

## Evaluation of Baroreflex Function in Young Spontaneously Hypertensive Rats

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### Summary

**Background:** The literature describes contradictory data regarding the onset of the baroreflex reduction in spontaneously hypertensive rats.

**Objective:** This investigation was undertaken to evaluate the baroreflex function in 13-week-old spontaneously hypertensive rats.

**Methods:** Male Wistar Kyoto (n=15) and spontaneously hypertensive rats (n=15) aged 13 weeks were studied. Cannulas were inserted in the abdominal aortic artery through the right femoral artery to measure mean arterial pressure and heart rate. Baroreflex function was calculated as the derivative of the variation of HR in function of the MAP variation ( $\Delta$ heart rate/ $\Delta$ mean arterial pressure) tested with a depressor dose of sodium nitroprusside (50 $\mu$ g/kg) and with a pressor dose of phenylephrine (8 $\mu$ g/kg) in the right femoral venous approach through an inserted cannula in awake spontaneously hypertensive rats and Wistar-Kyoto. Differences with p values < 0.05 were considered statistically significant.

**Results:** Spontaneously hypertensive rats:  $\Delta$ mean arterial pressure=43.5mmHg $\pm$ 5.2,  $\Delta$ heart rate=-59.7ppm $\pm$ 17.9 and  $\Delta$ heart rate/ $\Delta$ mean arterial pressure=1.3ppm/mmHg $\pm$ 0.1 tested with phenylephrine; Wistar Kyoto:  $\Delta$ mean arterial pressure=&56mmHg $\pm$ 3,  $\Delta$ heart rate=-114.9ppm $\pm$ 11.3 and  $\Delta$ heart rate/ $\Delta$ mean arterial pressure=#1.9ppm/mmHg $\pm$ 0.3 tested with phenylephrine; spontaneously hypertensive rats:  $\Delta$ mean arterial pressure=-45.6mmHg $\pm$ 8.1,  $\Delta$ heart rate=40.1ppm $\pm$ 11.6 and  $\Delta$ heart rate/ $\Delta$ mean arterial pressure=0.9ppm/mmHg $\pm$ 0.5 tested with sodium nitroprusside; Wistar Kyoto:  $\Delta$ mean arterial pressure=-39.8mmHg $\pm$ 6.2,  $\Delta$ heart rate=51.9ppm $\pm$ 21.8 and  $\Delta$ heart rate/ $\Delta$ mean arterial pressure=1.4ppm/mmHg $\pm$ 0.7 tested with sodium nitroprusside (\*p<0.05; #p<0.01; &p<0.001).

**Conclusion:** Our results showed that 13-week-old spontaneously hypertensive rats presented reduced baroreflex function when tested with phenylephrine. (Arq Bras Cardiol 2009;92(3): 205-209)

**Key words:** Hypertension; baroreflex/control; central nervous system; age of onset.

### Introduction

Several factors (neural, humoral, myogenic) are involved in the onset of hypertension and different animal models have been used to study this pathology, such as the renal hypertension model, the DOCA-salt hypertension model, the neurogenic hypertension model and the genetic model of hypertension in spontaneously hypertensive rats (SHR). SHR is a suitable model to study hypertension development as it is similar to humans with essential hypertension. These similarities include a genetic predisposition to high blood pressure with no specific etiology, increased total peripheral resistance without volume expansion and similar responses to drug treatment<sup>1</sup>.

In cardiovascular physiology, the baroreflex or baroreceptor reflex is one of the body's homeostatic mechanisms to maintain blood pressure. It provides a negative feedback loop in which the elevated blood pressure reflexively causes blood pressure to decrease; similarly, the decreased blood pressure depresses the baroreflex, causing blood 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<sup>2</sup>. Previous studies on the development of young SHR baroreflex function have yielded conflicting results, when compared to normotensive rats (Wistar-Kyoto - WKY)<sup>3</sup>. Morrison et al<sup>4</sup> indicated that the baroreceptor function is approximately the same between SHR and WKY aged 15 weeks. However, studies of Lundin et al<sup>5</sup> showed that SHR of the same age presented reduced baroreflex function. A precise knowledge of early development damage to the baroreflex function is essential to understand hypertension as a disease process<sup>6,7</sup>. In view of these contradicting data, we aimed at evaluating

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baroreflex function in 13-week-old SHR tested with sodium nitroprusside and phenylephrine and verify whether there was a reduction in the baroreflex in SHR at this age.

## Methods

### Animals

The animals were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Institutes of Health (publication no. 96-23, revised, 1996) and were approved by the Animal Ethics Committee of our University (number 1071/06). Experiments were performed on male spontaneously hypertensive rats (SHR) (13 weeks old, 260-300 grams,  $n=15$ ) and Wistar Kyoto (WKY) rats (13 weeks old, 260-300 grams,  $n=15$ ) obtained from the Centro de Desenvolvimento de Modelos Experimentais para a Medicina e Biologia (CEDEME). The temperature was maintained at 22°C, air humidity at nearly 60% and the light-dark cycle was controlled and established as twelve hours each. Animals had free access to food and water.

### Arterial pressure recording in awake rats

Under anesthesia with halothane, a polyethylene cannula (PE 10 connected to a PE 50) was inserted into the abdominal aorta through the right femoral artery for the measurement of the mean arterial pressure (MAP) and heart rate (HR). A second cannula was inserted into the femoral vein for drug administration. Both cannulas were tunneled subcutaneously and exposed on the back of the rat to allow access when the animal was conscious. The MAP was measured with a strain gauge transducer (Statham P23Db) connected to a low-level direct-current preamplifier coupled to a polygraph (Beckman model R-611). HR was derived from arterial pressure waves with a cardiometer (coupler type 9875B). Studies were performed in conscious rats 24 hours after the surgical procedures, to allow animals to recover from the anesthetic effects. The experiments were initiated approximately 30 minutes after the period of their adaptation to laboratory song and light conditions<sup>8</sup>.

### Baroreflex test

The baroreflex was tested with a pressor dose of phenylephrine (PE-bolus-8  $\mu\text{g/kg}$  IV; Sigma Chemical) and depressor doses of sodium nitroprusside (NPNa-bolus-50  $\mu\text{g/kg}$  IV; RBI)<sup>9</sup>. The baroreflex was calculated as the derivation of HR in function of the MAP variation ( $\Delta\text{HR}/\Delta\text{MAP}$ )<sup>10</sup>. There was an interval of at least 15 minutes between the infusions to allow the recovery of basal values.

### Statistical analysis

Values are reported as the means  $\pm$  standard deviation of means. HR, MAP,  $\Delta\text{HR}$ ,  $\Delta\text{MAP}$  and  $\Delta\text{HR}/\Delta\text{MAP}$  were compared between SHR and WKY. After the distributions were evaluated through the Kolmogorov normality test, the *Student's T* test was used to verify differences between normal distributions and the Mann-Whitney test was used to assess differences between non-parametric distributions. The significance level was set at  $p<0.05$  for all statistical tests.

## Results

Body weight was not significantly different between spontaneously hypertensive rats (SHR) ( $270\text{g}\pm 8$ ) and Wistar-Kyoto (WKY) ( $298\text{g}\pm 5$ ) ( $p>0.05$ ). SHR presented a higher heart rate (HR) ( $p<0.05$ ) and higher mean arterial pressure (MAP) ( $p<0.05$ ) (Table 1). According to the literature, the higher MAP and HR in SHR aged 13 weeks were to be expected.

$\Delta\text{HR}$  (Figure 1A) and  $\Delta\text{MAP}$  (Figure 1B) were significantly different between the groups when baroreflex sensitivity was tested with PE. The variation in cardiovascular parameters were reduced in SHR ( $\Delta\text{HR}$ :  $p<0.05$ ;  $\Delta\text{MAP}$ :  $p<0.001$ ). Figure 2A and Figure 2B showed no significant differences between SHR and WKY regarding  $\Delta\text{HR}$  ( $p>0.05$ ) and  $\Delta\text{MAP}$  ( $p>0.05$ ) when tested with NPNa. WKY showed higher variations in MAP, while SHR presented higher variations in HR.

Baroreflex function ( $\Delta\text{HR}/\Delta\text{MAP}$ ) was tested with PE and NPNa. A significant difference was observed between the groups, with WKY presenting higher values ( $p<0.01$ ) (Figure 3A). Moreover, mean values of  $\Delta\text{HR}/\Delta\text{MAP}$ , when tested with NPNa, were also higher in WKY ( $p>0.05$ ) (Figure 3B).

## Discussion

Our investigation was undertaken to evaluate baroreflex function in 13-week-old spontaneously hypertensive rats (SHR) tested with sodium nitroprusside (NPNa) and phenylephrine (PE) and verify whether there was any difference between this study model and Wistar-Kyoto (WKY) rats of the same age. Our data showed that baroreflex sensitivity, when tested with PE, was decreased in SHR aged 13 weeks. Mean arterial pressure variation ( $\Delta\text{MAP}$ ), heart rate variation ( $\Delta\text{HR}$ ) and  $\Delta\text{HR}/\Delta\text{MAP}$ , when tested with PE, were significantly higher in WKY. However,  $\Delta\text{HR}$  and  $\Delta\text{HR}/\Delta\text{MAP}$ , when tested with NPNa, were not significantly higher in WKY, while SHR showed higher  $\Delta\text{MAP}$ .

The mechanisms that cause the reduction in the baroreflex function in SHR are not completely understood<sup>11</sup>. Some studies demonstrated that the carotid body in the adult SHR is significantly larger than in normotensive rats<sup>12-14</sup>, whereas other studies indicated that the decreased baroreflex function in SHR is due to impaired levels of norepinephrine, epinephrine and dopamine in the carotid body<sup>14-16</sup> and medulla oblongata areas that regulate the cardiovascular system<sup>4,8</sup>. Furthermore,

**Table 1 - Comparative analysis of weight and cardiovascular variables in SHR and WKY**

Cardiovascular Variable	SHR	WKY
<sup>a</sup> Weight (grams)	269.7 $\pm$ 12	298 $\pm$ 14,6
MAP (mmHg)	169.07 $\pm$ 4.85*	107.03 $\pm$ 6.97
HR (ppm)	466.19 $\pm$ 1.33*	371.1 $\pm$ 42.7

MAP - mean arterial pressure; HR - heart rate; ppm - Pulses per minute; mmHg - millimeters of mercury. <sup>a</sup>Animals were weighted before the surgical procedures. \* $p<0.05$ .

## Original Article

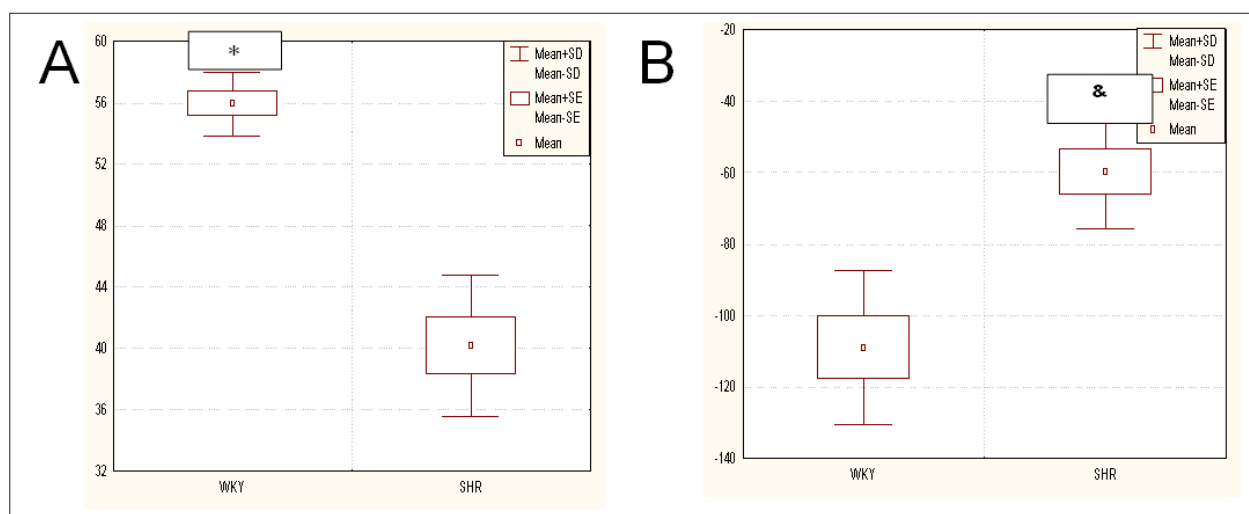


Figure 1 - Evaluation of baroreceptor function in SHR (n=15) and WKY (n=15) tested with PE: A - ΔHR (ppm) and B - ΔMAP (mmHg). \*p<0.05; \*\*p<0.001.

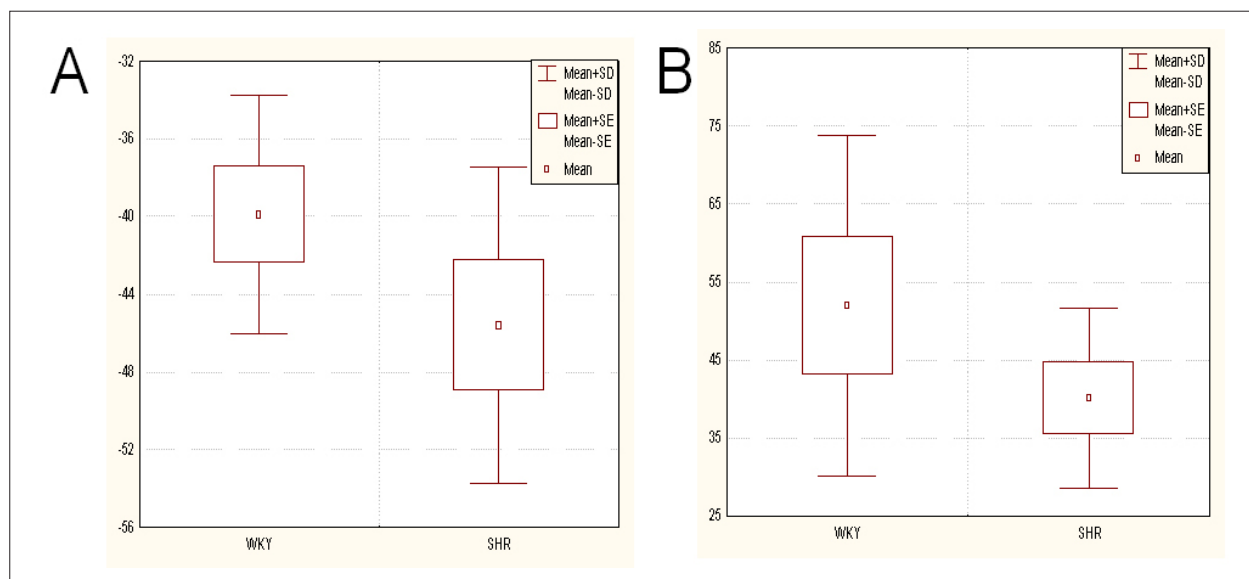


Figure 2 - Evaluation of baroreceptor function in SHR (n=15) and WKY (n=15) tested with NPNa: A - ΔHR (ppm) and B - ΔMAP (mmHg).

there have been reports that AT1 (angiotensin) receptor densities are increased in SHR, compared to the levels found in normotensive control rats<sup>17,18</sup>. Rossi et al<sup>19</sup> indicated that endogenous endothelin receptor mechanisms are involved in the hypertensive state observed in SHR. Moreover, recent investigations described the importance of oxidative stress<sup>20</sup>, nitric oxide<sup>21</sup> and the small GTPase rho-quinase<sup>22</sup> during baroreflex function development in SHR. Bertagnoli et al<sup>20</sup> suggested that exercise training reduces oxidative stress, which is associated to an improvement in baroreflex sensitivity in SHR. Waki et al<sup>21</sup> have shown that endogenous nitric oxide synthase activity in the medulla oblongata of SHR is increased when compared to WKY; it plays a major role in the preservation of the hypertension and decreases the cardiac baroreceptor reflex gain, which are features of this animal

model. According to Ito et al<sup>22</sup>, although the inhibition of endogenous rho-kinase in the medulla oblongata augments the baroreflex control of heart rate in WKY and SHR, it improves the impaired baroreflex function in SHR. Earlier studies that described a significant difference in blood pressure between WKY and SHR at 3 weeks of age<sup>23</sup> or at birth<sup>24</sup> were based on the assessment of a few animals. However, the age at which the baroreflex function starts to decrease in SHR has yet to be demonstrated.

In our findings, SHR presented lower depressor responses with PE, but higher depressor responses with NPNa, when compared to WKY. These contradictory data were unexpected. Gonzalez et al<sup>23</sup> electrically stimulated the aortic nerves of 15-week-old SHR using a wide range of stimulus frequencies. The depressor responses produced by these stimulations

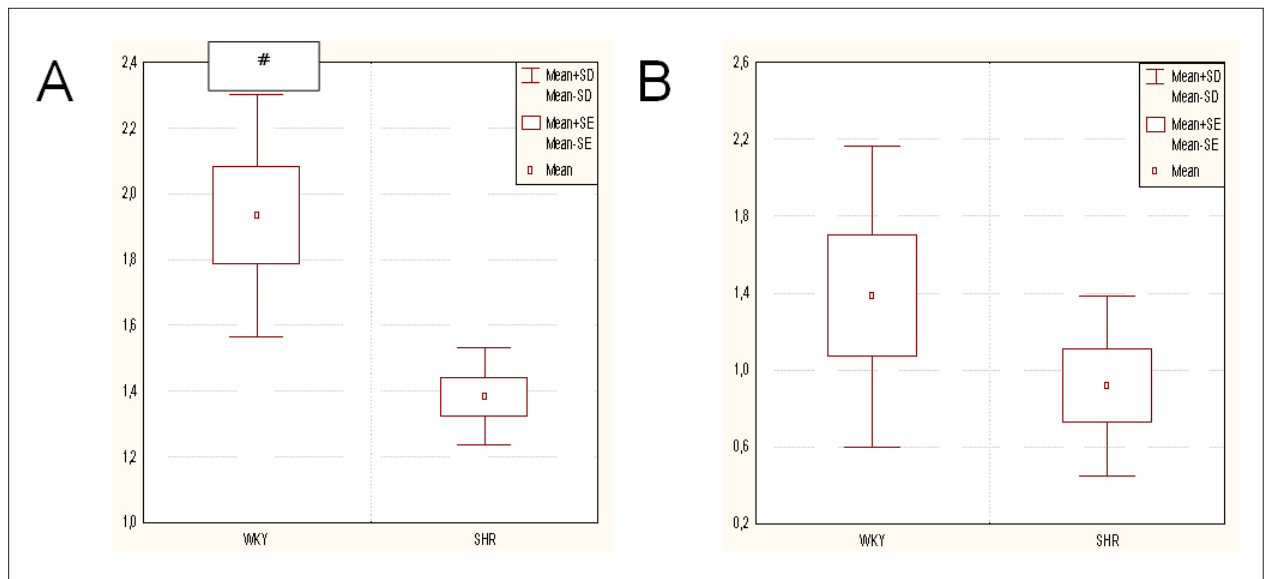


Figure 3 - Evaluation of baroreflex ( $\Delta\text{HR}/\Delta\text{MAP}$ , ppm/mmHg) in SHR (n=15) and WKY (n=15) tested with PE (A) and NPNa (B). \* $p < 0.05$ .

were significantly smaller in SHR than those in WKY. It was also reported in the same study that inhibition of the greater splanchnic nerve activity due to aortic nerve stimulation was found to be significantly smaller in SHR than in WKY and control of the sympathetic nerve activity was higher in SHR than in WKY. Judy et al<sup>24,25</sup> assessed the baroreceptor function through the spontaneous variation in MAP and sympathetic nerve activity in SHR and WKY between 5 and 40 weeks of age and verified that young SHR (5 weeks old) presented reduced baroreflex sensitivity. However, HR variation was not evaluated as in the present study.

Ohta et al<sup>26</sup> reported alterations in the afferent components of chemoreflex and baroreflex in 15-week-old SHR by aortic depressor nerve stimulation (depressor stimulus) and by carotid sinus nerve stimulation (pressor stimulus). They observed higher depressor responses in WKY and higher pressor responses in SHR. Lee et al<sup>27</sup> estimated baroreceptor responses in 6-10 month-old SHR by electrically stimulating the glossopharyngeal nerve and higher depressor responses were observed in SHR; however, bradycardia was reduced in SHR. In this study, our hypothesis was that SHR would present lower depressor and pressor responses; this fact was not observed regarding HR responses to intravenous NPNa. Methodological aspects may explain the differences in results among the aforementioned studies, as they used different procedures to evaluate pressor and depressor responses; moreover, we have not found in the literature any study that evaluated the baroreflex function tested with PE and NPNa in SHR younger than 14 weeks. Perhaps, SHR did not increase their MAP variability, similarly to WKY with PE, due to the fact that they presented high blood pressure.

Our findings showed that male 13-week-old SHR presented decreased baroreflex function. Nevertheless, conflicting data were described in the literature, considering the decreased baroreflex function observed in young SHR when evaluated through sympathetic activity control. Different studies showed

that baroreflex function in young SHR is approximately the same as<sup>4,5</sup> or lower than<sup>25,26</sup> the one observed in WKY. The study by Morrison and Whitehorn<sup>4</sup> demonstrated that the sympathetic hyper-reactivity in 15-week-old SHR did not correlate with the baroreceptor reflex, which was approximately similar to that of WKY, and they concluded that it could not confirm that the decreased baroreflex function in young SHR was due to their sympathetic hyperreactivity. Ricksten et al<sup>28</sup> recorded spontaneous variations in MAP, HR and rectified mean splanchnic nerve activity in conscious undisturbed 15 weeks old WKY and SHR. The variability in MAP was not significantly different, but HR variability tended to be lower in SHR. The variability in splanchnic nerve activity was not significantly different between SHR and WKY. Our data also showed lower HR variability in SHR, but the study by Ricksten et al<sup>28</sup> showed that the variability was not tested with drugs. Nagai et al<sup>29</sup> compared autonomic activity and baroreflex sensitivity in age-matched conscious groups of WKY and SHR aged 4-14 weeks. They verified that prazosin-sensitive and atropine-sensitive indices were associated with the elevation in blood pressure in both groups as well as that the pressor and depressor responses were not statistically different between SHR and WKY. It is possible that these results do not corroborate our data because different drugs were used.

Despite the fact that WKY presented higher cardiovascular parameter variability when  $\Delta\text{MAP}$  was tested with NPNa, SHR showed higher values than WKY. We have not found any evidence in the literature demonstrating which drug provides more reliable information about the baroreflex function. We believe that PE is a more reliable drug to evaluate baroreflex when compared to NPNa, as the latter causes venodilation and it may influence cardiac input and impair  $\Delta\text{HR}$ ,  $\Delta\text{MAP}$  and, consequently,  $\Delta\text{HR}/\Delta\text{MAP}$ <sup>30</sup>.

In conclusion, we showed that 13-week-old SHR presented significantly reduced baroreflex when tested with PE.

## Authors' contributions

All authors participated in the design of the study and the writing of the manuscript, as well as the approval of the final version of the manuscript.

## Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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## Study Association

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