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Original Contribution

ARTERIAL BLOOD PRESSURE VARIABILITY DURING SELECTIVE nNOS INHIBITION AFTER BILATERAL RENAL DENERVATION IN CONSCIOUS RATS

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ABSTRACT

In the current study we investigate the participation of the NO, produced by neuronal nitric oxide synthase (nNOS) as well as its interaction with the renal nerves in the modulation of blood pressure variability in rats. The experiments were carried out on conscious, male Wistar rats divided in the four groups: with intact renal nerves; with intact renal nerves and nNOS inhibition; with bilateral renal denervation; with bilateral renal denervation and nNOS inhibition. The blood pressure wave was registered in control period and after nNOS inhibition through previously implanted in a. femoralis catheter. The selective nNOS inhibition was performed by i.v. infusion of 7-Nitroindazole (7-NI), in dose 2 mg . kg $^{-1}$. h. The systolic (SAP), diastolic DAP and mean (MAP) arterial blood pressure spectrograms were derived in graphical programming environment Lab View 3.1.1. In the spectrograms, the spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band typical for rats (20-195; 195-605; 605-3000 mHz, respectively) were studied. Application of 7-NI changed only P_{HF} in SAP spectrograms in rats with intact renal nerves. However, nNOS inhibition in bilaterally renal denervated rats provoke a decrease of P_{LF} in SAP and MAP spectrograms ($p<0.05$), related to different humoral influences, as well as decreased mainly sympathetic mediated variations (P_{MF}). The interaction between renal nerves and nNOS produced nitric oxide play a significant role in the regulation of blood pressure variability in Wistar rats.

Key words: blood pressure variability, renal nerves, nitric oxide, nNOS, rats

INTRODUCTION

Blood pressure variability has been identified as an independent cardiovascular risk factor (1, 2). On the other hand the patterns of blood pressure variability may provide important insights into cardiovascular regulation (3, 4, 5). Three prominent peaks of power were frequently observed on the arterial blood pressure spectrum in different frequency bands, namely high, mid and low frequency components. High frequency (HF 0.6-3.00 Hz in rats) blood pressure variability linked to respiration has been suggested to involve fluctuations in cardiac output of purely mechanical origin (4), secondary to respiratory sinus arrhythmia (3). Mid frequency (MF) blood pressure fluctuations (0.2– 0.6 Hz in rats), the so-called Mayer waves, have been associated mostly with sympathetic modulation of vascular tone (6, 7, 8). Low frequency (LF) blood pressure oscillations (0.02– 0.2 Hz in rats) are related to a variety of factors, including catecholamines (9), the renin-angiotensin system (10, 11), heat stress (12). It has been established that myogenic vascular function also contributes to LF blood pressure variability in rats (13).

Nitric oxide (NO) is produced by the transformation of L-arginine to L-citruline trough catalysis by nitric nitric oxide synthase (NOS), (14). Three isoforms of NOS were discovered in the modulation of cardiovascular

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function: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) nitric oxide synthase (15). In control conditions eNOS and nNOS are constitutively expressed. The eNOS is predominantly present in endothelial cells (16). The nNOS expression was found in the brain and perivascular nerves (17, 18). The nNOS was detected also in non-neuronal cells including cardiomyocytes, endothelial cells, vascular smooth muscle cells, and skeletal muscle cells (19, 20, 21). It has been demonstrated a high level of nNOS protein in the kidney macula densa cells (22). Previous experience showed that the nitric oxide formed through nNOS in the brain neurons has a significant role in the regulation of cerebral functions as a neuromodulator or neurotransmitter (23, 24). Furthermore, the nitric oxide formed through nNOS in autonomic non-adrenergic, non-cholinergic inhibitory nerve, termed nitrergic, innervating vascular and other smooth muscles contributes to regulating muscular tone and contractility (20, 21). There is a great number of experimental data about the participation of NO generated in endothelia cells in the regulation of arterial blood pressure as well as in the modulation of fast oscillation of cardiovascular parameters (15, 13). However little is known about the involvement and role of NO produced by nNOS in the cardiovascular control and in regulation of blood pressure variability.

The renal sympathetic nerves participate in the regulation of arterial blood pressure by different mechanisms (25). It has been established that oscillations of blood pressure and sympathetic nerve is tightly coupled within a narrow range centered on 0.4 Hz in conscious rats (26). On the other hand it has been established that the renal sympathetic nerve activity modulated blood pressure oscillations in mid frequency spectral band (27). The sympathetic renal nerves are a main stimulus for activation of renin-angiotensin system. The available experimental data suggested interaction between renal nerves and NO produced by nNOS in kidney macula densa cell in the regulation of activity of renin-angiotensin system (28, 29). On the other hand it has been established that activity of renin-angiotensin system affected low frequency blood pressure oscillation (10, 11). The aim of our study was to investigate the role of NO produced by nNOS as well as its interaction with renal nerves in the regulation of blood pressure variability in conscious rats.

MATERIALS AND METODS

The experiments were carried out on conscious, male Wistar rats at age: 12-14 weeks. The animals were housed under standard conditions: constant temperature 22 ℃; 12/12 h light /dark cycle; free access to standard rat chow and tap water. The experiments were conducted in accordance with guidelines for the care and use of laboratory animals of the ethical commission at the Medical University - Sofia based on the Convention on Animal Protection.

The rats were divided in the following groups: with intact renal nerves (n=10); with intact renal nerves and selective inhibition of the neuronal nitric oxide synthase (n=10); with bilateral renal denervation (n=10); with bilateral renal denervation and selective inhibition of the neuronal nitric oxide synthase (n=10). The surgical preparations were performed under general anesthesia with pentobarbital sodium (Nembutal, Sigma) in dose 35 mg/kg b.w., applied intraperitoneally. Bilateral renal denervation (BRD) was performed through a flank incision. All visible nerves, supplying the kidney and around the renal artery and vein were stripped and then renal vessels were coated with 10% solution of phenol in ethanol. The bilateral renal denervated rats were allowed to recover for one week. One day before experiments under general anesthesia, polyethylene catheters (Portex) were inserted into the right femoral artery and vein for blood pressure measurement and drugs application respectively. The catheters were tunneled to the back of the neck and exteriorized. To avoid clotting the femoral and vein catheters were preliminarily flushed by 200 IU/ml heparin in sterile saline solution. The experiments were performed 24 hour after catheterization on conscious freely moving rats.

The blood pressure wave was monitored in 40 min control period and 20 min after selective neuronal nitric oxide synthase (nNOS) inhibition in the course of 40 min long experimental period. The selective neuronal nitric oxide synthase (nNOS) inhibition was performed by infusion of 7-Nitroindazole (7-NI), dissolved in 0.9% NaCl, in dose 2 $mg.kg^{-1}$. h, (30). During the control period infusion of vehicle as well as

in the equilibration and experimental period the infusion was performed in rate 25 μl/min.

The arterial blood pressure wave was registered directly in femoral artery by using blood pressure transducer Gould Statham P23ID, connected to computerized data acquisition system Biopac MP150WS. After analogue to digital conversion by peak and rate detector of the AcqKnowledge 3.8 software the values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure for each heart beat was determined. The obtained row data of investigated parameters was simultaneously re-sampled for 10 Hz. The SAP, DAP and MAP spectrograms were derived from 512 successive values in graphical programming environment Lab View 3.1.1, through Fast Fourier Transform (FFT) algorithm. In the spectrograms, the spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band typical of rats (20-195; 195-605; 605-3000 mHz, respectively) were studied.

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

The bilateral renal denervation (BRD) did not change mean values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure in Wistar rats. The selective nNOS inhibition by application of 7-NI did not provoke a change of mean values of arterial blood pressure in both Wistar rats with intact renal nerves and in rats with bilateral renal denervation, **(Table 1**).

The BRD in Wistar rats did not change fast oscillations in SAP, DAP and MAP, **(Figure 1).** In arterial blood pressure spectrograms of rats with intact renal nerves application of 7-NI provoked only decrease of P_{HF} in SAP spectrograms from 1.05±0.07 to 0.58±0.05 $mmHg^2$, ($p<0.05$). In rats with bilateral renal denervation 7-NI also decreased P_{HF} in SAP spectrograms from 1.02±0.24 to 0.44±0.07 $mmHg^2$, ($p<0.05$), but in addition provoked increase in P_{LF} from 2.46±0.06 to 3.53±0.46 $mmHg^2$, ($p<0.05$) and decrease in P_{MF} from 1.60±0.29 to 1.06±0.09 $mmHg^2$, ($p<0.05$). Selective nNOS inhibition also decreased P_{MF} in DAP and MAP spectrograms from 1.30±0.24 and 1.49±0.30 to 0.62±0.09 and 0.76±0.07 $mmHg^2$, respectively, ($p<0.05$).

Table 1. *Mean values of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure and heart rate (HR) in Wistar rats with intact renal nerves (W), in Wistar rats with bilateral renal denervation (WD) and during selective nNOS inhibition by 7-Nitroindazole (W+7NI); (WD+7NI) in dose 2 mg.kg^{-1}. h.*

	W	WD	W+7NI	WD+7NI
SAP (mmHg)	133.8±1.6	137.3±1.6	134.8±2.1	136.3±1.7
DAP (mmHg)	84.8±1.7	88.4±2.9	86.1±1.6	86.8±2.8
MAP (mmHg)	104.6±1.4	107.3±2.8	105.3±1.7	104.1±1.9

DISCUSSION

In the current study we investigated the participation of the NO, produced by nNOS as well as its interaction with the renal nerves in the modulation of blood pressure variability in rats. The available experimental data indicated that nNOS derivated NO participated in the regulation of microvascular tone (22, 23). Expression of nNOS has been established in the central nervous system (19, 20) and in the kidneys (24). These data suggest that nNOS derivated NO may participate in the regulation of arterial blood pressure as well as in its fast oscillation. Our data indicated that selective inhibition of nNOS in the course of 60 minutes in Wistar rats did not change mean values of arterial blood pressure neither its fast oscillations. The experimental data about the effect of selective inhibition of nNOS on arterial blood pressure are controversial. Some authors point out that the chronic inhibition of nNOS in rats led to increase of arterial blood pressure in rats (30, 31). Other experimental data, as well as, our experience did not establish a change in the blood pressure during the selective nNOS inhibition (32, 33). Differences of the effect of

nNOS inhibition on the mean values of arterial blood pressure may result from the different application method, dose and type of the nNOS inhibitors as well as the different experimental animal lines. It has been established that many factors are reflected in the LF fluctuations of arterial blood pressure (9, 12), including the activity of renin-angiotensin system (10, 11). The nNOS produced nitric oxide from macula densa cells in the kidney may affect the blood pressure variability by influencing the renin secretion (29). It has been established a buffer action of the renin-angiotensin system on LF spectral power (10, 11). Previously, we established a decreased plasma renin activity during the selective nNOS inhibition (34) in Wistar rats. However, in the present experiments nNOS inhibition did not affect the spectral power of LF oscillations in the arterial blood pressure spectrograms. We suggest that the level of decrease in the plasma renin activity is not sufficient to alter the LF spectral power of the arterial blood pressure or there is interference from other mechanisms and factors that compensate the absence of NO.

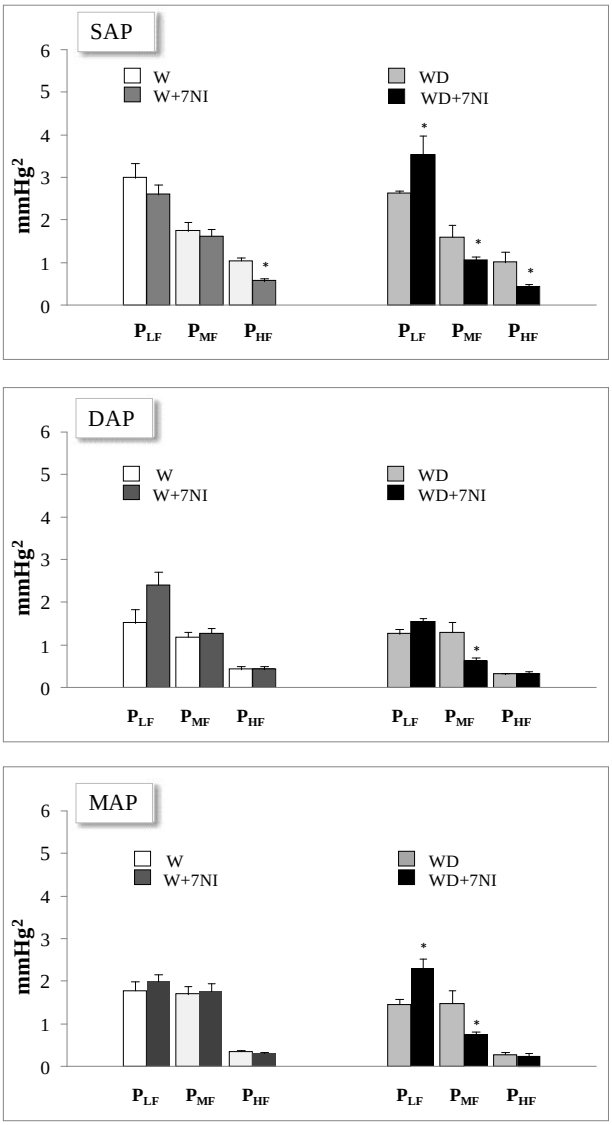

Figure 1. Spectral power distribution in low (P_{LF}), mid (P_{MF}) and high (P_{HF}) frequency band in spectrograms of systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure during selective neuronal nitric oxide synthase inhibition (nNOS) by intravenous application of 7-Nitoindazole (7NI) in dose 2 $mg.kg^{-1}.h$ in Wistar rats with intact renal nerves (W) as well in bilaterally renal denervated Wistar rats (WD).

* ($p<0.05$) show statistically significant effects as a result of selective nNOS inhibition

Another possible pathway of affecting the blood pressure variability by nNOS produced NO is its interaction with the sympathetic nerve activity. The available experimental data demonstrate tonic inhibition action of NO, produced by nNOS, on the sympathetic transmission (17). Our results show that the selective inhibition of nNOS in Wistar rats with intact renal nerves did not change significantly the power of sympathetically mediated variations (P_{MF}) in blood pressure spectrograms. One possible explanation for the absence of changes in the mainly sympathetic mediated mid frequency spectral power in arterial blood pressure spectrograms during the nNOS inhibition is the involvement of NO produced by eNOS in the mediation of MF blood pressure oscillations. From previous studies on the matter we established that nonselective NOS inhibition by L-NAME decreased the P_{MF} in arterial blood pressure spectrograms (35). This data indicates that NO participates in the generation of MF blood pressure fluctuations. Our result shows that nonselective nNOS inhibition in Wistar rats with intact renal nerves affected only P_{HF} in SAP spectrograms. The same effect of nNOS inhibition we observe in renal denervated rats. The variations of arterial blood pressure height frequency band involve fluctuations in cardiac output of purely mechanical origin (4), secondary to respiratory sinus arrhythmia (3). Neuronal NOS is found within cardiac myocytes, but its role in the control of cardiac function is not fully clarified (19). Our date indicate that NO produced by nNOS participate in the mediation of P_{HF} independently of renal nerves activity. In bilaterally renal denervated rats the selective nNOS inhibition decreased the MF spectral power in contrast to rats with intact renal nerves. Bilateral renal denervation interrupts the afferent and efferent renal nerves. The afferent sensor renal nerves transmitted information to the sites within the central nervous system associated with cardiovascular regulation (25). We suppose that the decrease of mid frequency spectral power during selective nNOS inhibition in bilateral renal denervated rats is a result of centrally mediated mechanisms provoked by the lack of afferent signals from kidney and absence of nNOS produced nitric oxide. Interestingly, the low frequency spectral power in SAP and MAP spectrograms increased during selective nNOS inhibition in rats with bilateral renal denervation.

Experimental data indicated that renal nerves as well as nNOS derivated nitric oxide have a stimulating action on renin secretion from macula densa cells (29). In our investigation about the role of interaction between renal nerves and nNOS produced nitric oxide in the regulation of plasma renin activity we found that selective nNOS inhibition in bilateral renal denervated Wistar rats led to a great decrease of plasma renin activity - 4.5 times more than the decrease established during nNOS inhibition in Wistar rats with intact renal nerves. We suppose that the increase of P_{LF} in SAP and MAP spectrograms may be a result of the tremendous decrease of plasma renin activity which led to a fall of buffer action of renin on low frequency oscillation of arterial blood pressure.

CONCLUSION

The interaction between renal nerves and nitric oxide produced by neuronal nitric oxide synthase play a significant role in the regulation of blood pressure variability in Wistar rats.

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