg kg⁻¹ b.w. L-NAME administration a non-selective ET_A/ET_B receptor inhibitor, bosentan (10 mg kg⁻¹ b.w.), was applied by an intravenous bolus injection (Leskinen *et al.* 1997). The dose of bosentan had been previously selected by us to be nonhypotensive (Girchev *et al.* 2004b). Ten minutes after the bosentan administration urine collection and blood pressure registration were started again. During concurrent L-NAME and bosentan administration, blood pressure increased to a similar extent as it had increased with L-NAME administration alone and was constant until the end of the experiment.

Vehicle administration

To eliminate any possible effect of the vehicle (0.9 % saline) after the end of the control period 200 µl sterile saline was applied as a bolus injection and after 20-min equilibration blood pressure was measured and urine was collected during a 40-min clearance period in 8 sham-operated and 8 renal denervated SHR as well as

in 8 sham-operated and 8 renal denervated Wistar rats.

Throughout the study, arterial blood pressure was measured directly in the femoral artery by Gould/Statham P23ID pressure transducer connected to computerized data acquisition system Biopac MP100WS. After analogue-to-digital conversion, by using peak and rate detector of the AcqKnowledge 2.0 software, values of systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) in every heart cycle were determined. HR was derived from the pulse intervals of arterial blood pressure waves.

A priming dose of 50 mg kg⁻¹ inulin (Sigma) and 12 mg kg⁻¹ paraaminohippuric acid (PAH, Merck) was applied as an intravenous bolus injection to rapidly attain high plasma levels of these substances. Immediately thereafter, a continuous intravenous infusion of inulin (1.8 mg kg⁻¹ min⁻¹) and PAH (0.70 mg kg⁻¹ min⁻¹) was started at a pump rate of 25 µl min⁻¹. Plasma and urine concentrations of inulin were determined by the anthrone method (Fuhr *et al.* 1955). Glomerular filtration rate (GFR) was estimated by the clearance of inulin. Urinary and plasma concentrations of PAH were determined by the modified method of Smith *et al.* (1945). Effective renal plasma flow (ERPF) was estimated from the clearance of PAH, and renal blood flow (RBF) was calculated as ERPF/(1 – hematocrit). Renal vascular resistance (RVR) was calculated as MAP/RBF.

Urine volume was assessed gravimetrically to establish urine flow rate (V). Urine osmolality was determined by using a vapor pressure osmometer (Vescor 5500A). Blood was taken at the end of the experiment. Hematocrit was obtained from all blood samples. Plasma and urine concentrations of sodium were determined by flame photometry (Corning 410 C). Plasma and urine chloride concentrations were determined by using a chloride analyzer (Corning 925). Plasma and urine concentrations of phosphate were determined spectrophotometrically using commercially available kits (SGM Italia). On the basis of these data the fractional excretions of sodium (FE_{Na}), chloride (FE_{Cl}) and phosphate (FE_P) were calculated (C_x.100 /GFR). At the end of the experiments, the rats were sacrificed by an overdose of sodium pentobarbital.

Data were tested for normality and unpaired Student's *t*-test or Wilcoxon's test for unpaired data were used when appropriate. One-way ANOVA followed by Bonferroni-Dunn *post hoc* test was used to compare control period values and response to L-NAME and bosentan. Two-way ANOVA followed by Tukey *post hoc*

Table 1. Changes in renal hemodynamics, blood pressure and heart rate due to L-NAME-induced nitric oxide synthase inhibition as well as concurrent nitric oxide synthase and ET_A/ET_B receptor inhibition in both sham-operated Wistar rats and SHR with intact renal nerves as well as in both Wistar rats and SHR one week after bilateral renal denervation

Wistar rats Intact renal nerves	Control n=14	L-NAME n=14	L-NAME+Bo n=13
<i>GFR</i>	0.80±0.11	0.79±0.07	0.81±0.06
<i>RBF</i>	3.89±0.37	3.99±0.43	3.86±0.36
<i>RVR</i>	26.7±0.9	32.4±1.4 *	32.9±1.6 *
<i>SAP</i>	137.1±1.4	156.4±3.0 **	157.7±5.0 **
<i>MAP</i>	105.0±1.4	129.7±3.3 **	127.2±4.3 **
<i>DAP</i>	83.7±1.1	112.2±3.6 **	109.5±4.0 **
<i>HR</i>	369.7±9.4	340.2±8.4 *	314.5±16.5 **
Wistar rats Renal denervation	Control n=12	L-NAME n=10	L-NAME+Bo n=10
<i>GFR</i>	0.75±0.03	0.72±0.08	0.71±0.04
<i>RBF</i>	3.81±0.19	3.77±0.23	4.05±0.32
<i>RVR</i>	26.1±1.1	33.1±1.4 **	29.8±1.0 *
<i>SAP</i>	135.6±4.1	153.9±4.0 **	157.9±9.8 **
<i>MAP</i>	101.7±2.2	125.6±3.9 **	125.0±7.6 **
<i>DAP</i>	79.4±1.6	106.4±3.8 **	106.8±6.6 **
<i>HR</i>	387.1±10.4	353.9±9.9 *	355.4±10.2 *
SHR Intact renal nerves	Control n=18	L-NAME n=11	L-NAME+Bo n=9
<i>GFR</i>	0.80±0.11	0.77±0.13	0.81±0.19
<i>RBF</i>	3.78±0.39	3.86±0.54	3.67±0.41
<i>RVR</i>	35.1±2.1	43.7±2.2 *	40.4±1.4 *
<i>SAP</i>	179.0±2.1 #	199.5±5.7 **	189.9±6.7 *
<i>MAP</i>	132.9±2.8 #	164.6±4.8 **	148.5±5.4 * +
<i>DAP</i>	106.1±3.0 #	140.9±4.4 **	124.0±4.8 ** +
<i>HR</i>	350.8±12.4	302.7±18.1 *	292.0±18.0 *
SHR Renal denervation	Control n=10	L-NAME n=10	L-NAME+Bo n=10
<i>GFR</i>	0.76±0.22	0.79±0.38	0.75±0.28
<i>RBF</i>	3.79±0.32	3.99±0.39	3.71±0.21
<i>RVR</i>	36.2±1.7	42.1±2.0 *	40.5±1.1 *
<i>SAP</i>	178.9±1.8 #	206.1±4.9 *	190.4±6.8 *
<i>MAP</i>	141.4±4.3 #	167.5±4.1 **	150.1±4.0 * +
<i>DAP</i>	116.4±5.2 #	142.3±3.8 **	126.4±5.4 * +
<i>HR</i>	336.0±13.0	279.5±18.3 *	288.9±10.5 *

Data represent mean ± S.E.M.; *n* is the number of experiments; SHR – spontaneously hypertensive rats; L-NAME - administration of 10 mg kg⁻¹ b.w. i.v. N^o-nitro-L-arginine methyl ester; L-NAME+Bo – concomitant administration of L-NAME and ET_A/ET_B receptor blocker bosentan 10 mg kg⁻¹ b.w. i.v.; GFR – glomerular filtration rate, ml min⁻¹ 100 g⁻¹ b.w.; RBF – renal blood flow, ml min⁻¹ 100 g⁻¹ b.w., RVR – renal vascular resistance, mmHg ml⁻¹ min⁻¹ 100 g⁻¹ b.w.; SAP – systolic arterial pressure, mmHg; MAP – mean arterial pressure, mmHg; DAP – diastolic arterial pressure, mmHg; HR – heart rate, beats min⁻¹; Statistically significant differences * p<0.05; ** p<0.01 as compared to control; + p<0.05 as compared to L-NAME; # p<0.05 as compared to normotensive rats.

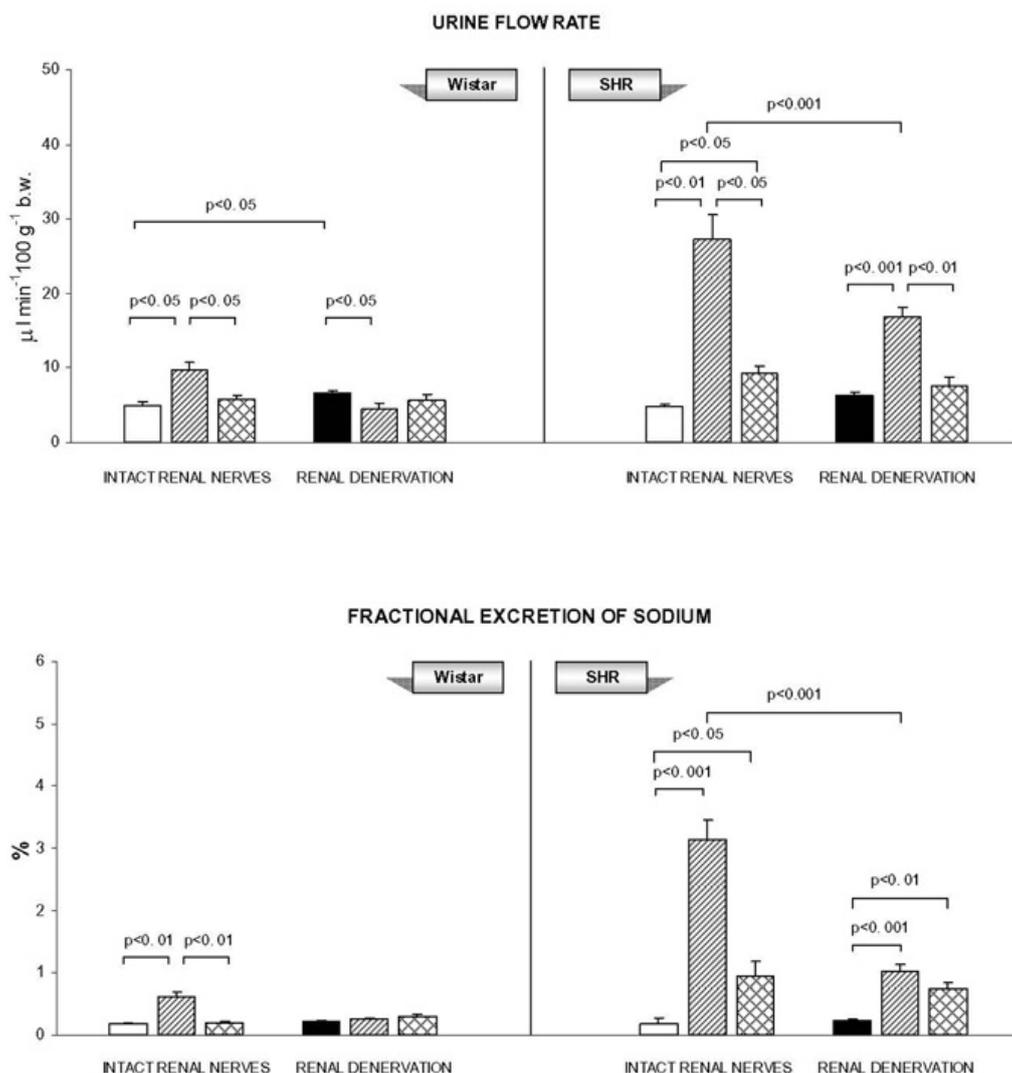


Fig. 1. Urine flow rate (upper panel) and fractional excretion of sodium - FE_{Na} (lower panel) in normotensive Wistar rats (left panel) and in spontaneously hypertensive rats - SHR (right panel) in control period with intact renal nerves (open bars) and in control period after renal denervation (solid bars), during NO synthase inhibition (hatched bars) and during concomitant NO synthase inhibition and endothelin A/B receptor blockade (cross-hatched bars).

analysis was used to evaluate the response to L-NAME and bosentan between different groups. All results are presented as mean \pm S.E.M. Differences at a level of $p < 0.05$ were considered statistically significant.

Results

In the rats with intact renal nerves as well as in the renal denervated normotensive and hypertensive rats renal excretory function and blood pressure were stable throughout the time control experiments. The investigated variables did not change after injection of the vehicle. Hematocrit did not differ between the groups. Baseline SAP, MAP and DAP were higher in the SHR than in the normotensive rats ($p < 0.05$). NOS inhibition with

L-NAME as well as concomitant NOS and ET_A/ET_B receptor inhibition with bosentan did not alter GFR or RBF in any of the groups investigated (Table 1).

In the normotensive rats with intact renal nerves, L-NAME induced an increase in SAP, MAP and DAP by 19.3 mm Hg (14.0 %), 24.7 mm Hg (23.5 %) and 28.5 mm Hg (34 %) respectively ($p < 0.01$). RVR increased by 21.3 % ($p < 0.05$), (Table 1). L-NAME administration resulted in an increase in urine flow rate from 5.01 ± 0.48 to $9.71 \pm 1.08 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.05$). FE_{Na} increased from 0.18 ± 0.01 to 0.62 ± 0.08 % ($p < 0.01$) (Fig. 1). FE_{Cl} increased from 0.23 ± 0.02 to 1.0 ± 0.11 % ($p < 0.01$). FE_P also increased from 4.50 ± 0.24 to 6.62 ± 0.31 % ($p < 0.01$) (Fig. 2).

The pressor effect of L-NAME on SAP MAP

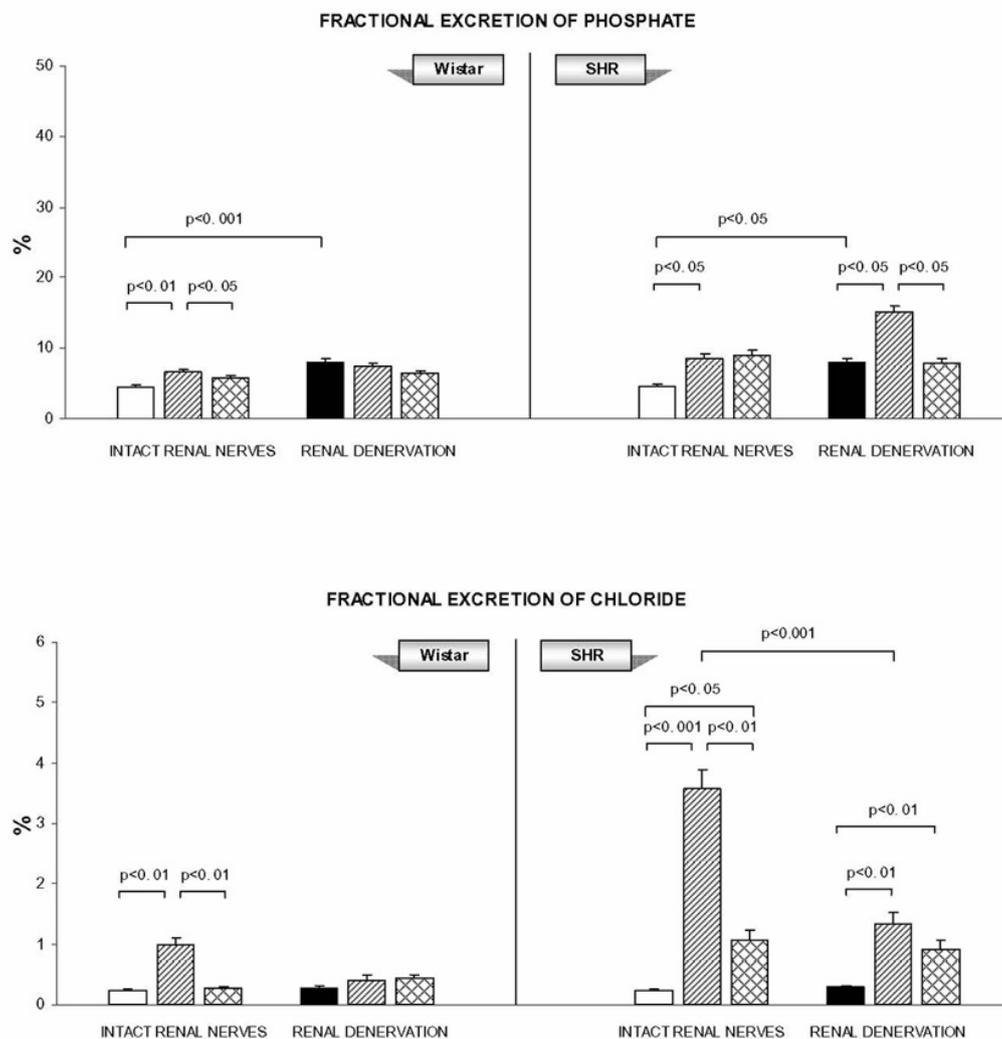


Fig. 2. Fractional excretion of phosphate - FE_p (upper panel) and fractional excretion of chloride - FE_{Cl} (lower panel) in normotensive Wistar rats (left panel) and in spontaneously hypertensive rats - SHR (right panel) in control period with intact renal nerves (open bars) and in control period after renal denervation (solid bars), during NO synthase inhibition (hatched bars) and during concomitant NO synthase inhibition and endothelin A/B receptor blockade (cross-hatched bars).

and DAP as well as the effect on RVR were not influenced by bosentan in the normotensive rats with intact renal nerves (Table 1). However, the effect of L-NAME on urine flow rate as well as on fractional excretion of sodium and chloride was markedly reduced by bosentan and the values returned to the control level. Urine flow rate was $5.78 \pm 0.45 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$, FE_{Na} was $0.19 \pm 0.02 \%$, FE_{Cl} was $0.27 \pm 0.03 \%$ and FE_p was $5.81 \pm 0.34 \%$ (Figs 1 and 2).

Chronic renal denervation did not change SAP, MAP, DAP, RVR, GFR, RBF or fractional excretion of sodium or chloride. Urine flow rate increased from 5.01 ± 0.48 to $6.52 \pm 0.44 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.05$), while urine osmolality decreased from 835.7 ± 52.7 to $626.4 \pm 40.0 \text{ mosm kg}^{-1} \text{ H}_2\text{O}$ ($p < 0.05$). FE_p increased from

4.50 ± 0.24 to $7.91 \pm 0.59 \%$ ($p < 0.001$). As a result of the denervation renal norepinephrine content decreased from 9.1 ± 0.62 to $0.51 \pm 0.02 \text{ nmol g}^{-1} \text{ renal tissue}$ ($p < 0.001$).

In the normotensive rats, subjected to bilateral renal denervation, L-NAME induced an increase in SAP, MAP and DAP by 18.3 mm Hg (13.4 %), 23.9 mm Hg (23.5 %) and by 27.0 mm Hg (34 %), respectively ($p < 0.01$). RVR increased by 26.8 % (Table 1). L-NAME administration decreased urine flow rate from 6.52 ± 0.44 to $4.47 \pm 0.70 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.05$). FE_{Na} was $0.25 \pm 0.03 \%$, FE_{Cl} was $0.39 \pm 0.05 \%$, FE_p was $7.49 \pm 0.33 \%$ and did not differ from the values after the renal denervation (Figs 1 and 2).

In the renal denervated normotensive rats the pressor effect of L-NAME on SAP, MAP and DAP as

well as the effects on RVR were not affected by bosentan (Table 1). Bosentan administration during NO synthase inhibition did not produce any changes in urine flow rate or fractional excretion of sodium, chloride or phosphate. Urine flow rate was $5.68 \pm 0.73 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$, FE_{Na} was $0.30 \pm 0.04 \%$ (Fig. 1), FE_{Cl} was $0.43 \pm 0.07 \%$ and FE_{P} was $6.42 \pm 0.37 \%$ (Fig. 2).

In the SHR with intact renal nerves, L-NAME induced an increase in SAP, MAP and DAP by 20.5 mm Hg (11.4 %), 31.7 mmHg (23.8 %) and by 34.8 mmHg (32.7 %), respectively ($p < 0.01$). RVR increased by 24.5 % ($p < 0.05$), (Table 1). L-NAME administration in the SHR dramatically increased urine flow rate from 4.81 ± 0.37 to $27.30 \pm 3.21 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.01$). FE_{Na} increased from 0.18 ± 0.01 to $3.13 \pm 0.42 \%$ ($p < 0.001$) (Fig. 1). FE_{Cl} also increased dramatically from 0.23 ± 0.02 to $3.58 \pm 0.32 \%$ ($p < 0.001$). FE_{P} increased from 4.59 ± 0.35 to $8.50 \pm 0.69 \%$, ($p < 0.05$) (Fig. 2).

The pressor effect of L-NAME on MAP and DAP in the SHR was reduced by bosentan by 16.1 mm Hg (9.8 %) and 16.9 mm Hg (12.0 %), respectively ($p < 0.05$) (Table 1). The effect of L-NAME on urine flow rate, FE_{Na} and FE_{Cl} was reduced by bosentan ($p < 0.01$), but the values remained significantly elevated compared to the control level: $9.20 \pm 1.06 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$; $0.94 \pm 0.25 \%$ and $1.08 \pm 0.15 \%$, respectively ($p < 0.05$). FE_{P} did not change and was 8.93 ± 0.73 (Figs 1 and 2).

Chronic renal denervation in the SHR did not change SAP, MAP, DAP, RVR, GFR, RBF or fractional excretion of sodium and chloride. Urine flow rate or urine osmolality did not change significantly either. FE_{P} increased from 4.59 ± 0.34 to $7.94 \pm 0.61 \%$ ($p < 0.05$). As a result of the denervation renal norepinephrine content decreased from 12.3 ± 0.58 to $0.43 \pm 0.02 \text{ nmol g}^{-1} \text{ renal tissue}$ ($p < 0.001$).

In the renal denervated SHR, L-NAME induced an increase in SAP, MAP and DAP by 27.2 mm Hg (15.2 %), 26.1 mm Hg (18.4 %) and 25.9 mm Hg (22.2 %), respectively ($p < 0.01$). RVR increased by 16.2 % ($p < 0.05$) (Table 1). Urine flow rate increased from 6.25 ± 0.48 to $16.81 \pm 1.37 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.001$). FE_{Na} increased from 0.23 ± 0.02 to $1.03 \pm 0.12 \%$ ($p < 0.001$), (Fig. 1). FE_{Cl} increased from 0.29 ± 0.03 to $1.34 \pm 0.18 \%$ ($p < 0.01$). EF_{P} also increased from $7.94 \pm 0.61 \%$ to $15.1 \pm 0.92 \%$ after L-NAME administration ($p < 0.05$) (Fig. 2).

The pressor effect of L-NAME on MAP and DAP in the renal denervated SHR was reduced by bosentan by 17.4 mm Hg (10.3 %) and 15.9 mm Hg

(11.1 %), respectively ($p < 0.05$), but HR or RVR did not change (Table 1). The effect of L-NAME on urine flow rate, FE_{Na} and FE_{Cl} was reduced by bosentan. Urine flow rate decreased to $7.59 \pm 1.10 \mu\text{l min}^{-1} 100 \text{ g}^{-1} \text{ b.w.}$ ($p < 0.01$), FE_{Na} was $0.75 \pm 0.10 \%$, (Fig. 1) and FE_{Cl} was $0.92 \pm 0.16 \%$ and the values remained significantly elevated as compared to the control level ($p < 0.01$). FE_{P} decreased to $7.81 \pm 0.71 \%$ ($p < 0.05$) (Fig. 2).

Discussion

The acute NOS inhibition in both normotensive rats and SHR produced a significant increase in urine flow rate, FE_{Na} , FE_{Cl} , and FE_{P} despite the fact that GFR and thus the filtered load of these electrolytes did not increase. It was previously demonstrated that acute systemic NOS inhibition with L-NAME leads to hypertension and natriuresis in rats with intact renal nerves (Baylis *et al.* 1997). The effect of NOS inhibition on water and electrolyte excretions could be due to the elimination of a direct action of NO on tubular sodium transport, pressure natriuresis or changes in RBF or GFR. It has been shown that natriuresis and diuresis, which are due to systemic NOS inhibition, are not entirely a result of a pressure effect and can be partially dissociated from the rise in blood pressure (Zhang and Baylis 1999). Intravenous infusion of L-NAME at a subpressor dose was shown to be diuretic and natriuretic (Liang *et al.* 2001). It has also been demonstrated that RBF autoregulation is maintained during an acute inhibition of NO synthesis, and that GFR does not change (Aberola *et al.* 1992). However, renal medullary circulation is a pressure-dependent vascular bed that is not able to autoregulate blood flow with changes in renal perfusion pressure (Cowley *et al.* 1992). The increase in sodium, chloride and phosphate excretions after NOS inhibition in our experiments is likely to be a result of some direct modulation of tubular transport as well as of pressure diuresis and natriuresis.

L-NAME-induced effects of renal excretory function in the normotensive Wistar rats were completely inhibited by bosentan. In contrast, bosentan did not affect arterial pressure. Since the kidney contains ET_{A} and ET_{B} receptors, the complete blockade of the actions of endogenous ET requires antagonism of both ET_{A} and ET_{B} receptors. It is well recognized that ET-1 exerts diuretic and natriuretic effects *via* ET_{B} receptors (Kohan 1996) but the participation of ET_{A} receptors is also suggested (Bailey *et al.* 2003, Girchev *et al.* 2004a). We have

recently demonstrated that the intravenous bolus injection of 10 mg kg⁻¹ b.w. bosentan decrease urine flow rate and sodium excretion without any changes in GFR or RBF (Girchev *et al.* 2004b). Based on our experimental data it becomes clear that endothelins play a pivotal role in the regulation of the renal excretory function during NOS inhibition since bosentan prevents renal changes due to L-NAME administration. L-NAME-induced effects of renal excretory function in SHR were markedly, though not completely, inhibited by bosentan. ET_A/ET_B receptor blockade in SHR was therefore able to prevent only a part of the urinary water and electrolyte excretion rise induced by an acute administration of L-NAME. Phosphate transport in the proximal tubule was affected by NO (Bacic *et al.* 2004). Since phosphate is reabsorbed entirely in the proximal tubule, urinary phosphate excretion was used to differentiate proximal from distal tubular effects of NOS and ET_A/ET_B receptor inhibition. The increase EF_{Na} during NOS inhibition in both normotensive rats and SHR was associated with an increase in FE_p, which indicates a decreased proximal tubular reabsorption. However, the fact that the magnitude of the increased output of sodium in SHR was greater than that of phosphate also suggests an involvement of the distal part of the nephron. The lack of normalization by ET_A/ET_B receptor blockade of renal excretory response to acute NOS inhibition in SHR may be related to an intrarenal deficit of ET. It was observed that ET-1 content in the renal papilla as well as urinary ET excretion were lower in SHR as compared to normotensive rats (Girchev *et al.* 2004a). Therefore, the participation of ET in the changes in renal excretory function due to NOS inhibition is diminished in SHR as compared to normotensive Wistar rats.

In contrast to rats with intact renal nerves, in renal denervated normotensive rats, urine flow rate decreased after NOS inhibition. NO might exert action in different ways – on the one hand, by inhibiting basal levels of fluid reabsorption, and on the other, by facilitating neurally stimulated fluid reabsorption (Wu and Johns 2002). Moreover, it was demonstrated that there was a tonic inhibitory action of NO on water excretion, which was independent of renal nerves, whereas its impact on sodium handling appeared to be dependent upon a background level of renal nerve activity (Wongmekiat and Johns 2001). Therefore, the natriuretic responses following NOS inhibition are more closely related to renal nerves. It was shown that NOS inhibition caused a reduction in proximal fluid

reabsorption and that this effect was abolished by renal denervation (Gabbai *et al.* 1995). Thus, more fluid is reabsorbed in the proximal tubule and the effect of NOS inhibition will be reduced. In our experiments, the effects of NOS inhibition were abolished by the renal denervation. The experiments performed demonstrated that the effects of NOS inhibition on renal excretory function are partly mediated through renal sympathetic nerves.

Interestingly, no responses to ET_A/ET_B receptor inhibition were observed in the renal denervated normotensive rats when NO synthesis was inhibited. Therefore, renal sympathetic nerve activity is necessary for the influence of endogenous ET on L-NAME-induced changes in renal excretory function. We have recently demonstrated that chronic renal denervation in normotensive rats is followed by a decrease in renal papillary ET-1 concentration (Girchev *et al.* 2004a). Such an influence of renal denervation on renal papillary ET-1 content may account for the differences in renal excretory function between rats with intact and rats with denervated kidneys observed in the present study.

In the normotensive rats with or without renal nerves, bosentan did not prevent the effect of L-NAME on blood pressure. However, in SHR with intact renal nerves and in renal denervated SHR it diminished changes in MAP and DAP induced by L-NAME. Therefore, the hypertensive response induced by NO synthase inhibition in SHR is partially due to the activation of endogenous ET and does not depend on renal nerves.

The main question addressed in this study was whether chronic renal denervation would modify the effects of NOS and ET_A/ET_B receptor inhibition on renal excretory function in SHR. In Wistar rats the action of NO became evident only in the presence of renal nerves. In renal denervated SHR, L-NAME induced an increase in urine flow rate, FE_{Na}, FE_{Cl} and FE_p. It is well known that tubuloglomerular feedback responses in WKY are not influenced by renal denervation, while in SHR enhanced tubuloglomerular feedback activity is attenuated by renal denervation (Takabatake *et al.* 1990). In addition, the results from the present study have shown that in renal denervated SHR the values of urine flow rate, FE_{Na} and FE_{Cl} are significantly lower as compared to the values after L-NAME administration in SHR with intact renal nerves (Figs 1 and 2). Therefore, the effects of L-NAME in renal denervated SHR differ from the effects of L-NAME in SHR with intact renal nerves

despite the fact that blood pressure in SHR with intact renal nerves was as high as that in renal denervated SHR. The interaction between NO and neural control of proximal fluid reabsorption did not occur in the genetically hypertensive rats (Wu *et al.* 1999). In stroke-prone SHR, irrespective of whether renal nerves were present or not, proximal tubular fluid reabsorption was not affected when NO production was either blocked or enhanced (Wu *et al.* 1999). This could explain the fact that the action of L-NAME is less influenced by renal denervation in SHR. However, there is evidence suggesting that NO may be of particular importance in buffering the action of sympathetic stimulation within renal medullary circulation (Zou and Cowley 2000) and especially in SHR (Bergström *et al.* 1996). The changes in medullary blood flow after NOS inhibition were associated with parallel changes in sodium and water excretion independent of alterations in renal cortical blood flow or GFR (Mattson *et al.* 1997). Our data show that under the conditions of chronic renal denervation the effects of L-NAME on urine flow rate, sodium and chloride excretion in conscious SHR are less pronounced as compared to normotensive Wistar rats.

We observed differences between the responsiveness to nonselective ET_A/ET_B receptor blockade of SHR with intact renal nerves on the one hand, and renal denervated SHR on the other. The diuretic effect of L-NAME was suppressed in both sham-operated and renal denervated SHR. However, in renal denervated SHR, bosentan did not prevent the effect of L-NAME on sodium and chloride excretions. Our experimental data showed that in SHR, the influence of renal sympathetic nerves on ET-induced effects during

NO synthase inhibition on renal excretion of sodium and chloride, is less clearly demonstrated than the effects on renal water excretion. It was reported that NO and renal nerves interaction is important for adequate perfusion of the medulla especially in SHR (Bergström *et al.* 1996). Moreover, our recent data showed that synthesis of ET-1 in the renal papilla of SHR is decreased as compared to that in normotensive WKY rats and that renal denervation did not alter papillary ET concentration in SHR whose tissue concentration had already been low (Girchev *et al.* 2004a). Therefore, this impaired effect of nonselective ET_A/ET_B receptor blockade on sodium and chloride excretion could be attributed to the low papillary ET-1 content observed in SHR.

In conclusion, the influence of renal nerves on NO induced effects on sodium and chloride excretion is less clearly manifested in SHR as compared to normotensive rats. The participation of endogenous endothelins in the changes of renal excretory function due to NOS inhibition is diminished in SHR compared to normotensive Wistar rats. Renal sympathetic nerves do not affect the ET-induced effects during NOS inhibition on renal sodium and chloride excretion as strongly as the ET-induced effects on renal water excretion in SHR. The hypertensive response induced by NOS inhibition in SHR is partially due to activation of endogenous ET but does not depend on renal nerves.

Acknowledgements

This study was supported by a grant 4/2004 from the Council of Medical Science, Medical University, Sofia, Bulgaria.

References

- ABEROLA A, PINILLA JM, QUESADA T, ROMERO JC, SALOM MG, SALAZAR FJ: Role of nitric oxide in mediating renal response to volume expansion. *Hypertension* **19**: 780-784, 1992.
- BACIC D, WAGNER CA, HERNANDO N, KAISLING B, BIBER J, MURER H: Novel aspects in regulated expression of the renal type IIa Na/Pi-cotransporter. *Kidney Int Suppl* **91**: S5-S12, 2004.
- BAILEY MA, HATON C, OREA V, SASSARD J, BAILLY C, UNWIN RS, IMBERT-TEBOUL M: ET_A receptor-mediated Ca²⁺ signaling in thin descending limbs of Henle's loop: Impairment in genetic hypertension. *Kidney Int* **63**: 12-76, 2003.
- BAYLIS C, BRAITH R, SANTMYRE BR, ENGELS K: Renal nerves do not mediate vasoconstrictor responses to acute nitric oxide synthesis inhibition in conscious rats. *J Am Soc Nephrol* **8**: 887-892, 1997.
- BERGSTRÖM G, RUDENSTAM J, TAGHIPOUR K, GÖTHEBERG G, KARLSTRÖM G: Effect of nitric oxide and renal nerves on renomedullary hemodynamics in SHR and Wistar rats, studied with laser Doppler technique. *Acta Physiol Scand* **156**: 27-36, 1996.

- COWLEY AW, ROMAN RJ, FENOY FJ, MATTSON DL: Effects of renal medullary circulation on arterial pressure. *J Hypertens* **10** (Suppl 7): S187-S193, 1992.
- FUHR J, KACZMARCZYK J, KRUTTGEN CD: Eine einfache colorimetrische Methode zur Inulinbestimmung. *Klin Wochenschr* **33**: 729-730, 1955.
- GABBAI FB, THOMSON SC, PETERSON O: Glomerular and tubular interactions between renal adrenergic activity and nitric oxide. *Am J Physiol* **286**: F1004-F1008, 1995.
- GIRCHEV R, BACKER A, MARKOVA P, KRAMER HJ: Impaired response of the denervated kidney to endothelin receptor blockade in normotensive and spontaneously hypertensive rats. *Kidney Int* **65**: 982-989, 2004a.
- GIRCHEV R, MARKOVA P, VUCHIDOLOVA V: Influence of nonselective ET_A/ET_B blockade on renal function in conscious rats: Effects of renal denervation. *J Physiol Pharmacol* **55**: 381-389, 2004b.
- GRISK O, RETTIG R: Interactions between the sympathetic nervous system and the kidneys in arterial hypertension. *Cardiovasc Res* **61**: 238-246, 2004.
- HERRERA M, GARVIN JL: Endothelin stimulates endothelial nitric oxide synthase expression in the thick ascending limb. *Am J Physiol* **287**: F231-F235, 2004.
- KOHAN DE: Endothelins: renal tubule synthesis and actions. *Clin Exp Pharmacol Physiol* **23**: 337-344, 1996.
- LECLERCQ B, JAIMES EA, RAI L: Nitric oxide synthase and hypertension. *Curr Opin Nephrol Hypertens* **11**: 185-189, 2002.
- LESKINEN H, VUOLTEENAHAO O, RUSKOAHO H: Combined inhibition of endothelin and angiotensin II receptors blocks volume load-induced cardiac hormone release. *Circ Res* **80**: 114-123, 1997.
- LI L, WATTS SW, BANES AK, GALLIGAN JJ, FINK GD, CHEN AF: NAD(P)H oxidase-derived superoxide augments endothelin-1-induced vasoconstriction in mineralocorticoid hypertension. *Hypertension* **42**: 316-320, 2003.
- LIANG M, BERNDT TJ, KNOX FG: Mechanism underlying diuretic effects of L-NAME as a subpressor dose. *Am J Physiol* **281**: F414-F419, 2001.
- LOZANOV V, PETROV S, MITEV V: Simultaneous analysis of aminoacid and biogenic polyamines by high-performance liquid chromatography after pre-column derivatization with N-(9-fluorenylmethoxy-carbonyloxy)succinimide. *J Chromatogr* **1052**: 201-208, 2004.
- MATTSON DL, LU S, COWLEY AW JR: Role of nitric oxide in the control of the renal medullary circulation. *Clin Exp Pharmacol Physiol*: **24**: 587-590, 1997.
- MATSUO G, MATSUMURA Y, TADANO K, HASHIMOTO T, MORIMOTO S: Involvement of nitric oxide in endothelin ET_B receptor-mediated inhibitory actions on antidiuresis and norepinephrine overflow induced by stimulation of renal nerves in anesthetized dogs. *J Cardiovasc Pharmacol* **30**: 325-331, 1997.
- MATSUOKA H, HISHIDA H, NOMURA G, VAN VLIET BN, TOSHIMA H: Hypertension induced by nitric oxide synthesis inhibition is renal nerve dependent. *Hypertension* **23**: 971-975, 1994.
- PLATO CF, GARVIN JL: Alpha₂-adrenergic-mediated tubular NO production inhibits thick ascending limb chloride absorption. *Am J Physiol* **281**: F679-F686, 2001.
- PLATO CF, POLLOCK DM, GARVIN JL: Endothelin inhibits thick ascending limb chloride flux via ET_B receptor-mediated NO release. *Am J Physiol* **279**: F326-F333, 2000.
- RICHARD V, HOGIE M, CLOZEL M, LOFFLER BM, THUILLEZ C: In vivo evidence of an endothelin-induced vasopressor tone after inhibition of nitric oxide synthesis in rats. *Circulation* **91**: 771-775, 1995.
- SAKUMA I, TOGASHI H, YOSHIOKA M, SAITO H, YANAGIDA M, TAMURA M, KOBAYASHI T, YASUDA H, GROSS SS, LEVI R: N^G-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? *Circ Res* **70**: 607-611, 1992.
- SMITH HW, FINKELSTEIN N, ALIMINOSA L, CROWFORD B, GRABER M: The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest* **42**: 388-404, 1945.
- TAKABATAKE T, USHIOGI Y, OHTA K, HATTORI N: Attenuation of enhanced tubuloglomerular feedback activity in SHR by renal denervation. *Am J Physiol* **258**: F980-F985, 1990.

-
- THOMSON SC, VALLON V: Alpha₂-adrenoceptors determine the response to nitric oxide inhibition in the rat glomerulus and proximal tubule. *J Am Soc Nephrol* **6**: 1482-1490, 1995.
- WONGMEKIAT O, JOHNS EJ: Role of nitric oxide and renal nerves in the renal responses to acute volume expansion in anaesthetized rats. *Exp Physiol* **86**: 47-54, 2001.
- WU XC, HARRIS PJ, JOHNS EJ: Nitric oxide and renal nerve-mediated proximal tubular reabsorption in normotensive and hypertensive rats. *Am J Physiol* **277**: F560-F566, 1999.
- WU XC, JOHNS EJ: Nitric oxide modulation of neurally induced proximal tubular fluid reabsorption in the rat. *Hypertension* **39**: 790-793, 2002.
- ZHANG XZ, BAYLIS C: Endothelin mediates renal vascular memory of a transient rise in perfusion pressure due to NOS inhibition. *Am J Physiol* **276**: F629-F634, 1999.
- ZOU AP, COWLEY AW Jr: Alpha₂-adrenergic receptor-mediated increase in NO production buffers renal medullary vasoconstriction. *Am J Physiol* **279**: R769-R777, 2000.
-

Reprint requests

Radoslav Girchev, Department of Physiology, Medical University, 1 "G Sofiiski" str., Sofia 1431, Bulgaria. Fax: +3592 9520345. E-mail: girchev@medfac.acad.bg