Comparison Of Cardiovascular Characteristics In Normotensive And Hypertensive Rat Strains

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Abstract

Background: Hypertensive rats serve as valuable tools for studies of dysregulations in cardiovascular functions before and during pathological elevation of blood pressure. They exhibit many defects in structure and function of heart and vessels which are often related to severity of hypertension.

Objective: The relationship of blood pressure level and manifestation of aberrations in selected cardiovascular and metabolic parameters were determined in 20-week-old normotensive Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR) and in their F1 offspring borderline hypertensive rats (BHR), and also in normotensive Wistar rats which are genetically less compatible with the other mentioned rat strains.

Methods: Systolic blood pressure and heart rate were measured in conscious rats by the non-invasive tail-cuff method. At the end of the treatment, rats were sacrificed, relative weight of their left heart ventricle and liver were determined and plasma concentration of glucose and triglycerides were measured. Thoracic aorta and superior mesenteric artery were isolated and prepared for isometric tension recording. Neurogenic contractions were elicited by electrical stimulation of perivascular adrenergic nerves.

Results: The level of systolic blood pressure in WKY rats (106.0±0.4 mmHg), BHR (149.5±2.5 mmHg) and SHR (186.4±3.9 mmHg) corresponded with the impairment of acetylcholine-induced relaxation of isolated thoracic aorta and with the increase in sensitivity of contractile responses to exogenous noradrenaline and to electrical stimulation of perivascular adrenergic nerves in mesenteric artery. However, rats of the normotensive strain Wistar (118.1±2.0 mmHg) exhibited arterial contractions similar to those obtained in hypertensive rats. Wistar rats had also the highest relative liver weight and plasma triglyceride concentration.

Conclusion: These observations indicate that when comparing non-related rat strains the higher magnitude of arterial contractions and abnormal lipid parameters may not correlate with hypertensive state.

Key words: hypertension, conduit arteries, sympathetic nervous system, endothelial dysfunction.

Introduction

Hypertensive rat strains serve as valuable tools for studies of dysregulations in cardiovascular functions before and during pathological elevation of blood...
pressure. The spontaneously hypertensive rat (SHR) has been widely used as a model of human essential hypertension, and the normotensive inbred Wistar-Kyoto (WKY) rats, derived from the same ancestral rat strain as the SHR, have been used as the closest genetic control for the SHR (1, 2). Hypertension in SHR is associated with multiple impairments in structure and function of heart and vessels and also with the features of metabolic syndrome like fasting hyperglycemia, hyperinsulinemia and dyslipidemia (25). Most of these defects are related to the final level of blood pressure in SHR and are not manifested in control WKY rats. However, when the comparisons are made with other non-related (normotensive) rat strains, the correlation between blood pressure and occurrence of these abnormalities might not exist because of different setting (adjustment) of their regulatory mechanisms. In spite of that, they are often used as normotensive control for SHR which may lead to incorrect conclusions regarding the direct relationship of high blood pressure and various cardiometabolic abnormalities in certain individuals (6, 7).

The goal of our study is to investigate the relationship of blood pressure level and manifestation of aberrations in selected cardiovascular and metabolic parameters in normotensive WKY rats and SHR and in their F1 offspring borderline hypertensive rats (BHR), and also in normotensive Wistar rats which are genetically less compatible with the other mentioned rat strains.

Materials and Methods

The animal protocols used in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic. All rats were housed at 22-24°C on a 12:12 h dark-light cycle (06.00-18.00 h lights on) and maintained on a standard laboratory rat chow and tap water ad libitum.

In the experiment, adult (20-week-old) male rats were used: normotensive Wistar and Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR) and borderline hypertensive rats (BHR) - F1 offspring of WKY males and SHR females. Systolic blood pressure and heart rate were measured in conscious rats by the non-invasive tail-cuff method. Before the experiment, all rats were fasted overnight and then sacrificed under CO₂ anesthesia. Samples of their blood were collected and used for measurement of plasma glucose and triglyceride concentration. Wet weight of left heart ventricle and of liver and length of tibia were determined for calculation of left heart ventricle weight-to-tibia length ratio and liver weight-to-tibia length ratio.

Functional studies were performed on isolated thoracic aorta and superior mesenteric artery. The arteries were cut into rings of 3.0-3.5 mm in width and suspended in 20 ml organ baths filled with oxygenated (95% O₂+5% CO₂) modified Krebs solution maintained at 37°C. The Krebs solution had the following composition (in mmol/l): NaCl 118, KCl 5, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 11, CaNa₂EDTA 0.03 and ascorbic acid 0.55. The arterial rings were set up for isometric tension recording using a force-displacement transducer Sanborn FT 10 (Sanborn, Baltimore, USA). The preparations were equilibrated under a resting tension of 10 mN for 60-90 min, and the solution was changed every 15 min.

To examine the endothelium-dependent vasorelaxation, the preparations of aorta were first precontracted by phenylephrine (10⁻⁶ mol/l). When the contraction reached a plateau, increasing concentrations of acetylcholine were applied in a cumulative manner (10⁻⁹-10⁻⁵ mol/l).

Adrenergic contractions were determined in endothelium-intact thoracic aortas and mesenteric arteries as the responses to cumulatively applied exogenous noradrenaline (10⁻¹⁰-3×10⁻⁵ mol/l).

In mesenteric arteries, neurogenic responses were induced by electrical stimulation of periarterial sympathetic nerves. The arterial rings were stimulated by two parallel platinum plate electrodes placed on either side of the preparation and connected to an electrostimulator ST-3 (Hungary). Frequency-
Response curves to electrical stimuli were obtained using square pulses of 0.5 ms in duration, at supramaximal voltage (> 30 V), applied at 1-32 Hz, for a period of 20 s. In our preliminary observations we found that the contractions of rat mesenteric arteries elicited by electrical stimulation (using the described parameters of stimulation) are blocked by phentolamine or tetrodotoxin, indicating that they are induced mainly by nerve-released (endogenous) noradrenaline.

The results are presented as means±S.E.M. Contractile responses are expressed in absolute values (in mN normalized to the cross sectional area of the respective ring preparation) or in relative values (in % of phenylephrine precontraction). Statistical evaluation was carried out by using one-way analysis of variance (ANOVA). The results were considered to be significant when $p<0.05$.

Results

Table I shows that in 20th week of age, significant differences in blood pressure and heart rate were detected between all rat strains. Increasing tendency in the values of these two parameters was found within the line of WKY rats - BHR - SHR. Normotensive Wistar rats had higher blood pressure than WKY rats and their heart rate was at the level of BHR.

The largest body weight values were found in normotensive Wistar rats while the smallest ones in SHR. Wistar and WKY rats had significantly lower relative weight of heart left ventricle than BHR and SHR. On the other hand, the highest relative liver weight was found in normotensive Wistar rats. WKY rats had significantly lower relative liver weight than BHR and SHR (Table I).

The levels of plasma glucose and triglycerides were highest in normotensive Wistar rats. Normotensive WKY rats had significantly higher plasma glucose levels than BHR and SHR (Table I).

Similarly, in superior mesenteric arteries the magnitude of contractions to exogenous noradrenaline and to electrical stimulation of perivascular adrenergic nerves corresponded to the level of blood pressure in WKY rats, BHR and SHR, the latter exhibiting the highest sensitivity and contractile force in response to these stimuli. Mesenteric arteries from normotensive Wistar rats developed contractile responses to exogenous noradrenaline similar to that in SHR (Figure 3) and their neurogenic contractions were at the level of BHR (Figure 4).

### Table I: Comparison of some cardiovascular and metabolic parameters in different normotensive and hypertensive rat strains in 20th week of age.

<table>
<thead>
<tr>
<th></th>
<th>Wistar rats</th>
<th>WKY rats</th>
<th>BHR</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mmHg)</td>
<td>118.1±2.0</td>
<td>106.0±0.4</td>
<td>149.5±2.5</td>
<td>186.4±3.9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>409.5±13.3</td>
<td>327.4±7.8</td>
<td>392.3±10.9</td>
<td>497.4±9.1</td>
</tr>
<tr>
<td>BW (g)</td>
<td>482.8±17.0</td>
<td>417.2±10.7</td>
<td>432.2±7.1</td>
<td>360.0±10.2</td>
</tr>
<tr>
<td>LVW/TL (mg/mm)</td>
<td>22.7±0.7</td>
<td>20.9±0.6</td>
<td>25.7±0.4</td>
<td>26.6±1.5</td>
</tr>
<tr>
<td>LiW/TL (mg/mm)</td>
<td>4.0±0.2</td>
<td>2.7±0.1</td>
<td>2.9±0.1</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>GLU (mmol/l)</td>
<td>11.9±1.2</td>
<td>9.8±0.4</td>
<td>6.9±0.3</td>
<td>7.2±0.5</td>
</tr>
<tr>
<td>TRIGL (mmol/l)</td>
<td>1.6±0.2</td>
<td>0.6±0.1</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Abbreviations: WKY Wistar-Kyoto, BHR borderline hypertensive rats, SHR spontaneously hypertensive rats, BP blood pressure, HR heart rate, BW body weight, LVW/TL left ventricular weight-to-tibia length, LiW/TL liver weight-to-tibia length, GLU glucose, TRIGL triglycerides. Values represent mean±SEM; n=6-8; *$p<0.05$ vs. SHR; †$p<0.05$ vs. BHR; ††$p<0.05$ vs. WKY.
Fig. 1: Dose-response curves of acetylcholine-induced relaxation in aortic rings from different normotensive and hypertensive rat strains in 20th week of age. *p<0.05 significantly different responses between particular rat strains as indicated by arrows.

Fig. 2: Dose-response curves of noradrenaline-induced contraction in aortic rings from different normotensive and hypertensive rat strains in 20th week of age. *p<0.05 significantly different responses between particular rat strains as indicated by arrows.
Fig. 3: Dose-response curves of noradrenaline-induced contraction in rings of superior mesenteric artery from different normotensive and hypertensive rat strains in 20th week of age. "p<0.05 significantly different responses between particular rat strains as indicated by arrows.

Fig. 4: Frequency-response curves of neurogenic contraction (induced by electrical stimulation of perivascular nerves) in rings of superior mesenteric artery from different normotensive and hypertensive rat strains in 20th week of age. "p<0.05 significantly different responses between particular rat strains as indicated by arrows.
Discussion

Our study brings some new findings regarding the relationship of high blood pressure and the manifestation of several cardiovascular and metabolic disturbances in normo- and hypertensive rat models. The genetic, environmental, and developmental aspects of cardiovascular dysfunction in SHR were widely described and analyzed in many experimental works and reviews (2, 3, 8). In this study we have confirmed that SHR, originally developed from the Wistar-Kyoto rat strain by selectively breeding of individuals for high blood pressure (1), exhibit various cardiovascular abnormalities like myocardial hypertrophy (indicated by higher relative heart weight), sympathetic hyperresponsiveness (demonstrated by tachycardia and high sensitivity of arterial contractile responses to noradrenergic stimulation), and endothelial dysfunction in large arteries. These aberrations are also evident in less amplitude in BHR which were obtained as offspring of normotensive WKY males and SHR females. From our presented results it is clear that within the line of rat strains WKY-BHR-SHR, the occurrence of the observed impairments is connected to the higher levels of blood pressure.

Similar tendencies in these relationships were found also in the study of Púzserová et al. (9) measuring the reactivity of femoral arteries from the mentioned groups of rats. These authors revealed that the degree of endothelial dysfunction is associated with the level of blood pressure in these rats, and that this aberration is not caused by impairment in production of nitric oxide (NO) which is the main endogenous vasodilating substance particularly in conduit arteries. On the contrary, the significant positive correlation between blood pressure and NO-dependent component of acetylcholine-induced relaxation in femoral artery was found (9). Together with other investigators, these authors suppose that the endothelial dysfunction in SHR and BHR is related rather to the decreased NO-independent part of relaxant response that is mediated by endothelium-derived hyperpolarizing factor(s) and/or prostacyclin (9-11). Moreover, the increased oxidative stress and the enhanced production of endothelium-derived contracting factors contribute to the impairment of endothelial function in hypertensive rat arteries (9, 12).

The hypertensive state in SHR is clearly associated with enhanced sympathetic tone in cardiovascular system. This was demonstrated by several functional studies confirming exaggerated cardiovascular responsiveness to sympathetic stimuli as well as by morphologically proved sympathetic hyperinnervation of cardiovascular structures. Increased activity of the sympathetic nervous system is evident even in juvenile SHR in prehypertensive phase of their development (13-15). It is caused by elevated endogenous level of the peptide nerve growth factor in vascular system of SHR before and during the period of pathological increase of their blood pressure (16-19). Experimentally induced neonatal sympathectomy using chemical or immunological intervention resulted in prevention of hypertension development (20, 21) which clearly confirmed the involvement of sympathetic nervous system in the development of high blood pressure in these rats. In our study, genetically determined sympathetic hyperinnervation in SHR caused the higher contractile responses of arterial mesenteric preparations to electrical nerve stimulation when comparing to WKY rats.

Moreover, increased contractions in aorta and mesenteric artery were observed also in response to exogenously applied noradrenaline, the main neuromediator of sympathetic nervous system, indicating that the altered postsynaptic processes contribute to the exaggerated sympathetic reactions as well. In BHR, the arterial responses to sympatheodrenergic stimuli were also significantly increased comparing to WKY rats but they were lower than that in SHR. The same is valid also for the level of their heart rate which was found to be in the middle of the values obtained in SHR and WKY rats. This supports the idea that in these rat groups the increasing level of blood pressure relates with the higher sympathetic responses in their cardiovascular system.

Different situation is in the group of genetically less
compatible Wistar rats which are often being used in studies as normotensive control to SHR also. We have found that individuals of this rat strain have slightly higher blood pressure than WKY rats but it is still much lower than that in hypertensive groups. Endothelium-dependent relaxation of thoracic aorta is not impaired in Wistar strain and it is at the level of WKY rats. However, cardiovascular sympathoadrenergic reactivity assessed indirectly from the level of heart rate and from neurogenic contractile responses of mesenteric arteries (induced by electrical stimulation of perivascular sympathetic nerves) is evidently closer to BHR. We also detected abnormally enlarged contractile responses to exogenous noradrenaline in isolated aortas as well as in superior mesenteric arteries from Wistar rats when compared to WKY rats, BHR and SHR. Similarly, in our previous study we found that contractile responses in conduit arteries of normotensive Wistar rats are alike or even larger than that of SHR (22). Such unexpected results may be related to the inherited alterations in morphology and biomechanical properties of arterial wall which was found to be thicker and more rigid in SHR than in Wistar rats (23, 24). The reduced compliance of arteries in SHR may also limit their contractile properties and can be considered as analogous to the arterial stiffness detected in human (pre)hypertensive individuals (25, 26). Taking into account these findings, it seems that the settings of cardiovascular regulation in the aforementioned rat strains is different and that despite of higher arterial contractions and sympathetic activity in cardiovascular system of Wistar rats in comparison to WKY rats, their blood pressure does not rise into hypertensive levels.

In conclusion, we have found that when comparing various genetically less compatible rat strains with different predisposition to hypertensive state the larger magnitude of arterial adrenergic contractions and plasma glucose and triglyceride concentrations may not positively correlate with the values of their blood pressure. These results also suggest that normotensive Wistar rats are markedly different in several features from the line of rat strains WKY rats BHR SHR and therefore should not be used as normotensive control for hypertensive rats from this line.

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Conflict of interest

The authors declare that there is no conflict of interests.
References


