



*Original Contribution*

**EFFECT OF RENAL NERVES AND NITRIC OXIDE ON PLASMA RENIN IN SPONTANEOUSLY HYPERTENSIVE RATS**

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**ABSTRACT**

We investigated whether renal nerves and nitric oxide (NO) act together in maintenance of plasma renin activity (PRA) in spontaneously hypertensive rats (SHR). The experiments were performed on conscious male normotensive Wistar rats (W) and SHR with intact renal nerves and after bilateral renal denervation (BRD). NO-synthase inhibition (NOSI) was achieved by intravenous L-NAME. Arterial blood pressure (ABP) was measured directly. Plasma renin activity (PRA) was determined using radioimmunoassay. ABP in SHR was higher compared to W ( $p < 0.01$ ), but heart rate (HR) did not differ. BRD did not affect ABP or HR in both W and SHR. NOSI increased ABP and decreased HR in W and in SHR ( $p < 0.01$ ) as well as in W and SHR after BRD ( $p < 0.01$ ). PRA did not differ between W and SHR. BRD decreased PRA only in W ( $p < 0.05$ ). NOSI decreased PRA in W ( $p < 0.01$ ) as well as in W denervated rats ( $p < 0.01$ ). In SHR, NOSI did not change PRA, but in renal denervated SHR, NOSI decreased PRA ( $p < 0.01$ ). NO influences PRA without involving renal nerves in normotensive rats, but in SHR renal nerves act with NO in the maintenance of PRA.

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

**INTRODUCTION**

Renin is a proteolytic enzyme released from the juxtaglomerular cells of the kidney. The determination of plasma renin activity (PRA) has been widely adopted to evaluate the renin-angiotensin system in disease state. Different factors are involved in the regulation of renin secretion: pressure sensitive mechanism, macula densa mechanism and sympathetic nervous discharge to the kidney.

Development of hypertension is associated with activation of multiple neuroendocrine systems, including the renin-angiotensin (1) and nitric oxide systems (2). Different studies have been performed to define the regulatory factors that participate in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR). 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 (3). It has been shown that despite normal PRA in SHR, renin-angiotensin system is involved in pathogenesis of hypertension (4). The critical role in pathogenesis of hypertension in SHR is played by the decreased bioavailability of NO and the reduced eNOS activity (5). It has been established that in addition to its vasodilator activity, NO influenced renin release (6, 7).

The aim of this study was to determine whether renal nerves, which are an important factor in the renin synthesis, act in concordance with nitric oxide in the maintenance of plasma renin activity in spontaneously hypertensive rats.

**MATERIALS AND METHODS**

Experiments were performed on normotensive Wistar (W) and spontaneously hypertensive rats (SHR) of the same age, 14-16 weeks. The study was performed in accordance with the Convention on Animal Protection. Normotensive and spontaneously

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hypertensive rats were studied with intact renal nerves (n=10) and 7 days after bilateral renal denervation (BRD), (n=10), which was performed after flank incision and dissection of renal vessels. All visible nerves, supplying the kidney and around the renal artery, renal vein and ureter, were cut. These structures were then stained with 10% phenol in ethanol solution. One week was allowed for the rats to recover. Polyethylene catheters (Portex) were placed into right femoral artery and vein for blood pressure measurement and drugs application respectively, 24 hours before experiments. All surgical preparations were performed under general anaesthesia with Nembutal in dose 35 mg/kg b.w. applied intraperitoneally.

In all experimental groups, blood pressure wave was monitored during 40 min control period, 20 min equilibration and 40 min experimental period. NO-synthase inhibition (NOSI) was achieved by intravenous bolus injection of 10 mg/kg N<sup>o</sup>-Nitro-L-arginine methyl ester (L- NAME). 20 min after L- NAME injection, the effects of NOSI were studied in the experimental period. Arterial blood pressure registration was performed through arterial catheter by using blood pressure transducer Gould Statham P23ID, connected to data acquisition system Biopac MP100WS. Systolic (SAP), diastolic (DAP), mean arterial blood pressure (MAP), and heart rate (HR) were calculated from blood pressure wave by *AcqKnowledge* 3.8 software. Blood samples needed for the measurements of PRA were collected through arterial catheter in EDTA coated tubes on ice at the end of the experiments. All blood samples were centrifuged and plasma was stored at -20°C until assayed. PRA was measured with radioimmunoassay (RIA) kit (DiaSorin).

Data are presented as mean  $\pm$  SEM and the significance of the differences between groups was assessed using unpaired t-test.  $p < 0.05$  was considered significant.

## RESULTS

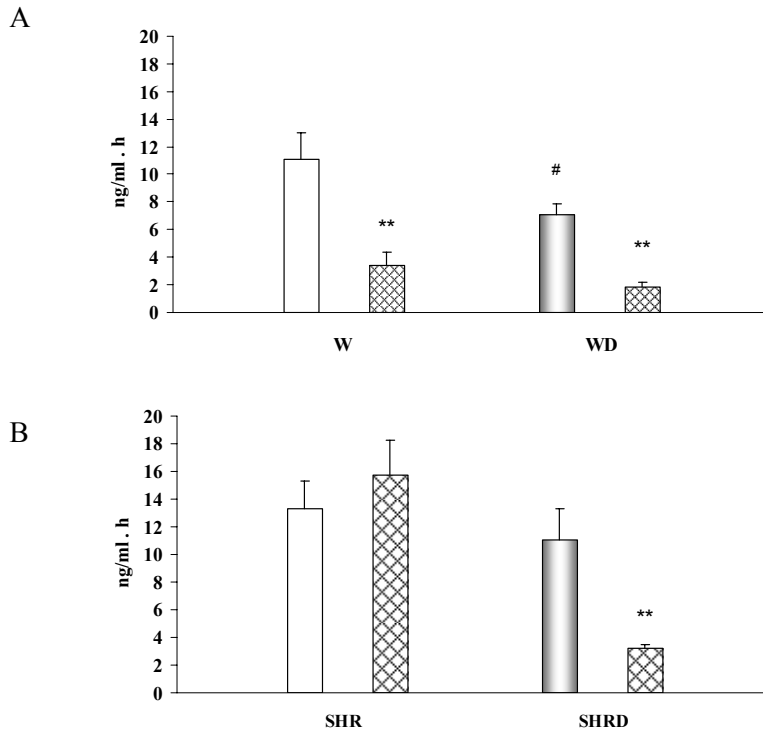
SAP, DAP, and MAP values in SHR were higher compared to normotensive Wistar rats ( $p < 0.01$ ). HR did not differ between normotensive and hypertensive animals (**Table 1**). BRD did not affect arterial blood pressure or HR in both normotensive and hypertensive rats. NOSI increased SAP, DAP and MAP and decreased HR in the

normotensive rats and in SHR ( $p < 0.01$ ) as well as in bilaterally renal denervated normotensive rats and SHR ( $p < 0.01$ ). PRA did not differ between normotensive and spontaneously hypertensive rats ( $11.07 \pm 1.94$  v.s.  $13.31 \pm 2.03$  n.s.). BRD decreased PRA only in Wistar rats to  $7.05 \pm 0.86$ ,  $p < 0.05$  (**Fig.1**). NOSI led to a decrease of PRA in normotensive rats with intact renal nerves to  $3.41 \pm 0.92$ ,  $p < 0.01$  (**Fig.1A**) as well as in Wistar denervated rats to  $1.86 \pm 0.32$ , ( $p < 0.01$ ). In SHR with intact renal nerves (**Fig.1B**) NOS inhibition did not change PRA ( $13.31 \pm 2.03$  v.s.  $15.78$ , n.s.), but in SHR with renal denervation NOS inhibition decreased PRA (from  $11.08 \pm 2.25$  to  $3.19 \pm 0.29$ ,  $p < 0.01$ ).

## DISCUSSION

In the present study we attempted to assess whether the effects of renal nerves on the PRA are accomplished by involving nitric oxide system in hypertensive state. Our results are in accordance with previously published data (4, 7), which also established the same level of PRA in SHR and normotensive rats. It was shown that high blood pressure in SHR is associated with a modest increase of baseline levels of Ang II and Ang II generating activity, in the absence of an increase of PRA. One possible explanation for this could be that "normal" PRA coinciding with high blood pressure may actually be inappropriately high. It was determined that administration of ACE inhibitors decreased blood pressure only in SHR but increased PRA in both SHR and normotensive rats. This suggested that in SHR exists imbalance between high blood pressure and activity of renin-angiotensin system.

Sympathetic nervous discharge to the kidney stimulates renin secretion through  $\beta$ -adrenergic receptors on the juxtaglomerular cells (8). The renal denervation eliminates interference of renal nerves and thus denervated kidney functions as a control for the changes in PRA, caused by hormonal factors and blood pressure. In our study BRD did not affect PRA in SHR but led to a decrease of PRA in normotensive Wistar rats. Our results suggest that in SHR, the increased renal sympathetic nerve activity is not a primary mechanism in the regulation of PRA. Most probably after renal denervation in SHR, effects of some hormonal factors and pressure-dependent mechanisms, responsible for renin secretion, are more sensitive compared to normotensive rats.



**Figure 1.** Panel A: Effects of NOS inhibition on the plasma renin activity in normotensive Wistar rats with intact renal nerves (W) and in renal denervated Wistar rats (WD); Panel B: Effects of NOS inhibition on the plasma renin activity in spontaneously hypertensive rats with intact renal nerves (SHR) and in renal denervated SHR (SHRD)

\*\* Significant differences as a result of NOSI, ( $p < 0.01$ )

# Significant differences as a result of BRD, ( $p < 0.05$ )

**Table 1.** Effects of NOS inhibition (10 mg/kg b.w. L-NAME) on systolic (SAP), diastolic (DAP), mean (MAP) arterial blood pressure and heart rate (HR) in normotensive Wistar rats with intact renal nerves (W) and after bilateral renal denervation (WD) as well as in spontaneously hypertensive rats with intact renal nerves (SHR) and after bilateral renal denervation (SHRD)

	SAP mmHg	DAP mmHg	MAP mmHg	HR b.p.m.
W	136.3±2.0	82.7±1.7	103.9±1.7	369.7±9.4
W+L-NAME	156.9±3.4**	112.2±3.6**	129.7±3.3**	340.2±8.4**
WD	133.3±3.8	79.0±1.6	101.07±2.3	366.2±18.9
WD+L-NAME	153.9±4.1**	106.0±3.8	125.62±4.0	343.9±10.0
SHR	180.6±3.0 <sup>SS</sup>	109.5±3.7 <sup>SS</sup>	134.4±3.6 <sup>SS</sup>	370.0±16.8
SHR+L-NAME	198.9±4.7**	139.7±3.7**	163.8±3.9**	314.5±15.6**
SHRD	179.0±1.8	112.7±4.0	138.64±3.7	344.4±11.3
SHRD+L-NAME	203.0±6.0**	140.3±3.8**	165.1±3.8**	258.3±26.2**

\*\* Significant differences as a result of NOSI, ( $p < 0.01$ )

<sup>SS</sup> Significant differences between normotensive Wistar rats and spontaneously hypertensive rats (SHR) ( $p < 0.01$ )

Previous investigations have indicated that NO plays an important role in the regulation of renin release. Persson et al. (6, 9, 11) demonstrated that bolus injection of L-NAME inhibited pressure-dependent renin release in conscious dogs. On the other hand, in experiments with direct recordings of sympathetic nerve activity in conscious rats is demonstrated straightforward evidence for L-

NAME-induced sympathetic activation (10). In our study NOSI increased SAP, DAP and MAP in Wistar rats as well as in SHR but PRA was affected only in normotensive rats. In this case the increased sympathetic nerve activity in SHR balanced the absence of NO mediating effects on the renin release. In agreement with this opinion NOS inhibition after bilateral renal denervation in SHR

displays decreased PRA in the same manner as in normotensive rats. This indicates that renal sympathetic nerves in SHR, acting together with NO, play an important role in the maintenance of plasma renin activity.

### CONCLUSION

NO influences PRA without involving renal nerves in normotensive rats, but in SHR renal nerves act together with NO in the maintenance of PRA.

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