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Original Contribution

CHANGES OF PLASMA RENIN ACTIVITY AFTER SELECTIVE NNOS SYNTHASE INHIBITION IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

PURPOSE: The present study investigates the role of nNOS in the regulation of plasma renin activity in spontaneously hypertensive rats (SHR).

METHODS: Experiments were carried out on conscious normotensive Wistar rats and on SHR. One day before experiment femoral artery for a direct arterial blood pressure (ABP) measurement, femoral vein for drug application and bladder for urine collection were catheterized. The ABP registration and urine collection were performed during two 40 min. periods: control period and after selective neuronal nitric oxide synthase (nNOS) inhibition with 7-Nitroindazole (7-NI) in dose 2 $mg.kg^{-1}$.h. Plasma renin activity (PRA) was determined by radioimmunoassay kit (DiaSorin). The urine flow rate was measured gravimetrically; sodium concentration was determined by flame photometry.

RESULTS: In SHR 7-NI decreased urine flow rate ($p<0.01$). The nNOS inhibition did not alter ABP and sodium excretion in both experimental groups. The PRA decreased after nNOS inhibition in Wistar rats as well as in SHR but in SHR decrease of PRA was attenuated ($p<0.05$).

CONCLUSION: The role of NO, produced by nNOS in the regulation of PRA in SHR is attenuated in comparison to its role in normotensive rats. This may by a result of involving of different mechanisms responsible for hypertension.

Key words: plasma renin activity, nNOS, SHR

INTRODUCTION

The renin-angiotensin system is a major factor in the regulation of renal sodium and water excretion, and therefore, in maintaining total body sodium and total body water. The renin-angiotensin system also maintains mean arterial blood pressure by controlling total body water and by the vasoconstrictor action of Ang II (1). In this way mean arterial blood pressure as well as sodium and water homeostasis is one of the key elements of the renal renin release. Renin release is also controlled by renal sympathetic nerve activity and circulating catecholamines.

The various physiological stimuls may influence renin release (2, 3, 4). Evidence were accumulated that endothelial factors are also involved in the regulation of renin secretion in renal juxtaglomerular cells (4, 5). It has been established, that nitric oxide (NO) participate in the regulation of renin secretion through various pathways. In most instances, NO appears as a tonic enhancer of renin secretion, acting via inhibition of cAMP degradation through the action of cGMP (5).

Nitric oxide (NO) generated from a neuronal or type 1 constitutive nitric oxide synthase (nNOS) that is heavily expressed in macula densa cells (MD) is activated during MD solute reabsorption (6). It normally counteracts the tubuloglomerular feedback (TGF) mediated vasoconstriction (6, 7). In spontaneously hypertensive rats (SHR), tubuloglomerular feedback activity is enhanced compared with that in age-matched normotensive Wistar-Kyoto rats (8, 9). It has been suggested that the renin-angiotensin system is responsible for the enhanced tubuloglomerular feedback activity in young SHR (10). On the other hand the blunting effect of MD-derived NO on tubuloglomerular feedback response is impaired in SHR (11). The established defect in NO generation in the juxtaglomerular apparatus could contribute to the heightened tubuloglomerular feedback responses enhanced

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renal vascular resistance, and hypertension (12). It has been suggested possible role of renal neuronal nitric synthase (nNOS) to mediate beta-adrenergic control of renin secretion (13). The available experimental data for the relationship between nNOS and renin secretion is controversial.

Therefore, the aim of present study was to investigate the role of nNOS derivate nitric oxide in the regulation of plasma renin activity in spontaneously hypertensive rats.

MATERIALS AND METHODS

Experiments were carried out on normotensive Wistar rats (W, n=8) and on spontaneously hypertensive rats (SHR, n=8) at the same age of 12-14 weeks; male in sex. The study was performed in accordance with the Convention on Animal Protection. The animals were housed under standard condition: constant temperature 22°C; 12/12 h light /dark cycle; free access to standard rat chow and tap water. In the SHR group were included only rats with systolic arterial pressure over 170 mmHg previously measured noninvasive by tile cuff method (Ugo-Basile). One day before experiments under general anesthesia (Nembutal – Sigma, in dose 35 mg/kg b.w., i.p.) femoral artery for a continuous blood pressure measurement and for withdrawal of blood for determination of plasma renin activity as well as femoral vein for drug application was catheterized. To avoid clotting the catheters were preliminary flushed and after that filed with 20 IU/ml heparin in sterile saline. Through a small suprapubic incision a modified polyethylene catheter was inserted in the bladder for urine collection in the short time intervals. The catheters were tunneled subcutaneously and exteriorized at the back of the neck. The experiments were performed on conscious freely moving animals 24 hours after surgical intervention. In the both experimental groups the arterial blood pressure registration and urine collection were performed during two 40 min long lasting periods; control period and 20 min after selective neuronal nitric oxide synthase inhibition. The selective neuronal nitric oxide synthase inhibition was achieved by intravenous infusion of 7-Nitroindazole (7-NI) in dose 2 mg. kg^{-1}. h (14) for 40 min. Arterial blood pressure wave was registered by Gould Statham transducer P23ID connected to computerized data acquisition system Biopac MP100WS through arterial catheter. Systolic (SAP), diastolic (DAP) and mean arterial blood pressure (MAP) were calculated from blood pressure wave by AcqKnowledge 3.8.software. The urine flow rate was determined gravimetrically. The urine concentration of sodium was measured by flame photometry (Corning 410C) and sodium excretion was calculated (U_{Na} .V). Blood samples needed for the determinations of plasma renin activity (PRA) were collected through arterial catheter in EDTA rinsed tubes on ice at the end of experiments. Blood samples were centrifuged and plasma was stored at -20 °C until assayed. PRA was measured by radioimmunoassay (RIA) kit (DiaSorin).

All results were present 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

In SHR systolic, diastolic and mean arterial blood pressure was significantly higher compared to normotensive rats: SAP was 182.4±3.4 vs. 134.3±3.2 mmHg; DAP was 112.82±4.43 vs. 85.61±3.69 mmHg; DAP was 139.27±4.19 vs. 104.34±3.30 mmHg ($p<0.01$). Application of 7-NI did not change SAP, DAP and MAP in normotensive rats, as well as in SHR.

There were no differences in the urine flow rate, sodium excretion and PRA between normotensive and spontaneously hypertensive animals **(Fig. 1)**.

The 7-NI did not change urine flow rate in normotensive rats (4.58±0.72 vs. 4.38±0.69 µl $.min^{-1}$.100 g b.w.), but in SHR caused decrease in urine flow rate from 4.78±0.69 to 2.70±0.45 µl$.min^{-1}$.100 g b.w., ($p<0.01$). The sodium excretion did not change neither in Wistar nor in SHR after nNOS inhibition. In Wistar rats U_{Na}.V was 252.07±15.97 nmol$.min^{-1}$ .100 g b.w. in control period and 239.25±27.8 nmol $.min^{-1}$.100 g b.w. during 7-NI infusion. In SHR, in control period and during 7-NI infusion U_{Na}.V was 262.11±58.9 and 281.96±25.99 nmol$.min^{-1}$.100 g b.w. respectively.

The 7-NI decreased PRA in the normotensive rats from 11.07±1.94 to 2.16±0.60 ng $.ml^{-1}$. h, ($p<0.01$) as well as in the spontaneously hypertensive rats from 13.31±2.03 to 6.44±1.72 ng $.ml^{-1}$.h, ($p<0.05$), **(Fig.1)**. In

Wistar rats the decrease of PRA after 7-NI application was more clearly demonstrated compared to SHR. The 7-NI decreased plasma renin activity in Wistar rats by 80.5±5.4% and by 51.6±9.6% in SHR, $p<0.05$.

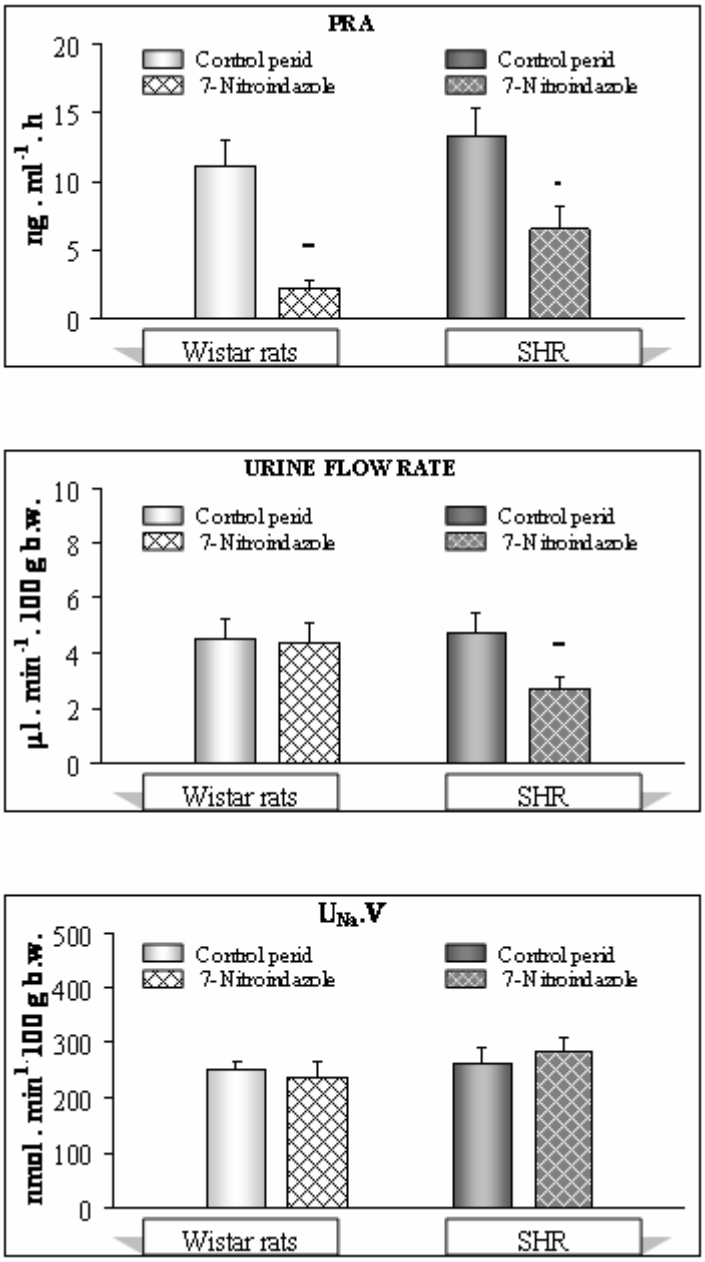

Figure 1. Plasma renin activity (PRA), urine flow rate and sodium excretion ($U_{Na}.V$) in normotensive Wistar rats as well as in spontaneously hypertensive rats (SHR) in control period and after nNOS synthase inhibition with 7-Nintroindasole (7NI), applied i.v. in dose 2 $mg.kg^{-1}.h^{-1}$)
* ($p<0.05$); ** ($p<0.01$) - shows statistically significant effects as a result of intravenously application of 7-Nintroindazole compared to control value

DISCUSION

In our experiment we examined the effect of application of selective nNOS synthase inhibitor 7-NI on plasma renin activity. Considering the marked systemic side effects of prolonged administration of NOS inhibitors, in vivo experiments, with acute or subchronic administration of NOS inhibitors are likely to be more informative about the physiological influence of NO on renin secretion (5). Our results show that urine flow rate, sodium excretion and PRA did not differ between normotensive and hypertensive animals in control conditions. Similar baseline values of PRA, urine flow rate and water and sodium excretion in Wistar and SHR were reported in

the investigations of Bolterman et al. (15) and Arendshort W.J. et al. (16). One possible explanation about lack of difference despite the higher blood pressure in SHR is higher expression of nNOS in the renal medulla of the SHR compared with Wistar rats. It is proposed that the higher expression of the neuronal isoform in the medullary tubular cells is a protective mechanism aimed to improve renal function of SHR (17).

Our data show that acute selective inhibition of nNOS with 7-NI greatly decrease PRA in Wistar rats about fivefold and did not change urine flow rate and sodium excretion. The situation in the SHR is somewhat different. The 7-NI decrease PRA about twofold, caused decrease in urine flow rate about two fold and did not change sodium excretion.

The difference in response of PRA to nNOS inhibition in SHR presumably involves nNOS that is located in the macula densa. It has been established dissociation between nNOS expression and response to NOS inhibition in the juxtaglomerular apparatus of SHR. This data demonstrate a defect in nNOS function in the juxtaglomerular apparatus of SHR, despite abundant transcriptional and translational expression (18).

One possible reason for established in our experiment decrease of urine flow rate in SHR after selective nNOS inhibition could have been greater fluid reabsorption occurred at distal parts of the nephron. There is evidence indicating that expression of medullary aquapurine (1-3) proteins is increased in the kidney in SHR compared with that in Wistar rats. This is associated with enhanced activity of arginine vasopressin/cAMP pathway (19). On the other hand endogenous NO tonically inhibits the activity of arginine vasopressin producing neurons (20). After nNOS inhibition this course of action of NO was eliminated.

Nitric oxide appears to act more as a general enhancer than a specific stimulator of renin secretion, since NOS inhibition interferes with all classic regulators of the renin system. It is difficult to separate direct and indirect effects of NO on the renin system. For example an effect of NOS inhibitors in vivo is the well-known elevation in blood pressure, and since renal renin secretion and renin gene expression are inversely related to the renal perfusion pressure, increases in blood pressure could account for the effect of NOS inhibitors on the renin system. The experiments suggest that changes in blood pressure may contribute to, but do not essentially mediate, the effects of NOS inhibitors on the renin system (5). In our experiments selective blockade of nNOS did not change arterial blood pressure, therefore the impact of pressure-dependent effect on renin release has been excluded.

Studies in intact SHR kidney suggest a diminished role for NO in tubular and vascular regulation and enhanced TGF responses. That has been ascribed to diminish blunting by macula densa-derived NO generated from nNOS. It has been suggested that NOS inhibition may alter proximal reabsorption and delivery of sodium to the macula densa and that this was a prominent factor in the change in renin secretion (21). The NOS inhibitors also attenuated renin secretion stimulated by β-adrenoreceptors or by macula densa blockade (22). It has been proved that SHR have increased sympathetic nerve activity (23) compared to normotensive rats. We hypothesize that in SHR diminished response in PRA to nNOS inhibition could possibly be a result of disability of NO to exhibit its inhibitory influence on enhanced renal sympathetic nerve activity.

We concluded that nitric oxide derived by nNOS participates in the regulation of plasma renin activity in normotensive as well as in spontaneously hypertensive rats without changing blood pressure and sodium excretion. The role of nitric oxide, produced by nNOS in the regulation of plasma renin activity in spontaneously hypertensive rats is attenuated in comparison to its role in normotensive rats. This may by a result of involving of different mechanisms responsible for development and establishment of hypertension.

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