Dimethylarginines and Serotonin in the Blood of Spontaneously Hypertensive Rats

M. A. Gilinsky, T. V. Latysheva, and G. M. Petrakova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 12, pp. 608-610, December, 2009
Original article submitted December 26, 2008

The serotoninergic system and nitric oxide system were studied in spontaneously hypertensive rats SHR and Wistar rats (control). The contents of serotonin, 5-hydroxyindoleacetic acid (serotonin metabolite), L-arginine, monomethylarginine, and asymmetric and symmetric dimethylarginines were measured in blood plasma. Serotonin content in hypertensive animals was much higher than in Wistar rats. No interstrain differences were found in the concentration of 5-hydroxyindoleacetic acid. The concentration of asymmetric dimethylarginine in SHR rats was higher than in Wistar rats. However, the concentration of monomethylarginine in SHR rats was lower than in Wistar rats. Our results and published data on the serotoninergic system indicate that SHR rats serve as a convenient model of hypertension.

Key Words: *rats; hypertension; serotonin; nitric oxide; dimethylarginines*

The serotoninergic system and nitric oxide (NO) system play an important role in the pathogenesis of atherosclerosis, arterial and pulmonary hypertension, coronary heart disease, heart failure, and renal failure [1,3,4,9,12]. It was hypothesized that these systems can interact with each other [15]. There are conflicting data on quantitative parameters of activity of these systems. It is interesting to evaluate and compare the data on control Wistar rats and spontaneously hypertensive SHR rats.

Here we measured the concentrations of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA, serotonin metabolite), and L-arginine (L-Arg, substrate for the synthesis of NO in the reaction catalyzed by NO synthase). The content of the following methylated forms of arginine was evaluated: monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). These amino acids serve as the endogenous modulators of NO synthesis and play an important role in the development of endothelial dysfunction, atherosclerosis, and hypertension [1,3,6].

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** m.a.gilinsky@physiol.ru. M. A. Gilinsky

MATERIALS AND METHODS

Experiments were performed on 8-month-old SHR rats (n=12) and Wistar rats (n=9, control). The animals were obtained from the vivarium of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Medical Sciences). The maintenance and experimental procedures with animals were performed in accordance with the Directives of the European Community (86/609/EC) and approved by the Biomedical Ethics Committee (Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences).

The methods of 5-HT and 5-HIAA assay in blood plasma were described previously [2]. The blood was collected in vacutainers with EDTA and centrifuged at 150g to separate the erythrocyte mass. Repeated centrifugation at 5000g was performed to obtain poor plasma and platelet pellet. After precipitation of proteins, acidified poor plasma (5 μl) was injected into a chromatograph. The platelet pellet was homogenized in 0.1 M HCl. Solid components were precipitated. The supernatant was injected into a chromatograph. The substances were separated in a column (2×65 mm) packed with Nucleosil C18 sorbent (5 μ). The detection was con-

0007-4888/09/1486-0849 © 2009 Springer Science+Business Media, Inc.

ducted using a glass-carbon electrochemical detector equipped with a LC-4A controller (BAS) at 6 V.

The major amino acids L-Arg, MMA, ADMA, and SDMA were isolated by solid-phase extraction on cationic cartridges (MCX-1ml-30 mg, Waters). The eluate collected from cartridges was dried and dissolved in water before chromatography. Amino acids were derivatized with a reagent on the basis of *o*-phthalic dialdehyde and mercaptopropionic acid [13]. Separation was performed on a Luna-2 column (C18, 3 μ, 2×100 mm; Phenomenex) using a 10A series chromatograph (Shimadzu) equipped with an autoinjector and RF-10Axl fluorescence detector. The serotonin chromatograms were analyzed using a MultiChrome hardware-software system (Ampersend). The amino acid curves were analyzed using LC-solution software (Shimadzu). The results were analyzed by nonparametric methods (significance level $p<0.05$).

RESULTS

Before the study, plasma samples were stored in a freezing chamber for several days. The freezing-defrosting procedure led to partial disintegration of platelets and release of additional amounts of 5-HT into the plasma. We evaluated the total concentration of 5-HT and 5-HIAA per 1 ml plasma and platelets.

5-HT concentration in spontaneously hypertensive animals was much higher than in control specimens (1873±157 and 906±69 ng/ml, $p=0.0005$). No differences in 5-HIAA concentration were found between animals of these strains (30.4±3.1 and 25.5±4.0 ng/ml, respectively, $p=0.34$). The 5-HIAA/5-HT ratio (the parameter reflecting the intensity of serotonin metabolism) was different in these rats ($p<0.01$).

Table 1 shows the concentrations of amino acids in blood plasma of SHR and Wistar rats. Insignificant differences were revealed in the contents of L-Arg and SDMA. Plasma MMA concentration in hypertensive rats was much lower than in control animals. The content of ADMA in plasma samples from hypertensive animals was higher than in the control. Some parameters of NO regulation also differed in these animals (Mann–Whitney test). The L-Arg/MMA ratio in SHR and Wistar rats was 281.7 and 113.8, respectively ($p<0.01$). The ADMA/SDMA ratio in animals of these strains was 2.27 and 1.53, respectively ($p<0.001$).

Control animals were characterized by a strong correlation between the ADMA/SDMA ratio and 5-HT concentration ($r=-0.82$, $p=0.007$). Moreover, a correlation was found between the contents of MMA and 5-HIAA ($r=0.79$, $p<0.02$). A significant correlation was revealed between the concentrations of MMA and 5-HIAA in hypertensive rats ($r=0.59$, $p<0.05$). The concentrations of MMA and SDMA correlated with the content of 5-HT ($r=0.64$, $p<0.03$; and $r=-0.65$, $p<0.03$, respectively).

It should be emphasized that plasma 5-HT concentration in hypertensive rats was 2-fold higher than in control animals. Previous studies showed that the content of 5-HT in patients with essential hypertension is below normal [14]. Some models of hypertension in rats are also characterized by reduced parameters of the serotoninergic system (as differentiated from SHR rats) [10]. By activity of the serotoninergic system, SHR rats constitute a specific population of experimental animals.

Published data indicate that MMA and ADMA are competitive antagonists of NO synthase that reduce the bioavailability of NO [8]. Previously, it was believed that SDMA does not play a role in the regulation of NO. Recent studies showed that SDMA can inhibit transmembrane transport of arginine, thus reducing bioavailability of the substrate for enzymatic synthesis of NO [5].

The concentration of MMA in human blood is one order of magnitude lower than the content of ADMA. Hence, MMA is often used as an internal standard [13]. We showed that the concentration of MMA in rat plasma is similar to that of dimethylarginines. The increase in ADMA concentration in SHR rats is one of the major causes of hypertension [11]. The results of our study indicate that MMA should be considered as an inhibitory factor of NO synthesis. However, ADMA plays the major role in the development of hypertension (Table 1).

Reciprocal influence of the serotoninergic system and NO-regulating system manifests in the relation-

TABLE 1. Concentrations of L-Arg and Methylarginines in Blood Plasma of SHR and Wistar Rats

Group	L-Arg	MMA	ADMA	SDMA
Wistar, $n=9$	58.0±16.6	0.52±0.01	0.23±0.02	0.15±0.01
SHR, $n=12$	84.0±6.6*	0.35±0.04*	0.29±0.01*	0.13±0.008*
	$p=0.12$	$p=0.003$	$p=0.013$	$p=0.22$

Note. *Significant differences from Wistar rats (control).

ship between platelet function and concentration of L-Arg in the blood. For example, platelet aggregation induced by oxidized lipoproteins is accompanied by the release of 5-HT. This process is inhibited by arginine [7]. In our experiments, this interaction probably manifests in a strong correlation between the ADMA/SDMA ratio and 5-HT concentration in control animals. This correlation was not found in hypertensive rats.

This work was supported by the Russian Foundation for Basic Research (grant No. 08-04-00832a).

REFERENCES

1. M. A. Gilinsky, *Uspekhi Fiziol. Nauk*, **38**, No. 3, 21-39 (2007).
2. M. A. Gilinsky, T. V. Latysheva, and L. P. Semenova, *Klin. Lab. Diagnost*., No. 6, 25-28 (2007).
3. I. V. Zotova, D. A. Zateishchikov, and B. A. Sidorenko, *Kardiologiya*, No. 4, 58-67 (2002).
4. Kh. M. Markov, *Uspekhi Fiziol. Nauk*, **32**, No. 3, 49-65 (2001).
5. S. M. Bode-Boger, F. Scalera, J. T. Kielstein, *et al*., *J. Am. Soc. Nephrol*., **17**, No. 4, 1128-1134 (2006).
6. R. H. Boger, *Clin. Chem. Lab. Med.*, **41**, No. 11, 1467-1472 (2003).
7. L. Y. Chen, P. Mehta, and J. L. Mehta, *Circulation*, **93**, No. 9, 1740-1746 (1996).
8. U. Förstermann, E. I. Closs, J. S. Pollock, *et al*., *Hypertension*, **23**, No. 6, 1121-1131 (1994).
9. F. Lechin, B. van der Dijs, and A. E. Lechin, *J. Appl. Res*., **5**, No. 4, 605-621 (2005).
10. W. Ni, K. Lookingland, and S. W. Watts, *Hypertension*, **48**, No. 1, 134-140 (2006).
11. P. Sicard, S. Delemasure, C. Korandji, *et al.*, *Free Radic. Res*., **42**, No. 3, 226-236 (2008).
12. C. C. Smith and D. J. Betteridge, *Atherosclerosis*, **130**, Nos. 1-2, 87-92 (1997).
13. T. Teerlink, R. J. Nijveldt, S. de Jong, and P. A. van Leeuwen, *Anal. Biochem*., **303**, No. 2, 131-137 (2002).
14. S. W. Watts, *Clin. Sci. (Lond.)*, **108**, No. 5, 399-412 (2005).
15. G. Wegener, V. Volke, and R. Rosenberg, *Br. J. Pharmacol*., **130**, No. 3, 575-580 (2000).