Intra-strain variations of baroreflex sensitivity in young Wistar-Kyoto rats

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Abstract

Purpose: To compare baroreflex sensitivity among conscious rats of the same strain.

Methods: Male WKY rats (eight weeks old) were studied. Cannulas were inserted into the abdominal aortic artery through the right femoral artery to measure mean arterial pressure (MAP) and heart rate (HR). Baroreflex gain was calculated as the ratio between variation of HR in function of the MAP variation (ΔHR/ΔMAP) tested with a depressor dose of sodium nitroprusside (SNP, 50µg/kg, iv) and with a pressor dose of phenylephrine (PE, 8µg/kg, iv). We divided the rats into four groups: 1) Low bradycardic baroreflex (LB), BG between -1 and -2 bpm/mmHg tested with PE; 2) High bradycardic baroreflex (HB), BG < -2 bpm/mmHg tested with PE; 3) Low tachycardic baroreflex (LT), BG between -1 and -2 bpm/mmHg tested with SNP and; 4) High tachycardic baroreflex (HT), BG < -2 bpm/mmHg tested with SNP. Significant differences were considered for p<0.05.

Results: Approximately 82% of the rats presented reduced bradycardic reflex while 22 showed attenuated tachycardic reflex. No alterations were noted regarding basal MAP and HR, tachycardic and bradycardic peak and HR range.

Conclusions: There was alteration in baroreflex sensitivity among rats of the same strain. Care should be taken when interpreting studies employing WKY as a control for the SHR.

In 1963, Okamoto and Aoki reported that they had selectively bred Wistar rats to be spontaneously hypertensive. Established as an inbred strain in 1969 at the National Institutes of Health (NIH), the spontaneously hypertensive rat (SHR) remains the most widely studied animal model of essential hypertension (i.e., persistent high blood pressure of unknown causation), and the strain presents similar characteristic to hypertension in humans. As controls for the SHR, most workers have employed normotensive descendants of Wistar rats that NIH investigators obtained in 1971 from the colony in Kyoto from which the SHR strain was originally derived (Wistar-Kyoto rats, WKY).

Baroreflex function is one of the body's homeostatic mechanisms to sustain arterial pressure. It offers a negative feedback loop in which the increased arterial pressure reflexively causes blood pressure to de-

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crease. Similarly, the decreased blood pressure depresses the baroreflex, causing arterial pressure to rise. The system relies on specialized neurons (baroreceptors) in the aortic arch, carotid sinuses and elsewhere to monitor changes in blood pressure and relay them to the brainstem. Subsequent changes in blood pressure are mediated by the autonomic nervous system.  

Over the past 20 years, it has been reported that arterial baroreflex function is related to the prognosis of acute cardiovascular infarction, arrhythmias, heart failure and stroke in humans. Clinical observations indicate that patients with lower baroreflex sensitivity exhibit shorter survival times with these diseases. Arterial baroreflex function plays an important role in the pathogenesis and prognosis of hypertension, atherosclerosis, aconitine-induced arrhythmia and LPS-induced shock. Conditions such as age, hypertension and radiation therapy to the neck diminish both arterial compliance and the baroreflex, and impaired baroreflex responses in these conditions are thought to reflect vascular stiffness. Baroreflex sensitivity is helpful in the diagnosis and prognosis of cardiac diseases.

In 1987, WKY from two different laboratories presented differences with respect to growth rate and blood pressure. Although it was previously shown that a portion of normotensive Sprague–Dawley rats spontaneously exhibit lower baroreflex sensitivity, no previous study investigated whether differences of baroreflex sensitivity occurred among other type of rats of the same strain. Therefore, in this study we compared the baroreflex sensitivity among young WKY from the same laboratory to determine if there are intra strain differences.

Methods

Animals

The experiments were performed in eight-week old male WKY rats from the same laboratory. Rats were housed individually in plastic cages under standard laboratory conditions. They were kept under a 12 h light/dark cycle (lights on at 06:30 h) and had free access to food and water. Housing conditions and experimental procedures were approved by the Institution's Animal Ethics Committee.

Surgical Preparation

One day before the experiments, the rats were anesthetized with ketamine (50 mg/kg ip) and xylaxine (50 mg/kg im) and a catheter was inserted into the abdominal aorta through the femoral artery for blood pressure and heart rate recording. Catheters were made of 4 cm segments of PE-10 polyethylene (Clay Adams, USA) heat bound to a 13 cm segment of PE-50. The catheters were tunneled under the skin and exteriorized at the animal's dorsum.

Arterial pressure and heart rate recording in awake rats

Approximately 24 h after surgery, the animals were kept in individual cages used in the transport to the experimental room. Animals were allowed 60 min to adapt to the conditions of the experimental room such as sound and illumination before starting blood pressure and heart rate recording. The experimental room was acoustically isolated and had constant background noise produced by an air exhauster. At least another 30 min were allowed before beginning experiments. Pulsatile arterial pressure (PAP) of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, USA) and an acquisition board (MP100A, Biopac Systems Inc, USA) connected to a computer. Mean arterial pressure (MAP) and heart rate (HR) values were derived from the PAP recordings and processed on-line.

Baroreflex test

The baroreflex was tested with a pressor dose of phenylephrine (PE-bolus-8 µg/kg iv; Sigma Chemical)
and depressor doses of sodium nitroprusside (SNP-bolus-50 µg/kg iv; RBI). Baroreflex gain was calculated as the ratio between variation of HR in function of the MAP variation ($\Delta$HR/$\Delta$MAP). There was at least 15 min between infusions to allow recovery of basal values. We also evaluated bradycardic and tachycardic peak, HR range and baroreflex gain according to pilot studies and from statistical analysis of pooled data.

### Statistical Analysis

Values are reported as the means ± standard error of means (SEM). HR, MAP, $\Delta$HR, $\Delta$MAP and $\Delta$HR/$\Delta$MAP were compared between LB and HB groups as well as between LT and HT groups. After the distributions were evaluated through the Kolmogorov normality test, Student’s t test was used to verify differences between normal distributions and the Mann-Whitney test was applied to assess differences between non-parametric distributions. Differences were considered significant when the probability of a Type I error was less than 5% ($P < 0.05$).

### Results

Among all the 33 WKY rats evaluated, based on baroreflex gain tested with PE, approximately 18% presented high bradycardic baroreflex gain (HB; < -2 bpm/mmHg). On the other hand, the majority of animals presented lower bradycardic baroreflex gain (LB; between -1 and -2 bpm/mmHg).

To verify whether other cardiovascular parameters were different between LB and HB groups we compared baseline MAP and HR, bradycardic and tachycardic peak, HR range and baroreflex gain tested with PE and SNP. There were no differences between groups regarding basal MAP and HR, bradycardic and tachycardic peak, HR range and the sympathetic component of baroreflex gain. However, there was a difference in relation to the parasympathetic component of baroreflex gain (Table 1).

PE-induced increase in MAP was increased in the LB group. Moreover, bradycardic reflex responses to intravenous PE was decreased in LB group (Table 2).

We also compared SNP-induced decrease in MAP and tachycardic responses to i.v. SNP between LB and HB groups. MAP decrease in response to SNP was similar between groups and the same was observed with regard to tachycardic reflex responses (Table 2).

When baroreflex gain was tested with SNP, among all 34 WKY rats analyzed, approximately 78% presented higher baroreflex gain (HT; < -2 bpm/mmHg)

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### Table 1. Baseline mean arterial pressure (MAP) and heart rate (HR), bradycardic and tachycardic peak, HR range and baroreflex gain (BG) in HB (n=27), LB (n=6), HT (n=9) and LT (n=26) groups. Mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group HB</th>
<th>Group LB</th>
<th>$P$</th>
<th>Group LT</th>
<th>Group HT</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>113.9±1.94</td>
<td>110.3±4.24</td>
<td>0.4446</td>
<td>118.8±3.15</td>
<td>112.15±1.98</td>
<td>0.0943</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>317.33±7.64</td>
<td>296.67±24.02</td>
<td>0.2769</td>
<td>308.7±17.52</td>
<td>315.3±7.7</td>
<td>0.6097</td>
</tr>
<tr>
<td>Bradycardic peak (bpm)</td>
<td>248.4±7.84</td>
<td>216.17±14.26</td>
<td>0.083</td>
<td>255.6±16.74</td>
<td>239.9±7.9</td>
<td>0.3588</td>
</tr>
<tr>
<td>Tachycardic peak (bpm)</td>
<td>446.43±9.8</td>
<td>418.33±14.75</td>
<td>0.2056</td>
<td>412.3±17</td>
<td>455.6±8.4</td>
<td>0.0176</td>
</tr>
<tr>
<td>HR range (bpm)</td>
<td>197.08±8.1</td>
<td>202.17±22.19</td>
<td>0.8113</td>
<td>145.75±10.1</td>
<td>213.9±7.29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BG (bpm x mmHg⁻¹) PHE</td>
<td>-1.14±0.06</td>
<td>-2.36±0.09</td>
<td>&lt;0.0001</td>
<td>-1.2±0.19</td>
<td>-1.39±0.12</td>
<td>0.4295</td>
</tr>
<tr>
<td>BG (bpm x mmHg⁻¹) SNP</td>
<td>-2.66±0.217</td>
<td>-2.125±0.17</td>
<td>0.2581</td>
<td>-1.39±0.124</td>
<td>-2.96±0.16</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
while the other part (around 22%) presented lower baroreflex gain (LT; between -1 and -2 bpm/mmHg).

We observed differences with respect to the sympathetic component of the baroreflex gain, tachycardic peak and HR range but there was no difference between groups regarding basal MAP and HR and bradycardic peak. PE-induced increase in MAP provided no differences between group nor were the bradycardic reflex responses to increase in arterial pressure.

The decrease in MAP in response to SNP was similar between HT and LT groups. However, tachycardic reflex responses to decrease in arterial pressure was reduced in LT group.

Discussion

We found that rats of the same strain could be divided into two groups based on baroreflex gain (HB vs. LB, based on the parasympathetic responses and; HT vs. LT, based on the sympathetic responses). Comparison between groups demonstrated that the parasympathetic component of the baroreflex gain and bradycardic reflex response to increase in arterial pressure were reduced in approximately 82% of the rats studied. Furthermore, the sympathetic component of the baroreflex gain and tachycardic reflex in response to decrease in arterial pressure were reduced in approximately 22% of the rats investigated. The absence of difference between the groups regarding basal MAP and HR reject the possibility of influence of stress or pain after surgery.

Baroreceptor reflex was estimated by bolus infusion and we verified HR changes in response to arterial pressure elevation or reduction caused by i.v. infusion of SNP or PE, respectively. According to our findings, approximately 82% of 33 WKY rats (LB) presented reduced bradycardic reflex response to increase in arterial pressure and decreased baroreflex gain tested with the α1-adrenergic agonist PE. Nevertheless, when we compared basal MAP and HR, bradycardic and tachycardic peak, HR range and baroreflex gain tested with SNP no differences were observed. Our data indicate that the majority of WKY rats present damaged gain of reflex bradycardia, the parasympathetic component of baroreflex sensitivity, whereas a small part showed significant increased gain of the reflex bradycardia. The mechanisms that cause the reduction in the baroreflex function in rats are not understood.13 Some studies demonstrated that the carotid body was larger in rats with impaired baroreflex14-16, whereas other studies indicated that the decreased baroreflex function is due to impaired levels of norepinephrine, epinephrine and dopamine in the carotid body16-18 and medulla oblongata areas that regulate the cardiovascular system.19 Furthermore, there have been reports that AT1 (angiotensin) receptor densities are involved in models of damaged baroreflex function.20,21 Such mechanisms may be involved regarding alteration of baroreflex function among those rats of the same strain.

Approximately 78% of the animals in this study presented increased gain of reflex tachycardia. Great attention has focused on the role of the sympathetic activity regarding the onset of hypertension in a SHR, which WKY is its control. Previous studies have shown that there is elevated sympathetic drive to the vessels in adult SHR and have suggested that this is relevant in the maintenance of increased blood pressure.22,23 This elevation in sympathetic output may

TABLE 2. Decrease and increase in mean arterial pressure (MAP, mmHg) and tachycardic and bradycardic reflex (HR, bpm) in response to sodium nitroprusside (SNP, 50µg/kg iv) and phenylephrine (PE, 8µg/kg iv), respectively, in HB (n=27), LB (n=6), HT (n=9) and LT (n=26) groups. Mean±SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group HB</th>
<th>Group LB</th>
<th>Group HT</th>
<th>Group LT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in MAP (mmHg)</td>
<td>37.83±4.1</td>
<td>48.8±1.7</td>
<td>46.56±2.1</td>
<td>46.12±2.5</td>
<td>0.9153</td>
</tr>
<tr>
<td>Bradycardic reflex (bpm)</td>
<td>-89.3±9.9</td>
<td>-55.2±3.6</td>
<td>-62.4±5</td>
<td>-54.9±7.8</td>
<td>0.4664</td>
</tr>
<tr>
<td>Decrease in MAP (mmHg)</td>
<td>-45.3±3</td>
<td>-39.8±1.9</td>
<td>-39.58±1.9</td>
<td>-43.4±2.4</td>
<td>0.29</td>
</tr>
<tr>
<td>Tachycardic reflex (bpm)</td>
<td>98.2±11</td>
<td>98.5±11.8</td>
<td>111.73±5</td>
<td>61.5±8</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
not be primarily a consequence of changes in either baroceptor reflex or chemoreflex function but rather is a product of a modification of the central neural circuitry involved in generating the sympathetic output. In view of the above considerations, although there was no alteration with respect to basal MAP and HR between LT and HT groups, we cannot discard the possibility that rats with increased tachycardic reflex are more susceptible to present higher sympathetic nerve activity since we did not measure it. Futures studies are necessary to confirm this hypothesis.

The HB group presented decreased PE-induced increase in MAP compared with the LB group and no difference was noted with respect to blood pressure reduction in response to SNP. This may be explained because a lower baroreflex gain in the HB group would not adequately reduce the heart rate and cardiac output during PE infusion, which could contribute to the higher pressor response.

The variation in baroreflex sensitivity is surprising because WKY rats are an inbred strain. This variation in baroreflex sensitivity in an inbred strain, in which practically all alleles are homozygous and the individuals are quite similar, is supported by a previous study, which showed that WKY from two different laboratories presented differences regarding growth rate and blood pressure.

In our research the baroreflex function was evaluated in conscious rats, since baroreflex activity is blunted under anesthesia reducing the range of HR, which outcomes in an analysis of a restricted portion of the baroreflex response. Our investigation provides valid information regarding the discrepancy of baroreflex function among rats of the same strain, in this case the WKY strain. It would be interesting to compare this cardiovascular reflex in rats of other strains such as SHR, SHR stroke prone (SHRSP), Wistar rats and in other animals such as rabbit and mouse.

Baroreflex dysfunction is a clinical situation indicating impairment of autonomic function and predicts cardiovascular diseases. The data is relevant since baroreceptor reflex is largely studied in different models and strains of rats aiming to prevent hypertension development in humans, because reduced baroreflex function is indicative of cardiovascular disease.

In conclusion, we found differences of baroreflex function among WKY rats. The majority of animals (82%) presented attenuated parasympathetic activity of the baroreflex (reduced bradycardic responses to increase in arterial pressure and reduced baroreflex gain tested with PE) while 22% presented decreased sympathetic activity of the baroreflex (reduced tachycardic responses to decreased arterial pressure and reduced baroreflex gain tested with SNP). Interpretation of studies employing "the WKY strain" as a control for the SHR may be much more problematic than previously recognized.

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