ROLE OF NITRIC OXIDE, PRODUCED BY nNOS IN THE REGULATION OF WATER AND ELECTROLYTE EXCRETION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Summary

It has been established that there is a higher expression of neuronal isoform of nitric oxide synthase (nNOS) in the renal medulla in spontaneously hypertensive rats (SHR) compared to normotensive rats. In the present study there was investigated the role of the nitric oxide, produced by nNOS in the regulation of water and electrolyte excretion in spontaneously hypertensive rats. Experiments were carried out on conscious, male, normotensive Wistar rats and spontaneously hypertensive rats at the age of 12-14 weeks. Appropriate surgical preparation was undertaken for catheters implantation. The urine flow rate, sodium and chloride concentrations were measured. The investigated parameters did not differ between Wistar rats and SHR in control condition. The selective nNOS inhibition did not change urine flow rate in normotensive rats but in SHR nNOS inhibition provoked a decrease of urine flow rate by 50.9%. Neuronal nitric oxide synthase inhibition did not change sodium and chloride excretion neither in Wistar rats nor in SHR.

Key words: Spontaneously hypertensive rats (SHR), Neuronal nitric oxide synthase (nNOS), Urine flow rate, Electrolyte excretion

Introduction

Nitric oxide (NO) has been intensively studied in recent years and suspected in many renal physiological and pathophysiological processes. Production of this biologically active substance starts from L-arginine, mediated by group of enzymes called nitric oxide synthases (NOS). There have been identified at least three different isoforms of this enzyme in the mammalian body by using indirect immunofluorescence technique with polyclonal antibody (1). Two NOS isoforms are tonically active, continuously present and termed constitutive NOS: endothelial NOS (eNOS) and neuronal (nNOS) The endothelial NOS is located in the cytoplasm of endothelial cells, cardiomyocytes and renal arterioles (2). Expression of eNOS is the major factor for production of NO, responsible for the regulation of vascular tone. The neuronal isoform of NOS, which was first identified in the cytosol of brain tissue (3), has also been identified particularly in the macula densa (MD) cells of the juxtaglomerular apparatus. These isoforms are calcium dependent, they persist in the tissues but are not active until intercellular calcium concentration is enough for interacting with calmodulin. Their activity is modulated by physiological and pathological stimuli. The third type of NOS, the inducible NOS (iNOS), has been found in a wide range of renal structures. Glomerular, proximal tubular, distal convoluted and mesangial cells have all been shown to contain iNOS (4). Furthermore, iNOS has been identified in endothelial cells of renal vessels, mainly in preglomerular arteries. It is calcium independent, which effects are transient because of rapid inactivation.

The activity of the isoforms of NOS is modulated by transcriptional or post transcriptional mechanisms. Although the effects of general NO inhibition by structural analogues of L-Arginine have been thoroughly investigated, very little is known about the physiological importance of NO produced specifically by the different isoforms.
The major role of NO in the kidney is regulation of glomerular filtration (5, 6), stimulates renin release (7, 8), influences tubuloglomerular feedback mechanism (9), regulates sodium and water excretion, inhibits release of noradrenalin from renal sympathetic nerves (10) and interacts with biologically active substances such as adenosine (11), angiotensin (12), endothelins (13), antidiuretic hormone (14), prostaglandins (15). Due to its high diffusibility, NO produced in a particular segment of nephron or in the renal vessels could affect the function of surrounding structures. Consequently, NO does not have to be produced in a nephron segment to have an effect (16). In addition there are evidences indicating that NO can act as a modulator of neurotransmission within the central nervous system, in sympathetic ganglia and in peripheral neuroeffector junctions (17).
The spontaneously hypertensive rat is a commonly used rat genetic model of raised blood pressure because it shares many of the cardiovascular abnormalities manifested in humans with essential hypertension (18, 19). The development of hypertension in this strain is usually associated with the impairment of endothelial function (19) and increased overall sympathetic nerve activity (20). In SHR has been reported a decreased bioavailability of NO in the vasculature and kidneys (21, 22) due to an excess of reactive oxygen species, leading to its bioinactivation (21). It has been established higher expression of nNOS in renal medulla in SHR compared to normotensive rats which is possibly a protective mechanism responsible for improving renal function in SHR (23).
The aim of the present study was to investigate the role of nitric oxide, produced by neuronal nitric oxide synthase in the regulation of water and electrolyte excretion in spontaneously hypertensive rats.

Materials and methods

Experiments were conducted in accordance with the ethical commission for Medical University - Sofia guide for the care and use of laboratory animals. Studies were performed on 10 male Wistar rats and 10 male SHR, 12 – 14 weeks old, weighing 280–300 g. The animals were housed in cages in a temperature-controlled room 22 ºC, with a 12 h/12 h light–dark cycle. Tap water and rat chow were provided ad libitum. The SHR group consisted of rats with systolic arterial blood pressure over 170 mmHg previously measured by tile cuff method (Ugo-Basile 58500). One day before experiments under general anesthesia (Nembutal – Sigma, in dose 35 mg/kg b.w. i.p.) catheter was implanted in femoral vein (ID: 0.58mm; OD: 0.96 mm, Portex) for drug application. To avoid clotting the catheter was flushed with 20 IU/ml heparin in sterile saline. The modified polyethylene catheter was inserted in urinary bladder for urine collection in short time intervals. The experiments were performed on conscious freely moving animals 24 hours after surgical intervention. Urine was collected during 40 minute control period. After a control period selective neuronal nitric oxide synthase inhibition (nNOSI) was performed by intravenous infusion of 7-Nitroindazole (2 mg. kg^{-1}. min $^{-1}$), dissolved in 0.9 % warmed NaCl. The urine collection was started again 20 minutes after beginning of nNOSI infusion for a period of 40 minutes. In the present study we investigated urine flow rate, determined gravimetrically, sodium concentration, measured by flame photometry (Corning 410C) and chloride concentration, determined by chloride analyzer (Corning 925). The excretion of sodium (U_{Na}.V) and chloride (U_{Cl}.V) was calculated.

All results were presented as mean ± SEM. Student's t-test was used for comparison between two means. Differences at a probability level of $p<0.05$ were considered significant.

Results

The systolic arterial blood pressure in SHR was statistically significantly higher compared to normotensive rats: 182.4±3.4 vs. 134.3±3.2 mmHg, ($p<0.01$). Urine flow rate, sodium and chloride excretion did not differ between Wistar rats and SHR in control period, (Fig.1). In Wistar rats urine flow rate was 4.58±0.72 µl.min^{-1}.100 g b.w and in SHR was 4.78±0.69 µl.min^{-1}.100 g b.w. Sodium excretion was 252.07±15.94 and 262.11±58.90 nmol.min^{-1}.100 g b.w. in Wistar rats and SHR respectively. In control period chloride excretion in Wistar rats was 76.32±6.16 nmol.min^{-1}.100 g b.w. and in SHR was 90.55±19.43 nmol.min^{-1}.100 g b.w. The selective inhibition of nNOS with 7-Nitroindazole did not change urine flow rate in Wistar rats: 4.38±0.69 µl.min^{-1}.100 g b.w., but in SHR caused a decrease to 2.70±0.45 µl.min^{-1}.100 g b.w, ($p<0.01$). During 7-Nitroindazole infusion sodium and chloride excretion did not change in Wistar as well as in SHR. Sodium excretion during 7-Nitroindazole infusion was 239.25±27.81 nmol.min^{-1}.100 g b.w. in Wistar rats and 281.96±25.99 nmol.min^{-1}.100 g b.w. in SHR. In normotensive rats and SHR chloride excretion was 87.06±13.49 and 107.80±23.21 nmol.min^{-1}.100 g b.w. respectively.

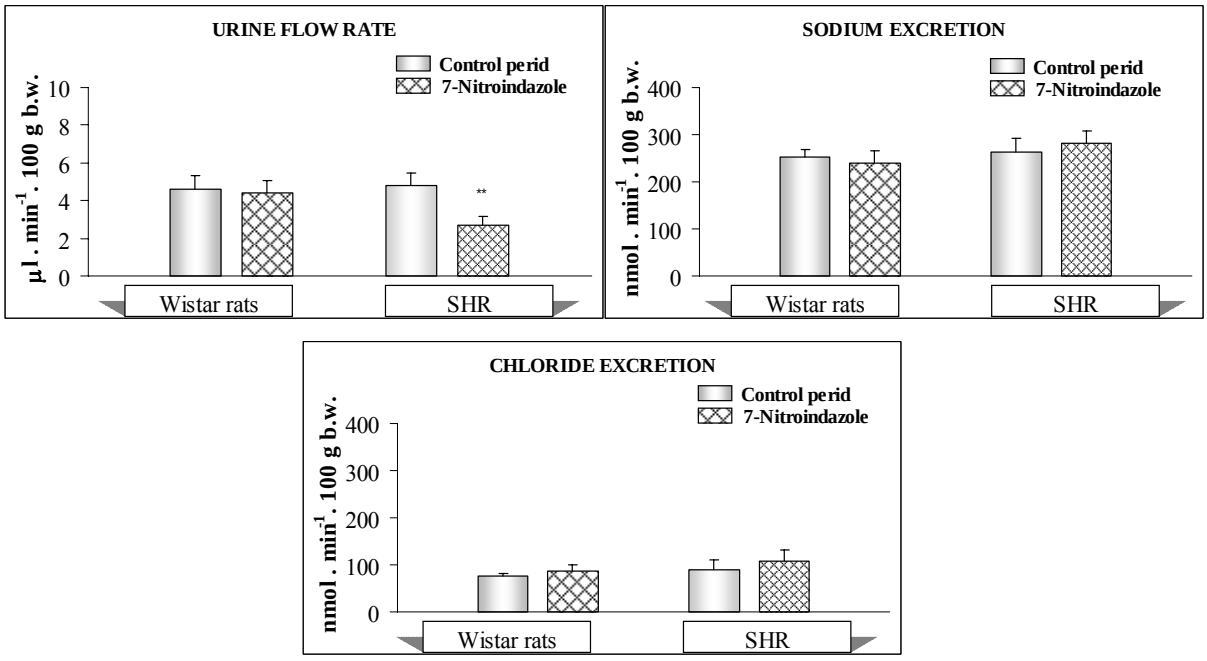

Figure 1. Urine flow rate, sodium and chloride excretion in normotensive (Wistar rats) and spontaneously hypertensive rats (SHR) in control period and during selective neuronal nitric oxide inhibition by 7-Nitroindazole (2 mg . kg^{-1}. min^{-1})
**** p<0.01 statistically significant difference as a result of 7-NI application**

Discussion

We established in our experiment differences in urine flow rate during selective nNOSI between normotensive and spontaneously hypertensive rats. Interestingly, despite the observed decrease of urine flow rate in SHR the electrolyte excretion was not affected as a result of nNOSI. In spite of the fact that SHR are characterized with permanent increase of arterial blood pressure, their renal excretory function did not display differences compared to normotensive rats. It has been supposed that different factors are involved in the regulation of renal function in hypertensive state. Using immunohistochemistry and Western blot, Fernandez et al. established higher expression of nNOS in renal medulla compared to normotensive rats (23). On the other hand the activity of nNOS in SHR in renal medulla is about ten times higher compared to renal cortex (24). It was suggested that the increased expression of nNOS in the medullar tubular cells is a possible protective mechanism responsible for improving renal function in SHR (23).

There are a number of surveys, showing the unique role of NO in the regulation of renal medullar perfusion, sodium homeostasis and arterial blood pressure. Experimental data consider that NO, produced by macula densa cells, affects tubuloglomerular feedback mechanism, intrarenal hemodynamics (9) and vascular tone of afferent arteriole (25, 26). Based on experimental data for distribution of nNOS in the kidney it can be concluded that NO, produced by nNOS in macula densa is also involved in the regulation of macula densa-mediated arteriole tone. This substance participates in the modulation of afferent arteriolar response to the changes of renal perfusion only when TGF is intact or more sensitive (27). It has been proved that TGF in spontaneously hypertensive rats is more sensitive compared to normotensive rats (28). In addition SHR have increased sympathetic nerve activity (29) and diminished renal NO bioavailability (21) compared to Wistar rats. This is probably compensated by the increased expression of nNOS, located predominantly in the outer renal medulla and the papillary region.

Experimental data suggest that increased NOS activity contributes to preglomerular vasodilation and subsequent glomerular hyperfiltration (30). Based on micropuncture experiments, the systemic administration of NO synthesis inhibitors lead to considerable increase in both afferent and efferent arteriolar resistances, which causes a fall in glomerular plasma flow, although the single nephron glomerular filtration rate is relatively protected (25). The effects on glomerular dynamics and glomerular filtration rate have been more variable, with some studies showing decreases in glomerular filtration rate and the ultrafiltration coefficient. Collectively, these studies have shown that NO plays an important role in regulating renal microvascular function (25). The reduction of the glomerular ultrafiltration coefficient during NOS inhibition is a result of changes in the mesangial cell tone caused by NO. This fact has been proved after in vitro studies, demonstrating that Angiotensin II-mediated mesangial contraction could be inhibited by NO. Recent studies in anesthetized rats have shown that after the selective inhibition of nNOS with 7-Nitroindazole, there is a considerable reduction of medullary blood flow. In addition, the results were similar as after unselective inhibition of NOS. Then it was concluded that NO generated by nNOS is mainly responsible for adequate perfusion of the medulla whereas the activity of nNOS and other isoforms are required to maintain cortical blood flow (31).

In our study after the selective inhibition of nNOS with 7-Nitroindazole, there was a decrease of urine flow rate only in the group of SHR. We hypothesize that the effects of a reduce urine flow rate in spontaneously hypertensive rats can be a result of the intimate interaction between antidiuretic hormone (ADH) and NO, derived by nNOS. ADH regulates water transport, by increasing dramatically the permeability for water in collecting duct after expressing Aquaporine-2 (32). Experimental studies, using isolated perfused rat cortical

collecting ducts have shown that NO inhibits ADH-stimulated osmotic water permeability by increasing cGMP via soluble guanylate cyclase, activating cGMP-dependent protein kinase and decreasing cAMP (33). Normally, the reduced bioavailability of NO in SHR is compensated by nNOS located in macula densa cells which is thought to improve renal function in these animals (23). It has been proved that SHR have increased sympathetic nerve activity (20) compared to normotensive rats. We hypothesize that part of the effects of NO in SHR could possibly be a result of nNOS, derived by perivascular sympathetic nerve endings. In addition, recent experimental data report that NO modulates neurotransmission and is a co-mediator in peripheral neuroeffector junctions (17). We consider that after the selective blockade of nNOS with 7-Nitroindazole there is a considerable decrease of NO in the group of SHR which leads to ADH-stimulated osmotic water permeability and statistically significant reduction of urine flow rate observed in that experimental group. In contrast, normotensive rats, which do not have deficit of NO bioavailability, no statistically significant change in urine flow rate can be reported.

The influence of NO upon sodium excretion has been studied intensively but the results are controversial. In vitro experiments have shown that NO affects tubular transport directly by decreasing sodium reabsorbion in proximal and collecting ducts. Nitric oxide in the proximal tubule possibly mediates the effects of Angiotensin II on tubular reabsorbtion (26). In the collecting duct, an NO-dependent inhibition of solute transport is suggested (26). In contrast, studies of the role of NO on renal sodium excretion in vivo have given rise to several contradictory results. The differences in experimental conditions such as species, anesthesia, doses, routes, types of NOS inhibitors renal blood flow, as well as blood pressure and hormonal status could possibly be the cause of these varied results. In our study we did not report statistically significant difference in sodium and chloride excretion in spontaneously hypertensive rats and Wistar rats. We consider that NO, derived by nNOS, does not possibly take part in the process of sodium and chloride reabsorbtion and secretion or it does not interact intimately with the biologically active substances that regulate these processes.

We concluded that the nitric oxide, produced by nNOS, in spontaneously hypertensive rats participated only in the regulation of water excretion, without changing sodium and chloride excretion.

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