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Original Contribution

PLASMA RENIN ACTIVITY DURING SELECTIVE OR NONSELECTIVE NITRIC OXIDE SYNTHASE INHIBITION AFTER BILATERAL RENAL DENERVATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

The aim of the present study was to investigate the role of interaction between renal nerves and nitric oxide produced by different nitric oxide synthases in the regulation of plasma renin activity (PRA) in conscious spontaneously hypertensive rats.

Experiments were carried out on conscious normotensive Wistar rats and spontaneously hypertensive rats (SHR) with intact renal nerves and after bilateral renal denervation at age 12-14 weeks, male in sex. One day before experiments femoral artery for a direct blood pressure measurement and femoral vein for drug application were catheterized. The arterial blood pressure registration was performed during two 40 min long lasting periods: control period and 20 min after nonselective or selective neuronal nitric oxide synthase inhibition. The nonselective NO-synthase inhibition was achieved by intravenous infusion of L-NAME in dose 10 mg/kg b.w. The selective neuronal nitric oxide synthase inhibition was achieved by intravenous infusion of 7-Nitroindasole in dose 2 $mg.kg^{-1}$. h.

Our data suggest that renal nerves alone in SHR did not play a significant role in the regulation of plasma renin activity. Renal nerves compensate lack of effect of NO, produced by all NOS isoforms in regulatory mechanism of PRA in SHR. The NO, produced only by nNOS is a key factor in the regulation of PRA in SHR and its effects are independent of renal nerves activity.

Key words: plasma renin activity, renal nerves, nitric oxide, nNOS, SHR

INTRODUCTION

The renin-angiotensin system plays an important role for the maintaining of the blood pressure, volume and electrolyte homeostasis in organism. The activity of the renin-angiotensin system in the circulation is mainly depended on the activity of the protease renin, which is a key regulator of the system. Renin synthesis and secretion is modulated by multiple systemic factors and local control mechanisms (1). The attention to the nitric oxide (NO) as a possible physiological regulator of renin secretion is provoked of finding, that main site of renin production, the renal juxtaglomerular region is identified with high capacity for NO formation (2). The high level of expression of neuronal nitric oxide synthase (nNOS) has been established in macula densa cells, whereas expression of endothelial nitric oxide synthase (eNOS) has been found in endothelial cells of the afferent arterioles (3). It is already well established that NO formed within the kidney acts a potential vasodilatator that determined the basal tone of the renal arteriole independently of the myogenic autoregulation of renal blood flow (4). The evidence for functional role for NO produced by juxtaglomerular cells has been determined by studying of tubuloglomerular feedback system. Endogenous NO significantly modulates the

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feedback response by exerting a specific buffer function (5, 6). It has been established that SHR have increased tubuloglomerular feedback (TGF) and diminished role for NO produced from nNOS for the regulation of TGF (7). It is commonly accepted that the renal sympathetic nerves play an important role in the plasma renin activity regulation. It is well accepted that renal sympathetic nerve activity is elevated in SHR compared to normotensive rats and that increased renal sympathetic nerve activity contributes to the development of hypertension (8).

The aim of the current study was to investigate the role of interaction between NO produced by the different isoforms of NOS and renal symphathetic nerves in the regulation of PRA in SHR.

MATERIALS AND METHODS

The experiments were carried out on conscious, male, normotensive Wistar rats (W) and spontaneously hypertensive rats (SHR) at the same age 12-14 weeks. In the SHR group were included only rats with systolic arterial pressure over 170 mmHg, previously measured noninvasive by tile cuff method (Ugo-Basile). The study was performed in accordance with the Convention on Animal Protection. Experiments were performed on animals with intact renal nerves and on billateraly renal denervaded rats. Bilateral renal denervation was performed by standard procedure (8). Each kidney was exposed by a dorsal flank incision. The kidneys were denervated by striping all visible nerves, fat and connecting tissue from the renal vessels, and by coating the renal arterial wall with 10% phenol in ethanol solution to ensure the destruction of any remaining nerves. One week after bilateral renal denervation was allowed for the rats to recover. One day before experiments the catheters (Portex) were placed into right femoral artery and vein for blood pressure measurement and drugs application respectively. All surgical preparations were performed under general anesthesia with Nembutal in dose 35 $mg.kg^{-1}$ b.w., applied intraperitoneally. Nonselective NO-synthase inhibition was achieved by intravenous bolus injection of 10 $mg.kg^{-1}$ b.w. N^{ω}-Nitro-L-arginine-methyl ester (L-NAME). The selelective neuronal nitric oxide synthase inhibition was achieved by intravenous infusion of 7-Nitroindasole (7-NI) in dose 2 mg . kg^{-1}. h.

The normotensive Wistar rats (W) as well as spontaneously hypertensive rats (SHR) were divided into the following groups: with intact renal nerves W (n=10) and SHR (n=10); rats subjected to bilateral renal denervation: WD, (n= 10) and SHRD (n=10); rats with intact renal nerves and nonselective nitric oxide synthase (NOS) inhibition: W+L-NAME, (n=9) and SHR+L-NAME (n=9); rats with intact renal nerves and selective neuronal nitric oxide (nNOS) inhibition: W+7NI, (n=9) and SHR+7NI, (n=9); rats subjected to bilateral renal denervation and nonselective nitric oxide inhibition: WD+L-NAME, (n=9) and SHRD+L-NAME, (n=9); rats subjected to bilateral renal denervation and selective nitric oxide (nNOS) inhibition: WD+7NI, (n=9) and SHRD+7NI, (n=9).

The experiments were performed on freely moving conscious animals 24 hours after catheters implantation. In all experimental groups, blood pressure wave was monitored during 40 min control period, 20 min equilibration and 40 min experimental period. 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) calculated from blood pressure wave by AcqKnowledge 3.8. software. Blood samples needed for the determinations of plasma renin activity (PRA) were collected through arterial catheter in EDTA rinsed tubes on ice on 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 were significantly higher ($p<0.01$) in comparison to normotensive rats, **(Table 1)**. Bilateral renal denervation did not affect arterial blood pressure in both

normotensive and hypertensive rats. Nonselective NO-synthase inhibition increased SAP, DAP and MAP in the normotensive rats and in SHR ($p<0.01$) as well in bilaterally renal denervated normotensive rats and SHR, ($p<0.01$**), (Table 1).** Plasma renin activity did not differ between normotensive rats and SHR (11.07 ± 1.24 ng.ml $^{-1}$. h vs. 13.31 ± 1.71 ng.ml $^{-1}$. h.). Bilateral renal denervation decreased PRA only in Wistar rats to 7.05 ± 0.86 ng/ml^{-1}.h, ($p<0.05$), **(Fig. 1).** Nonselective nitric oxide synthase inhibition by L-NAME decreased PRA in normotensive Wistar rats with intact renal nerves by 72%, ($p<0.01$), **(Fig. 1)** as well in Wistar denervated rats by 73%, ($p<0.01$). In SHR with intact renal nerves L-NAME application did not change PRA, but in SHR with bilateral renal denervation L-NAME decreased PRA by 76%, ($p<0.01$), **(Fig. 1).** The selective nNOS inhibition, achieved by application of 7-NI did not change SAP, DAP and MAP in normotensive and spontaneously hypertensive rats with intact renal nerves as well as in bilaterally renal denervated rats. The 7-NI application led to decrease of PRA in all experimental groups. PRA decreased in normotensive rats by 82%, ($p<0.001$); in Wistar rats with bilateral renal denervation from 7.05 ± 0.86 ng.ml $^{-1}$. h to 0.48 ± 0.12 ng.ml^{-1}. h, ($p<0.01$); in SHR with intact nerves from by 48%, ($p<0.05$) and in SHR with bilateral renal denervation from 11.08 ± 2.25 to 3.82 ± 0.97 ng.ml^{-1}. h, ($p<0.05$), **(Fig. 1).**

Table 1. *Systolic (SAP) diastolic (DAP) and mean (MAP) arterial blood pressure in normotensive Wistar rats (W) and spontaneously hypertensive rats (SHR) with intact renal nerves, after bilateral renal denervation (WD; SHRD) and during nonselective inhibition of nitric oxide synthase by i.v. bolus application of N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) in dose 10 mg.kg $^{-1}$ b.w., as well as selective inhibition of neuronal nitric oxide synthase (nNOS) with 7-Nitroindazole (7-NI), applied i.v. in dose 2 mg.kg $^{-1}$. h.*

	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)
W	133.8±1.6	84.8±1.7	104.6±1.4
W+L-NAME	156.9±3.4**	112.2±3.6**	129.7±3.3**
W+7-NI	134.8±2.1	86.1±1.6	105.3±1.7
SHR	185.1±3.5 ♦♦	120.5±3.1 ♦♦	148.0±3.1 ♦♦
SHR+L-NAME	198.9±4.7**	139.7±4.7**	163.8±3.9**
SHR+7-NI	184.1±3.4	117.7±3.2	144.1±3.7
WD	137.3±1.6	88.4±2.9	107.3±2.8
WD+L-NAME	153.9±4.1**	106.0±3.8**	125.62±4.0**
WD+7-NI	136.3±1.7	86.8±2.8	104.1±1.9
SHRD	181.76±1.6	121.03±3.3	146.7±2.6
SHRD+L-NAME	203.0±6.0**	140.3±3.8**	165.1±3.9**
SHRD+7-NI	183.6±3.1	121.6±2.6	142.3±2.8

** $p<0.01$ – statistically significant effect as a result of L-NAME application in comparison to control values

♦♦ $p<0.01$ – statistically significant differences between normotensive Wistar rats and spontaneously hypertensive rats (SHR)

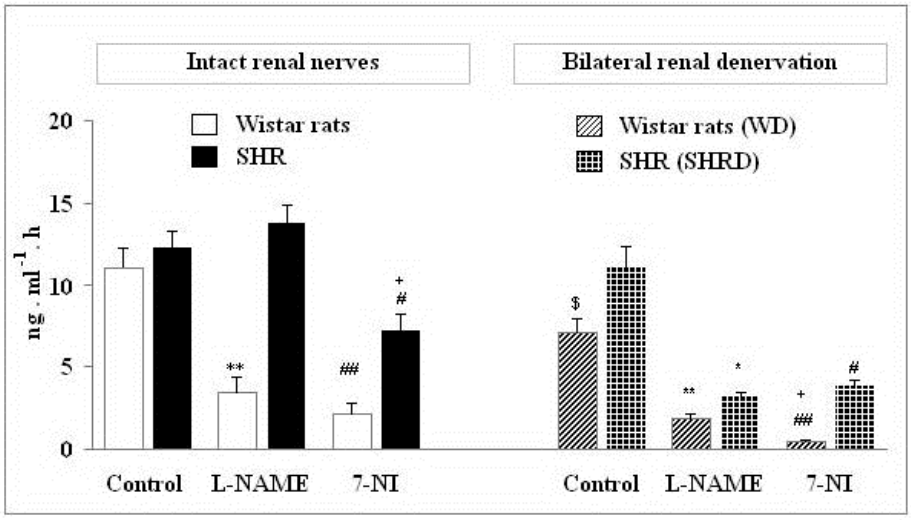

Figure 1. Plasma renin activity in normotensive and spontaneously hypertensive rats with intact renal nerves, after bilateral renal denervation and during nonselective inhibition of nitric oxide synthase (NOS) by i.v. bolus application of N^{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) in dose 10 mg.kg $^{-1}$ b.w., as well as selective inhibition of neuronal nitric oxide synthase (nNOS) with 7-Nitroindazole (7-NI), applied i.v. in dose 2 mg.kg $^{-1}$. h.

* $p<0.05$; ** $p<0.01$ – statistically significant effect as a result of L-NAME application in comparison to control values

# $p<0.05$; ## $p<0.01$ – statistically significant effect as a result of 7-NI application in comparison to control values

$ $p<0.05$- statistically significant effect of bilateral renal denervation

\+ $p<0.05$ – statistically significant differences between effects provoked by selective and nonselective NOS inhibition

DISCUSION

The available experimental data about physiological role of nitric oxide (NO) in the control of renal renin synthesis and secretion are controversial (9, 10). In the present series of experiments we studied the influence of NO on the regulation of plasma renin activity and try to clarify which isoform of NOS plays important role in this regulation in SHR. We also investigated the importance of NO produced by different NOS and renal sympathetic nerves in the regulation of level of the plasma renin activity. NO has attracted considerable interest as a possible controller of renin secretion, since both macula densa cells and endothelial cells are sites of substantial NO formation (1, 3). In our experiments nonselective inhibition of NO did not change PRA in SHR in comparison to normotensive rats in which PRA decreased as a result of NOS inhibition. We presume that the absence of NO production in SHR as a result of nonselective NOS inhibition is equilibrated through the participation of other physiological mechanism included in the regulation of PRA. It is a possible that pressure sensitive mechanism (11) is involved in the compensation of lack of NO in the regulation of PRA, because nonselective NOS inhibition led to fast and sustained increase of blood pressure. However, as a result of NOS inhibition arterial blood pressure increased in both normotensive and spontaneously hypertensive rats but PRA decreased only in normotensive Wistar rats. These results suggested that pressure sensitive mechanism is not responsible for compensation the lack in the regulation action of NO on PRA in SHR. It is well accepted that the sympathetic renal nerves activity is another significant control factor in the regulation of renin release (11). The sympathetic nervous discharge to the kidney stimulates the renin secretion via β-adrenergic receptors on the juxtaglomerular cells (12). Indeed, in normotensive Wistar rats bilateral renal denervation decreased plasma renin activity. We examined if the established increased symphathetic nerve activity in SHR (13) was able to compensate the lack of stimulation effects of NO on the PRA. After bilateral renal denervation nonselective inhibition of NOS in SHR decreased PRA. This

result indicated that renal sympathetic nerves compensate absence of NO production in regulation of PRA in SHR. Interestingly bilateral renal denervation did not change the PRA in SHR alone. We suggested that renal nerves and nitric oxide in SHR interplay in the regulation of PRA. It is known that NO produced by nNOS affects the renal sympathetic nerve activity on pre- or postjunctional level (14). On the other hand it is established that there is an increased expression of nNOS in the juxtaglomerular apparatus, which appears to be a main site of renin production (2). In SHR it has been established enhanced tubuloglomerular feedback mechanism and a diminished buffering role of nNOS derivated NO (7). In this connection we investigated the role of nNOS produced NO on the level of PRA in SHR as well as its interaction with the renal nerves. Our results displayed that in contrast to the nonselective inhibition of NOS, selective nNOS inhibition in SHR decreased the plasma renin activity in both intact and renal denervated SHR. This data lead us to the conclusion, that NO produced by nNOS participates in the regulation of PRA without the involvement of renal nerves.

CONCLUSION

1. The renal nerves alone in SHR did not play a significant role in the regulation of plasma renin activity however they compensate the lack of NO production from all NOS isoforms in the mechanisms that regulate the plasma renin activity in SHR.
2. The nitric oxide, produced only by nNOS is a key factor in the regulation of PRA in SHR and its effects are independent from the renal nerves activity.

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