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HEART RATE VARIABILITY DURING nNOS INHIBITION IN SPONTANEOUSLY HYPERTENSIVE RATS

P. Markova*, B. Iliev, D. Popov, R. Girchev

Department of Physiology, Medical Faculty, Medical University, Sofia

ABSTRACT

PURPOSE: The current study investigated role of nitric oxide (NO), produced by neuronal nitric oxide synthase (nNOS) on heart rate variability (HRV) in spontaneously hypertensive rats (SHR).

METODS: The selective neuronal nitric oxide synthase (nNOS) inhibition was performed by application of 7-Nitroindazole (7NI) in conscious normotensive Wistar rats (n=10) and in SHR (n=10), intravenously in a dose 2mg/kg/h. Blood pressure wave was registered directly through a femoral artery catheter in a control period and during nNOS inhibition. In spectrograms of heart rate, derived by FFT algorithm, spectral power (P) in the low (LF), mid (MF) and high (HF) frequency band were studied. Throughout relation, P_{MF}/P_{HF} the sympatho-vagal balance was determined.

RESULTS: The nNOS derivate NO in Wistar rats decreased P_{MF} from 1.39±0.34 to 0.43±0.03 ms^2, $p<0.05$ and altered P_{MF}/P_{HF} ratio from 0.80±0.1 to 0.44±0.03, ($p<0.05$). In SHR, nNOS inhibition did not affect HRV.

CONCLUSIONS: The absence of inhibiting action of nNOS-derived NO on sympathetic neurotransmission in normotensive rats led to a decrease of sympathetically mediated oscillations in heart rate and to displacement of sympatho-vagal balance. The lack of effect of nNOS-derived NO inhibition in SHR on the HRV may be a result of the impaired nNOS signaling pathway in SHR.

Key words: power spectral analysis, nNOS, SHR

INTRODUCTION

Heart rate variability (HRV) is a reliable criterion for evaluation of various physiological factors modulating the normal rhythm of the heart. The spectral analysis of HRV is widely applied to assess the state of the autonomic nervous system, responsible for the heart regulation, as well as the balance between the sympathetic and parasympathetic activity in the heart rate control (1).

Nitric oxide (NO) is a diffusible signal molecule, synthesized by a number of cell types throughout the body. NO biosynthesis from the substrate L-arginine is performed by three distinct isoforms of nitric oxide synthase (NOS), found in a variety of tissues (liver, brain, heart) and cell types (macrophage, endothelial, microglial) (2). The endothelial (eNOS) and neuronal, (nNOS) nitric oxide synthase isoforms constitutively expressed. The third, inducible (iNOS) NOS isoform is triggered by an inflammatory immune response. The role of nitric oxide signaling in cardiovascular system has been defined in processes such as neural transmission, blood vessels dilation and regulation of cardiac function (3). However, the exact site and specific action of NO in regulation of cardiac functions have remained elusive. Although NO is a highly diffusible, it has been established, signaling via eNOS and nNOS is compartmentalized and eNOS and nNOS modulate differently the cardiac function (4, 5). Recent data have demonstrated, nNOS is present in the Ca^{2+} storage of cardiac myocytes (6, 7), in the peripheral vagal and sympathetic nerves (8, 9) and in the autonomic control regions of the central nervous system (10), suggesting this isoform may play a significant role in the control of cardiac function and his excitability. The available

***Correspondence to:** *Petya Markova*
Department of Physiology, Medical Faculty,
Medical University-Sofia 1431,boul. "G. Sofiiski" 1
e-mail: pp.markova@gmail.com

experimental data established nNOS-derived NO affects the cardiac excitability through its action as a neuromodulator of the postganglionic sympathovagal transmission (11-13).

In hypertension, cardiac autonomic impairment is an important contributor to the pathophysiological phenotype of the disease. A lot of evidence support the observation that the activity of the sympathetic nervous system is dramatically increased both in hypertensive patients (14, 15) and in the spontaneously hypertensive rats (SHR), (16, 17). The mechanism causing enhanced neurohumoral activation, established in SHR is unknown, but may be directly related to the nitric oxide NO–cGMP disruption, modulating norepinephrine release in central autonomic nuclei (18) and peripheral varicosities (11).

The aim of the current study was to determine the role of nitric oxide, produced by neuronal nitric oxide synthase in heart rate variability modulation in spontaneously hypertensive rats.

MATERIALS AND METHODS

Experiments were carried out on conscious, male normotensive Wistar rats (n=10) and spontaneously hypertensive rats SHR, (n=10) at the same age: 12-14 weeks. 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 animals were housed under standard conditions: constant temperature 22 °C; 12/12 h light /dark cycle; free access to standard rat chow and tap water. SHR were selected by systolic arterial pressure over 170 mmHg, measured noninvasively by tail cuff method (Ugo-Basile). For drug application and for blood pressure measurement the femoral vein and the femoral artery were catheterized. 24 hours before the experiments under general anesthesia (Nembutal– Sigma) applied *i.p*, in a dose 35 mg/kg b.w. The catheters were tunneled subcutaneously and exteriorized at the back of the neck of the rats. To avoid blood clotting, the catheters were flushed by 200 IU/ml heparin in 0.9% sterile saline. In the next day, after recovery of the animals from the surgical intervention, experiments were performed on conscious freely moving animals. Arterial blood pressure wave was registered in control and experimental periods directly in the femoral artery by blood pressure transducer Gould Statham P23ID, connected to data acquisition system Biopac MP100WS. During a control period (40 min), sterile saline was infused in a rate 25µl/min, throughout previously implanted venous catheter. The selective neuronal nitric oxide synthase (nNOS) inhibition was performed by infusion of 7-Nitroindazole in dose 2 mg/kg/h. The effect of nNOS inhibition was studied 20 min after the start of the infusion (25µl/min) in course of 40 minutes. The heart rate (HR) was calculated from the inter-pulse interval (IPI), determined from the arterial blood pressure wave, by peak and rate detectors of the AcqKnowledge 3.8 software in terms of time between diastolic minimums of each heart beat. The obtained row data were resampled at 10 Hz. The spectrograms were derived from 512 successive values through virtual instrument developed in graphic programming environment Lab VIEW 3.1.1., by Fast Fourier Transform algorithm. 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) in $msec^2$ were studied (19). Sympatho-vagal balance was determined by relation of power of mid to high frequency band P_{MF}/P_{HF} in the spectrograms.

Statistical analysis was performed by Student's t-test. The results are presented as mean±SEM. Differences less than $p<0.05$ were considered statistically significant.

RESULTS

The systolic arterial blood pressure in SHR was higher in comparison to Wistar rat: 185.1±3.5 vs. 133.8±1.6 mmHg, ($p<0.01$). In the control period, mean values of heart rate (HR) did not differ between normotensive and spontaneously hypertensive rats: 344.3±6.6 and 338.3±7.2 b.p.m. Selective nNOS ihibition by 7NI did not change HR neither in Wistar (345.1±5.7 b.p.m.), nor in SHR (342.7 ±8.9 b.p.m.).

The spectral power P_{LF} and P_{HF} did not differ between Wistar rats and SHR. The P_{MF} in SHR was reduced compared to Wistar rats: 0.42±0.06 ms^2 vs. 1.39±0.34 $msec^2$, ($p<0.01$). As a results of the decreased mid frequency oscillations in SHR the relation P_{MF}/P_{HF} displaced to low level. In SHR, P_{MF}/P_{HF} ratio was 0.38±0.01 vs. 0.80±0.10 in Wistar rats, ($p<0.01$).

In normotensive Wistar rats, the application of 7NI did not change the low frequency spectral power: 3.90±0.59 and 3.88±0.42 ms^2, as well as the high frequency spectral power: 1.12±0.15 and 1.23±0.08 ms^2. However, the selective nNOS inhibition provoked a decrease of P_{MF} from 1.39±0.34 to 0.43±0.03 ms^2, $p<0.01$. The P_{MF}/P_{HF} ratio in normotensive Wistar rats altered from 0.80±0.1 to 0.44±0.03, ($p<0.01$) during the selective nNOS inhibition.

In contrast to Wistar rats, nNOS inhibition in SHR did not change power distribution in spectrograms. The P_{LF}, P_{MF} and P_{HF} in control period and during nNOS inhibition in SHR were: 4.30±0.18 ms^2 and 4.03±0.33 ms^2; 0.42±0.06 ms^2 and 0.48±0.06 ms^2; 1.24±0.14 ms^2 and 1.17±0.11 ms^2. The sympatho-vagal balance in SHR was not affected by the 7NI application. During nNOS inhibition, P_{MF}/P_{HF} ratio was 0.37±0.04. **(Fig. 1)**

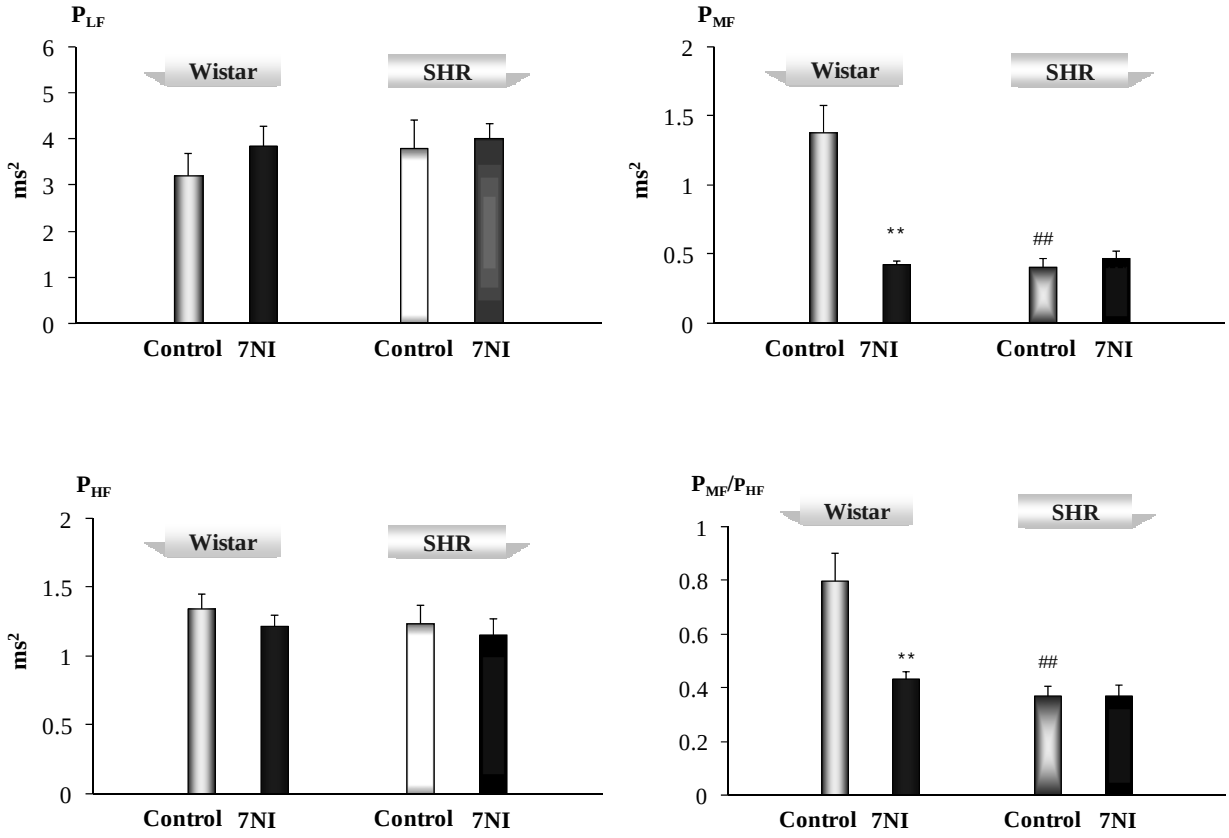

Fig.1. Spectral power distribution in low (P_{LF}), mid (P_{MF}), high (P_{HF}) frequency band and sympatho-vagal balance (P_{MF}/P_{HF}) in normotensive Wistar rats and in spontaneously hypertensive rats (SHR) in control period (Control) and during selective nNOS inhibition by intravenous application of 7Nitroindazole (7NI) in a dose 2 mg/kg/h.
($p<0.01$) show statistically significant differences between Wistar rats and SHR
** ($p<0.01$) show statistically significant effects as a result of selective nNOS inhibition

DISCISSION

In our study, we have found different response of heart rate variability to nNOS inhibition between normotensive Wistar rats and spontaneously hypertensive rats (SHR). The differences between Wistar and SHR in control conditions in the HRV established in the current study are in correspondence to our (20) and other results discussed in detail previously (21, 22). The SHR strain, a frequently used genetic animal model of essential hypertension, is associated with autonomic imbalance in the central and peripheral nervous system (23). It has been established, bradycardic response and acetylcholine release of the vagal nerve stimulation are reduced in the SHR at the level of the cardiac postganglionic neurons (24). On the other hand, it has been observed, the norepinephrine release from the right atria in response to field stimulation in SHR is higher compared to normotensive rats (25). This gives direct evidence that the sympathetic nervous system

is hyper-reactive in the SHR at cardiac level. In SHR reduced mid frequency variations, related mainly to the cardiac sympathetic modulation, indicate decreased heart rate ability to respond to deviations of increased sympathetic nerve activity. The displaced sympatho-vagal balance, determined by HR spectral characteristics is in agreement with the established autonomic imbalance in SHR.

Defective NOS signaling via eNOS has been widely implicated in the vascular aspect of hypertension. Cumulative evidences now suggest neuronally synthesized NO may also play a role in pathophysiology of hypertension (26). The signaling pathway responsible for the nNOS-derived NO inhibiting sympathetic neurotransmission is currently not completely elucidated. Available experimental data indicate soluble guanylate cyclase (sGC), the main target protein of NO, is markedly desensitized/downregulated in hypertension (26, 27). The increase of norepinephrine release in the normotensive rats and lack of effect in the SHR during guanylate cyclase inhibition suggested functional uncoupling of NO to its second messenger cGMP (22). We suppose that the absence of the inhibiting action of nNOS-derived NO on sympathetic neurotransmission in normotensive Wistar rats led to a decrease of sympathetically mediated mid frequency oscillations in heart rate and to displacement of sympatho-vagal balance. In SHR, nNOS inhibition did not affect HRV heart rate variability. This may be a result of the impaired nNOS signaling pathway in SHR accepted as a contributing factor to the cardiac autonomic dysfunction.

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REFERENCES

1. Saul, JP., Beat-to-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *News Physiol Sci*, 5:32–37, 1990.
2. Stuehr, DJ., Griffith OW., Mammalian nitric oxide synthases. *Adv Enzymol Relat Areas Mol Biol*, 65:287-346, 1992.
3. Pacher, P., Beckman, JS., Liaudet, L., Nitric oxide and peroxynitrite in health and disease., *Physiol Rev*, 87:315-424, 2007.
4. Barouch, LA., Harrison, RW., Skaf MW., Rosas GO., Cappola TP., Kobeissi ZA., et al., Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature*, 416(6878):337–339, 2002.
5. Ziolo, MT., Bers, DM., The real estate of NOS signaling: location, location, location. *Circ Res*, 92(12):1279–1281, 2003.
6. Petroff, MG., Kim,,SH., Pepe S., Dessy, C., Marban, E., Balligand, JL., et al., Endogenous nitric oxide mechanisms mediate the stretch dependence of Ca2+release in cardiomyocytes. *Nat Cell Biol*, 3(10):867–873, 2001.
7. Xu, KY., Huso DL., Dawson, TM., Bredt, DS., Becker, LC., Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci U S A*, 96(2):657–662, 1999.
8. Choate, JK., Danson, EJ., Morris, JF., Paterson, DJ., Peripheral vagal control of heart rate is impaired in neuronal NOS knockout mice. *Am J Physiol Heart Circ Physiol*, 281(6):H2310-H2317, 2001.
9. Schwarz, P., Diem, R., Dun, NJ., Förstermann, U., Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circ Res*, 77(4):841-8, 1995.
10. Patel, KP., Zhang, K., Zucker, IH., Krukoff, TL., Decreased gene expression of neuronal nitric oxide synthase in hypothalamus and brainstem of rats in heart failure. *Brain Res*, 23:734(1-2):109-15, 1996
11. Danson, EJ., Choate, JK., Paterson, DJ., Cardiac nitric oxide: emerging role for nNOS in regulating physiological function. *Pharmacol Ther.*, 106: 57–74, 2005.
12. Mohan, RM., Heaton, DA., Danson, EJF., Krishnan, SPR., Cai, S., Channon, KM., Paterson DJ., Neuronal nitric oxide synthase gene transfer promotes cardiac vagal gain of function. *Circ Res.*, 91:1089–1091, 2002.
13. Wehling-Henricks, M., Jordan, MC., Roos, KP., Deng, B., Tidball, JG., Cardiomyopathy in dystrophin-deficient hearts is prevented by expression of a neuronal nitric oxide synthase transgene in the myocardium. *Hum Mol Genet.*, 14: 1921–1933, 2005.
14. Sendeski, MM., Consolim-Colombo, FM, Leite, CC., Rubira, MC., Lessa P., Krieger EM., Increased sympathetic nerve activity correlates with neurovascular compression at the rostral ventrolateral medulla. *Hypertension*, 47:988–995, 2006.

15. Flaa, A., Mundal, HH., Eide, I., Kjeldsen, S., Rostrup, M., Sympathetic activity and cardiovascular risk factors in young men in the low, normal, and high blood pressure ranges. *Hypertension*, 47:396–402, 2006.
16. Fisher, LD., Tucker, DC., Air jet noise exposure rapidly increases blood pressure in young borderline hypertensive rats. *J Hypertens*., 9:275–282, 1991.
17. Vonend, O., Okonek, A., Stegbauer, J., Habbel, S., Quack, I., Rump, LC., Renovascular effects of sympathetic cotransmitters ATP and NPY are age-dependent in spontaneously hypertensive rats. *Cardiovasc Res*., 66:345–352, 2005.
18. Krukoff, TL., Central regulation of autonomic function: no brakes? *Clin Exp Pharmacol Physiol*., 25: 474–478, 1998.
19. Malpas, SC., Neural influences on cardiovascular variability: possibilities and pitfalls. Am J Physiol *Heart Circ Physiol*., 282(1):H6-20, 2002.
20. Markova, P., Girchev, R., Blood pressure and heart rate variability in conscious spontaneously hypertensive rats after renal denervation. *Comptes rendus de'lAcademie bulgare des Sciences*, 57(9): 83-88, 2004.
21. Malliani, A., Pagani, M., Lombardi, F, Furlan R, Guzzetti S, Cerutti S. Spectral analysis to assess increased sympathetic tone in arterial hypertension. *Hypertension*, 17: III36–III42, 1991.
22. Persson, PB., Spectrum analysis of cardiovascular time series. *Am J Physiol*., 273(4 Pt 2):R1201-1210, 1997.
23. Brook, RD., Julius, S., Autonomic imbalance, hypertension, and cardiovascular risk. *Am J Hypertens*., 13:112S–122S, 2000.
24. Heaton, DA., Li, D., Almond, SC., Dawson, TA., Wang, L., Channon, KM., Paterson, DJ., Gene transfer of neuronal nitric oxide synthase into intracardiac Ganglia reverses vagal impairment in hypertensive rats. *Hypertension*, 49(2):380-388, 2007.
25. Li, D., Wang, L., Lee, CW., Dawson, TA., Paterson DJ., Noradrenergic cell specific gene transfer with neuronal nitric oxide synthase reduces cardiac sympathetic neurotransmission in hypertensive rats. *Hypertension*, 50(1):69-74, 2007.
26. Chowdhary, S., Townend, JN., Nitric oxide and hypertension: not just an endothelium derived relaxing factor! *J Hum Hypertens*., 15:219–227, 2001.
27. Payne, JA., Reckelhoff, JF., Khalil, RA., Role of oxidative stress in age-related reduction of NO–cGMP-mediated vascular relaxation in SHR. *Am J Physiol Regul Integr Comp Physiol*. 285: R542–R551, 2003.