

# Effects of Sinoaortic Denervation on Blood Pressure, Pulse Interval, Sympathetic Nerve Activity and Diaphragmatic Electromyogram Variability in Conscious Rats

MR Ushizima<sup>1</sup>, ED Moreira<sup>1</sup>, ET Costa<sup>2</sup>, P Castiglioni<sup>3</sup>, EM Krieger<sup>1</sup>, M Di Rienzo<sup>3</sup>, IA Cestari<sup>1</sup>

<sup>1</sup>Heart Institute (InCor) HC-FMUSP, São Paulo, Brazil

<sup>2</sup>Biomedical Engineering Department, FEEC-UNICAMP, Campinas, Brazil

<sup>3</sup>Laboratorio di Ricerche Cardiovascolari, Centro di Bioingegneria, Fnd Don C Gnocchi, Milan, Italy

## Abstract

*The effects of sinoaortic denervation on systolic and diastolic blood pressure (SBP, DBP), pulse interval (PI), renal sympathetic nerve activity (RSNA) and respiratory activity (RA) were investigated in conscious Wistar rats by wide-band spectral and coherence analysis. Signals were recorded during 90 minutes 6 hour after sinoaortic denervation (SAD, n=8) and in sham operated rats (n=8). After SAD animals showed higher BP mean and variability, heart rate and RSNA and lower PI variability and respiratory frequency. The rhythmic component centered at 0.4 Hz seen in RSNA in sham rats was blunted after SAD. The results suggest that PI variability and the RSNA component at 0.4 Hz depend on baroreceptors and may contribute to regulate BP within a physiological range of variability.*

## 1. Introduction

The relationship between arterial blood pressure (BP) and heart rate (HR) is complex and involves neural baroreceptors and chemoreceptors mechanisms. Under normal conditions blood pressure fluctuates within a physiological range. However, under pathophysiological conditions, impairment of regulatory mechanisms may change the balance between excitatory and inhibitory factors and may lead to hypertension and poor blood pressure regulation. Activation of baroreceptors have tonic inhibitory effects on the cardiovascular system that decrease sympathetic activity, increase parasympathetic activity and then decrease heart rate and blood pressure [1]. The removal of baroreceptors performed in sinoaortic denervation may be utilized as an useful model to understand the cardiovascular regulatory mechanisms of BP.

## 2. Methods

Sixteen Wistar rats were assigned to two groups undergoing SHAM (n=8) or SAD (n=8) operation.

## 2.1. Sinoaortic Denervation (SAD)

SAD was performed as described elsewhere [2]. Briefly, under ether anesthesia a 3 cm midline incision was made and sternocleidomastoid muscles were reflected laterally exposing the neurovascular sheath. The common carotid arteries and vagal trunk were isolated and the aortic depressor fibers and other fibers traveling with the sympathetic nerve or as an isolated aortic nerve were cut. To complete SAD, the sinus nerve as well as all carotid branches and the carotid body were cut.

## 2.2. Instrumentation

Arterial and venous cannulas were implanted one day before signal recording. While the rat was under pentobarbital (40 mg/kg) anesthesia, polyethylene cannulas filled with heparin in saline were inserted into the carotid artery for blood pressure registration and into jugular vein for drug and saline administration. Blood pressure, renal sympathetic nerve activity and diaphragmatic muscle EMG were recorded during 90 minutes. Pulse interval was estimated considering the interval between consecutive diastolic events. All recordings were performed 6 hours after the completion of surgery to allow the animals to recover from anesthesia. Bipolar platinum electrodes were implanted around the left renal sympathetic nerve and carefully isolated from the tissue by silicone rubber (SilGel 604, Waker). The sympathetic electroneurogram was amplified (10,000 to 100,000 times), filtered between 100 to 3000 Hz, rectified and low pass filtered (50 Hz) and integrated in each cardiac cycle to estimate RSNA [1, 3]. Bipolar electrodes were implanted in the diaphragmatic muscle for electromyographic (EMG) recording. EMG signals were full wave rectified and low pass filtered (50 Hz) to obtain respiratory activity (RA). Respiratory frequency (RF) was estimated considering the beginning of the inspiratory phase of respiration detected in the RA. The free ends of the RSNA and EMG electrodes and of the arterial and venous cannulas were tunneled

subcutaneously and exteriorized at the neck region.

### 2.3. Wide band analysis

Wide band analysis was performed as described in [4] to estimate not only the fast fluctuations with period of seconds but also slower components with period of hours. Sampled beat-to-beat events obtained from each cardiac cycle were interpolated by a cubic spline at 4 ms and re-sampled at 13,9 Hz.

A single spectrum from the first 65,536 points (~90 minutes) of each time series was obtained after progressive smoothing performed by wide band analysis based on Fast Fourier transform (FFT). Wide band power spectral density (WBPSD) was calculated for each time series in the frequency range between 0.0002 to 3 Hz.

The coherence between two time series was calculated by the wide band coherence analysis (WBCA) in the frequency range between 0.0017 to 3 Hz. The full time series was partitioned into eight segments of 8,192 points and the linear trend of each segment was removed. Previous to the FFT a 10% cosine tapering was applied and wide band smoothing was used.

### 2.4. Statistical analysis

Results are presented as the mean  $\pm$  standard deviation (SD) and variability as the standard deviation. T-test was used for group comparisons (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). The individual spectra were log transformed and averaged for SHAM and SAD animals. The individual coherence values were averaged for the whole groups after hyperbolic arctangent transformation of the coherence moduli. In the frequency domain differences between groups were evaluated by t-test.

## 3. Results

The SBP and DBP in SHAM and SAD rats are presented in Figure 1. SBP and DBP were higher after SAD (28% and 41%, respectively). Their variabilities increased 138% and 61%, respectively. Results of spectral analysis are shown in Figure 5.

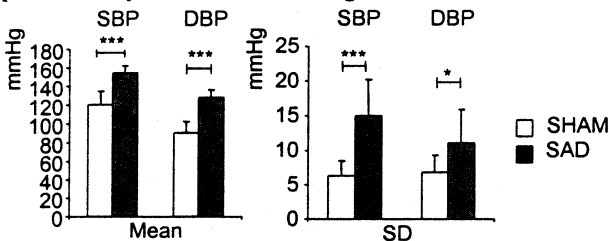


Figure 1. Left panel - Average systolic (SBP) and diastolic blood pressure (DBP) in SHAM (n=8) and sinoaortic denervated rats (SAD, n=8). Right panel - SBP and DBP variabilities.

The power of SBP after SAD was increased in the band between 0.7 to 1.5 Hz and between 0.001 to 0.15

Hz. The power of DBP was increased in the band between 0.0015 to 0.05 Hz and between 0.8 to 1.5 Hz and decreased from 0.2 to 0.5 Hz.

Pulse interval (Figure 2) was 12% lower after SAD and its variability decreased by 54%. The power of PI decreased in the frequency band between 0.0002 to 0.001 Hz and between 0.007 to 2 Hz (Figure 5).

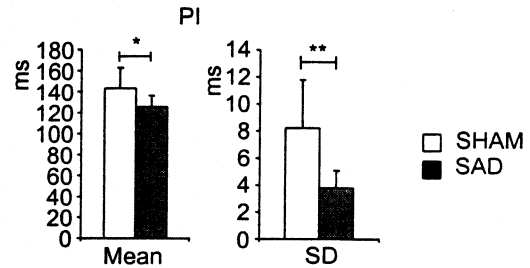


Figure 2. Left panel - Average pulse interval (PI) in SHAM (n=8) and sinoaortic denervated rats (SAD, n=8.). Right panel - PI variability.

RSNA increased 8% after SAD (Figure 3). No differences were found between groups in power spectral density of RSNA. However, rhythmic fluctuation characterized by a rhythmic fluctuation with central frequency around 0.4 Hz was blunted after SAD.

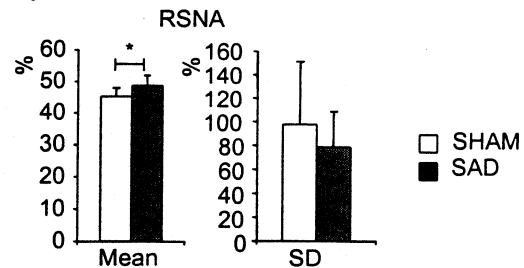


Figure 3. Left panel - Average renal sympathetic nerve activity (RSNA) in SHAM (n=8) and sinoaortic denervated rats (SAD, n=8). Right panel - RSNA variability.

RF was 22% lower after SAD (Figure 4) and the central frequency of the respiratory peak was shifted from 1.7 Hz to 1.35 Hz.

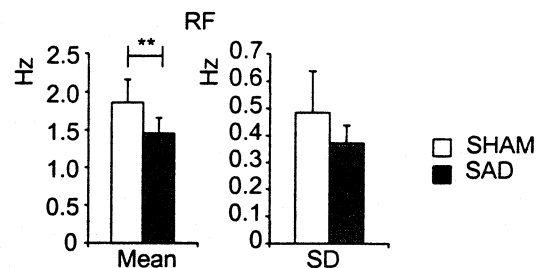


Figure 4. Left panel - Respiratory frequency (RF) in SHAM (n=8) and sinoaortic denervated (SAD, n=8) rats. Right panel - RF variability

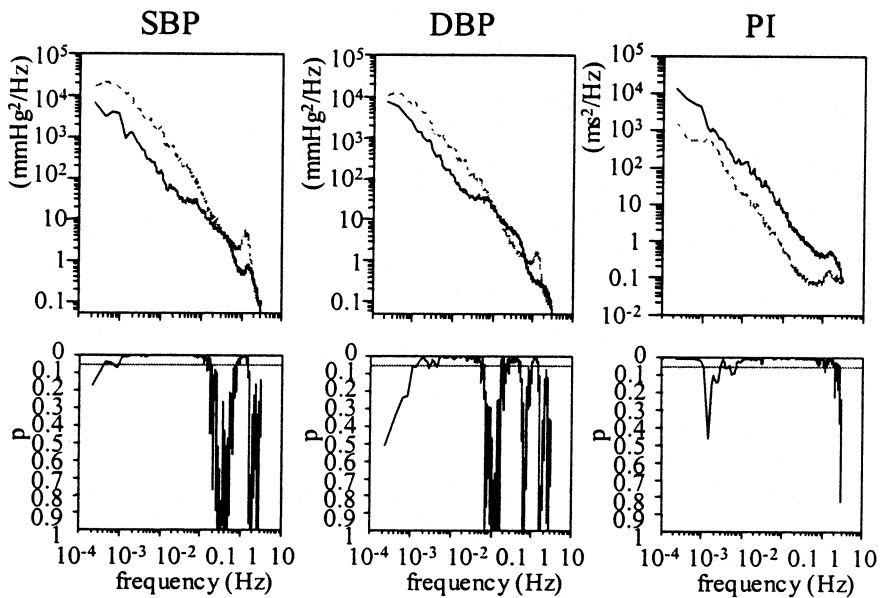


Figure 5. Average spectra obtained in SHAM (solid line, n=8) and SAD (dashed line, n=8) rats in the upper panel. Statistical significance is shown in the lower panel where the dashed line represents  $p = 0.05$ .

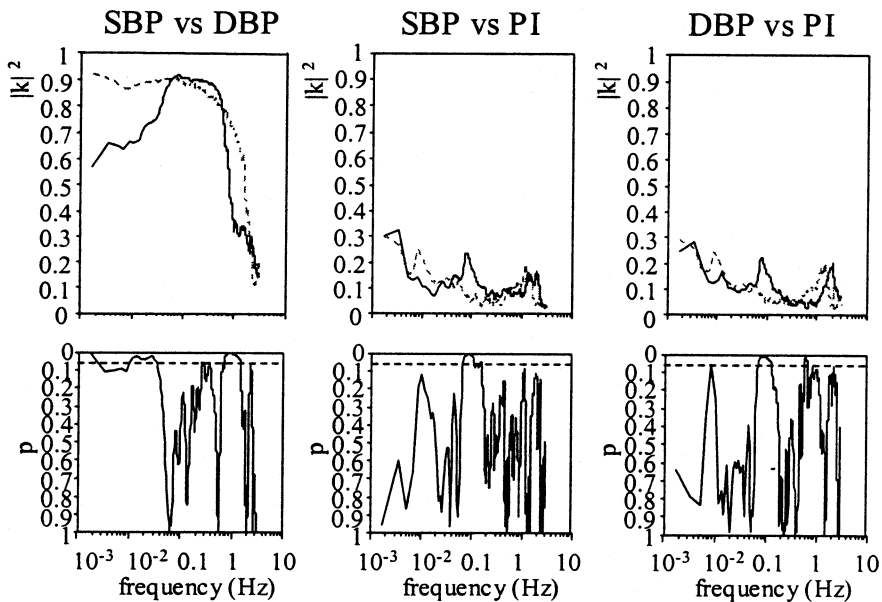


Figure 6. Average coherence data obtained in SHAM (solid line, n=8) and SAD rats (dashed line, n=8) in the upper panel. Statistical significance is shown in the lower panel where the dashed line represents  $p = 0.05$ .

The coherence between SBP vs DBP, SBP vs PI and DBP vs PI are shown in Figure 6. After SAD coherence was reduced between SBP vs PI was from 0.06 to 0.1 Hz and between SBP vs RSNA from 0.2 to 0.7 Hz. There was an increase in coherence between SBP vs RA and between PI vs RA around 1 Hz after SAD.

#### 4. Discussion

Blood pressure is under simultaneous excitatory and

inhibitory influences of neural and humoral mechanisms. Sympathetic activation and/or parasympathetic deactivation contribute to the excitation of the cardiovascular system and consequent increase in heart rate and blood pressure. SAD removes the afferent baroreceptors plus chemoreceptors information from the aortic arch and from carotid region. In our study, blood pressure, sympathetic nerve activity and respiratory activity were investigated in SHAM and sinoaortic

denervated rats. Sympathetic activity was determined from multi-fiber recordings which may be modified by the number of nervous fibers in the bundle, simultaneous fiber spikes, electrode coupling and isolation, making it difficult to compare nerve activities between groups [3]. A method of normalization of nerve activity was utilized which considered 3% of the cardiac cycles with the minimum nerve activity as zero activity and 3% of the cardiac cycles with the maximum nerve activity as 100% activity [1, 3]. Although this normalization allowed comparison of mean RSNA between groups, it may interfere with the variabilities calculated and this may partially explain why no differences in power spectral densities were found when groups were compared. After SAD, BP and its variability and the HR were higher when compared to SHAM. The increased BP variability after SAD is represented in the frequency domain by a predominant region of increased power of BP in the fast components (periods of seconds) and also in the slower components (periods of hour). After SAD PI variability is lower and this is represented by the decreased power of most of the components of PI spectra.

## 5. Conclusion

Baroreceptors act mainly buffering BP variability and increasing PI variability in the investigated frequency range. The results suggest that a baroreceptor dependent PI variability and RSNA rhythmic component at 0.4 Hz may contribute to regulate BP within a physiological range of variability.

## Acknowledgements

The first author of this study was supported by a scholarship of FAPESP, CAPES and CNPq.

## References

- [1] Irigoyen, MC, Moreira, ED, Ida, F, Pires, M, Cestari, IA, Krieger, EM, Changes of Renal Sympathetic Activity in Acute and Chronic Conscious Sinoaortic Denervated Rats. *Hypertension*, 1995; 26(2):1111-1116.
- [2] Krieger, E.M., Neurogenic Hypertension in the Rat, *Circ. Res.*, 1964;15:511-521.
- [3] Lundin, S, Ricksten, SE, Thorén, P, Renal sympathetic activity in spontaneously hypertensive rats and normotensive controls as studied by three different methods. *Acta Physiol. Scand.*, (1984);120:265-272.
- [4] Di Rienzo, M, Castiglioni, P, Parati, G, Mancia, G, Pedotti, A, Effects of Sinoaortic Denervation on Spectral Characteristics of Blood Pressure and Pulse Interval Variability: A Wide-Band Approach. *Med. Biol. Eng. Comp.*, 1996;34:1 - 9.

Address for correspondence.

Mauro Roberto Ushizima, PhD.  
Bioengineering Division, Heart Institute (InCor) HC-FMUSP  
Av. Dr. Enéas de Carvalho Aguiar, 44 – SS  
05403-000 São Paulo – SP  
Brazil  
mauro.ushizima@incor.usp.br