

## **Characteristic Enhancement of Blood Pressure V-shaped Waves in Sinoaortic-denervated Rats in a Conscious and Quiet State**

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Short title: Blood pressure V-shaped waves in sinoaortic-denervated rats.

## **Summary**

A hemodynamic feature of chronic sinoaortic-denervated (SAD) rats is the increase in blood pressure variability (BPV) without significant changes in the average level of blood pressure (BP). The current study was designed to investigate the changes in BP V-shaped waves (V waves) in SAD rats. Sprague-Dawley (SD) rats were divided into 2 groups: SAD rats and sham-operated rats (n = 13). Hemodynamics measurements were obtained in conscious, freely moving rats, four weeks after sinoaortic denervation or sham operation. V wave indices were evaluated in rats in both conscious and quiet states. Additionally, normal and high BPV was simulated by the production of V waves with different amplitudes. The results showed that the V wave amplitude was dramatically increased, with a significantly prolonged duration and reduced frequency in SAD rats. V wave BPV in SAD rats was significantly increased, though BP remained unchanged. The twenty-four hour BPV in all rats was positively correlated with amplitude, duration time and V wave BPV and negatively correlated with frequency. The systolic BP spectral powers in the low frequency range (0.38-0.45 Hz) were significantly reduced in the V waves of SAD rats. Moreover, there was a remarkable increase in mean BPV and a normal mean BP after simulating high BPV in SAD rats. These results suggest that enhancement of V waves might be a waveform character of BP in SAD rats in both the conscious and quiet states. These types of V waves appear to be related to a depression of sympathetic regulation of BP induced by sinoaortic denervation.

## **Key words**

Rat • Sinoaortic denervation • Nerve • Blood pressure • Blood pressure variability

## Introduction

Increased blood pressure variability (BPV) is an independent risk factor for end-organ damage in hypertension (Parati *et al.* 2006, Chowdhury *et al.* 2014, Leoncini *et al.* 2013, Miao *et al.* 2006). Animals that have undergone sinoaortic denervation are commonly used as models for studying high BPV. Long-term monitoring of conscious, sinoaortic denervated (SAD) animals has shown increased BPV, with no change in the average level of blood pressure (BP) (Norman *et al.* 1981; Flues *et al.* 2012, Sirvente *et al.* 2014). In fact, studies have suggested that BPV contributes more to end-organ damage than the level of BP itself (Miao *et al.* 2006). The possible mechanisms for end-organ damage induced by high BPV in SAD animals are direct endothelial lesions (Shen *et al.* 2006), inflammation (Zhang *et al.* 2003), oxidative stress (Zhang *et al.* 2015), renin-angiotensin system activation (Miao *et al.* 2003, Zhang *et al.* 2011), and increased cardiomyocyte apoptosis (Tao *et al.* 2004). Because of the marked feature of high BPV in SAD rats, it is reasonable to speculate that high BPV itself produces direct biophysical effects on the body, resulting in dysfunction. Simulating high BPV may be a better way of studying BPV itself; however this requires a more thorough understanding of the BP waveform. It has been reported that BP values were measured on the basis of BP wave recordings. Furthermore, pulse wave analysis was used to assess central aortic pressure. This is helpful when investigating the effect of anti-hypertensive agents on end organs (Avolio *et al.* 2010, Manisty and Hughes 2013). The four different patterns of the nocturnal BP profile are identified as normal dipping, nondipping, extreme dipping, and reverse dipping. These BP profile patterns are useful for assessing prognosis for organ damage (Afsar *et al.* 2015, Bendzala *et al.* 2015, O'Brien *et al.* 2003, Pickering *et al.* 2006).

However, there is little information regarding BP waveforms in high BPV and substantive knowledge of the BP waveform characteristic that simulates high BPV is lacking, thus the above speculation has not been examined further.

BPV is regulated by neural (Ramirez *et al.* 1985, Dai *et al.* 1997), humoral and vascular factors (Shen and Su 2000) and influenced by genetic and environmental factors (Xu *et al.* 2013). All of these factors ultimately impact the blood vessels in SAD animals. The BP waveforms of these animals may exhibit characteristic changes induced by high BPV; however, the characteristic changes in BP waveforms, when they occur, would be masked in moving animals. Therefore, the aim of this study is to examine whether the BP waveform characteristic appears in SAD rats in both conscious and quiet states. These results may contribute to our knowledge of SAD rats and be useful for simulating high BPV.

## **Materials and Methods**

### *Animals*

Male, Sprague-Dawley (SD) rats, weighing 220-250 g, were supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd. Rats were housed in a 12 h light/12 h dark cycle at a controlled temperature of  $24 \pm 2$  °C and humidity of 45%-60% and had free access to food and water. The Animal Care Ethics Committee of Taishan Medical University (2013004) approved all animal experiments.

### *Preparation of the SAD rat model and experimental group*

Sinoaortic denervation was performed according to the method described by Krieger with minor modifications (Krieger 1964, Liu *et al.* 2013, Yu *et al.* 2013). The SD rats were adapted to the housing conditions for 7 days prior to any procedures. Animals were anesthetized with pentobarbital sodium (40 mg/kg; Merck & Co., Inc, New Jersey, USA). A midline incision was made on the neck and bilateral sinoaortic denervation was performed. The superior laryngeal was isolated along the vagus nerve and a 5 mm segment was removed. The common carotid artery and the vagal trunk were isolated, and the rest of the tissue, including the aortic nerve, sympathetic trunk, and arterial sheath tissue were all cut by removing a 5 mm segment. The superior cervical ganglion was then removed, and the adventitia and its adjacent tissues in the carotid bifurcation were carefully isolated in the direction of the head. A 10% phenol in ethanol solution was applied to the isolated internal, external, and occipital arteries to remove any residual of nerve endings in the sinus caroticus region.

For the sham operation (Sham) a midline neck incision was made; the neck muscles were isolated bilaterally and phenol was applied to each common carotid artery to control for any potential effects of phenol in SAD rats. Rats were divided into 2 groups: an SAD group (n = 13) and a Sham group (n = 13). SAD was considered complete when the heart rate decreased by  $< 20$  beats per minute after a BP elevation of  $\geq 50$  mmHg after intravenous injection of phenylephrine (2–5 mg/kg). A heart rate decrease of 60–100 beats per minute has been discovered in sham-operated rats (Yu *et al.* 2013).

#### *Hemodynamic monitoring of conscious rats*

BP and heart rate were continuously recorded in conscious, freely moving rats as previously described (Liu *et al.* 2013, Yu *et al.* 2013, Shang *et al.* 2011, Xie *et al.* 2003). Briefly, 4 weeks after SAD or sham operations, rats were anesthetized with pentobarbital sodium and a floating catheter (PE10; Biotrol, Paris, France) was inserted into the lower abdominal artery via the left femoral artery; another catheter (PE50) was inserted into the left femoral vein for intravenous injection. After 2 days of recovery, rats were placed in individual cylindrical cages with food and water. A biosignal analytical system (model MPA 2000, Shanghai Alcott Biotech Co., Ltd, Shanghai, China) was connected to the arterial catheter to record BP. After a 14 h habituation, beat-to-beat BP and heart period (HP) values were acquired online for 24 hours. Offline analysis was performed to obtain related hemodynamic parameters. The beat-to-beat BP mean values were used as indexes of BP and the standard deviations of beat-to-beat BP values as indexes of BPV (Liu *et al.* 2013, Yu *et al.* 2013, Shang *et al.* 2011, Xie *et al.* 2003).

### *Determination of arterial baroreflex sensitivity (BRS)*

BRS was measured in conscious rats, after recording normal BP for 24 hours, using a previously described method (Smyth *et al.* 1969, Song *et al.* 2010, Yu *et al.* 2013). Briefly, a bolus of phenylephrine (3-5  $\mu\text{g}$  /kg; Sigma Chemical Co., St. Louis, MO, USA.) was administered via the femoral vein and BP was recorded. The dosage of phenylephrine was adjusted to elevate systolic BP(SBP) by 20-40 mmHg. There was a delay (about 1 second) between the increase in BP (stimulus) and the prolongation of the HP (response). HP (Y-axis) was plotted against SBP (X-axis) for linear regression analysis with 2 to 8 shifts. The slope with the largest correlation coefficient was taken as the BRS (msec/mmHg). The mean of two measurements served as the final result.

### *Measurement of amplitude, duration time and frequency of V waves*

BP was recorded from 9:00 a.m.-11:00 a.m. A surveillance camera (model 7028, Anni Digital Technology Co., Ltd, Shenzhen, China) was used to watch the rats during BP recording. Rats that did not move or engage in activity for 5 minutes were considered to be in a conscious and quiet state. Uncompressed BP of V waves in these rats were selected and analyzed offline using MPA 2000 software (Version 3.04.04.25). An entire V wave was taken as the point at which the BP started to drop until the point at which it was restored to the initial level. The amplitude of a V wave was represented by the difference between the maximum and minimum BP. The duration was taken as the time at which the BP of V wave started to drop to the time at which it was restored to the initial level. The frequency was calculated as the number of V waves that appeared in 5 minutes. The V wave amplitude and

duration time means over 5 minutes were taken as the final results. In this study, a V wave was considered valid only if the amplitude of diastolic BP(DBP) was more than 10 mmHg.

#### *Detection of the hemodynamic parameters of V waves*

The beat-to beat BP and BPV values for the V waves that appeared over 5 minutes in conscious, quiet rats were calculated using MPA 2000 software. The mean values of these parameters were taken as the final results.

#### *Power spectrum analysis of V waves*

The power spectrum of all V waves that appeared over 5 minutes in conscious, quiet rats was analyzed using MPA 2000 software. Calculations of SBP spectral powers in the low frequency (LF: 0.38-0.45 Hz) range and HP spectral powers in the high frequency (HF: 1.04-1.13 Hz) range were performed (Howarth *et al.* 2005, Cerutti *et al.* 1991) and mean SBP and HP powers were taken as the final results.

#### *Simulation of BPV in SAD and Sham rats*

The simulation system consisted of a flow control pump (model BT-100B, Huxi Instrument Company, Shanghai, China), a pressure roller pump (model PERIPRO-2H-220, Guangzhou Coslan instrument co. Ltd, Guangzhou, China), a pressure variability roller pump (model PERIPRO-2H-220, Guangzhou Coslan instrument co. Ltd, Guangzhou, China) and hemodynamic monitor (model MPA2000, Shanghai Alcott Biotech Co., Ltd, Shanghai, China). Heparinized whole blood from rats was allowed to flow into the simulation system and the

pressure pump was used to simulate BP. BPV in Sham rats was simulated by the producing repeated V waves each with an amplitude of about 15 mmHg, a duration times of about 2 s and a frequency of about 1 wave/5 seconds. BPV in SAD rats was simulated with repeated V waves of the same duration and frequency as above, but with amplitudes of approximately 40 mmHg. The amplitude, duration, and frequency were adjusted with a pressure variability roller pump (n = 4 simulations). BP results were recorded for 5 minutes via an MPA 2000 system. Mean BP (MBP) and mean BPV (MBPV) were obtained using the offline analysis detailed above.

#### *Statistical analysis*

Data are presented as means  $\pm$  SD. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Comparison of two groups was conducted with a t test or nonparametric test. Correlation analysis of two variables was performed using linear regression. Statistical significance was accepted at  $P < 0.05$ .

## Results

### *Twenty-four-hour hemodynamic data from SAD and Sham rats*

Hemodynamic changes were examined over a 24-hour period in order to describe the hemodynamic characteristics of SAD rats. SBP, DBP and MBP were not significantly different in SAD compared with Sham rats, however SBPV, DBPV and MBPV were significantly higher in SAD compared to Sham rats ( $P < 0.01$ ) (Fig. 1). Additionally, BRS was significantly lower in SAD than in Sham rats. Thus, the SAD rats exhibited high BPV with no change in BP, indicating that the SAD model was successfully generated in the current study (Yu *et al.* 2013, Yang *et al.* 2011).

### *Amplitude, duration time, and frequency of V waves in SAD and Sham rats in a conscious and quiet state*

The amplitude, duration time, and frequency were measured in order to identify the V wave characteristics (Figs. 2F, 2G) of rats in conscious, quiet states. The amplitude of SBP, DBP, and MBP of V waves was significantly higher in SAD rats compared to sham-operated rats ( $P < 0.01$ ). Also, the duration time of V waves was longer ( $P < 0.01$ ), and the frequency of V waves was lower ( $P < 0.05$ ) in SAD compared to sham-operated rats (Figs. 2A-2E)

### *Correlation analysis of the 24-hour hemodynamic parameters and the amplitude, duration time and frequency of V waves*

In both SAD and Sham rats, the 24-hour SBPV, DBPV, and MBPV were significantly positively correlated with the respective amplitudes of SBP, DBP, and MBP in V

waves ( $P < 0.01$ ) and with the respective duration times of V waves ( $P < 0.01$ ) while the 24-hour BPV was strongly negatively correlated with V wave frequency ( $P < 0.01$ ). The 24-hour SBP, DBP, and MBP were not correlated with any of the respective V wave parameters above (Table 1).

#### *Hemodynamic parameters of V waves in SAD and Sham rats*

Hemodynamic parameters were examined in SAD and Sham rats in a conscious and quiet state in order to investigate BP and BPV changes of V waves. The results showed that the SBPV, DBPV, and MBPV of V waves in the SAD group were significantly higher ( $P < 0.01$ ) than in the Sham group, though there was no significant difference in SBP, DBP, and MBP of V waves between these groups (Fig. 3).

#### *Correlation analysis between 24-hour hemodynamic parameters and BP and BPV of V waves in SAD and Sham rats,*

The 24-hour SBPV, DBPV, and MBPV were significantly positively correlated with the SBPV, DBPV, and MBPV of V waves, respectively, in both SAD and Sham rats, ( $P < 0.01$ ). However, the 24-hour SBP, DBP, and MBP were not correlated with the SBP, DBP, and MBP of V waves, respectively (Table 2).

#### *V wave Power spectrum changes in SAD and Sham rats*

Power spectrum analysis was performed to describe changes in autonomic nerve function in V waves of rats in a conscious and quiet state. SBP spectral powers in the LF

range decreased markedly in SAD compared with Sham rats (Fig. 4). There were no significant differences in HP spectral powers between SAD and Sham rats in the HF range.

*Simulated Hemodynamic parameters for SAD and Sham rats*

Simulated MBP for SAD rats was almost the same as the simulated MBP for Sham rats (Fig. 5A). However, simulated MBPV for SAD rats was markedly increased compared to that for Sham rats (Fig. 5B).

## Discussion

In this study, it was demonstrated that V waves are enhanced in conscious, quiet SAD rats. The enhancement of V waves in these rats was exhibited by larger amplitude, longer duration time, and lower frequency. Moreover, there was high BPV and no change in BP of V waves in SAD rats. The 24-hour BPV and the V wave BPV were significantly positively correlated in rats. The enhancement of V waves resulting from sinoaortic denervation may be related to a depression in the sympathetic regulation of BP. Furthermore, simulation of high BPV in SAD rats was produced when the corresponding V waves were simulated.

Since BP waveform characteristics can be disturbed by activity, the waveforms in this study were observed while rats were in a quiet state. The BP waveforms in conscious, quiet SAD rats were characterized as enhanced V waves. This suggests that V wave enhancement may be a waveform characteristic of BP in conscious, quiet SAD rats. The following evidence supports the V wave enhancement observed in this study: First, the V wave amplitude was dramatically increased, the duration time was significantly prolonged and the frequency was obviously reduced in SAD rats. In addition, the 24-hour BPV was positively correlated with V wave amplitude and duration time and negatively correlated with V wave frequency. Secondly, the SBPV, DBPV, and MBPV of V waves were significantly increased in SAD rats, however there were no significant changes in SBP, DBP, and MBP of V waves. Interestingly, these hemodynamic changes in V waves are consistent with the increased BPV and unchanged BP observed in SAD rats (Mancia and Parati 2003, Xie *et al.* 2005, Yu *et al.* 2013, Yang *et al.* 2011). Moreover, the 24-hour BPV was positively correlated

with the BPV of V waves in rats, but was not correlated with BP of V waves, indicating that the BPV of V waves reflected the 24-hour BPV. Third, simulation of high BPV was achieved when the corresponding V waves were simulated. On the basis of these findings, all V wave amplitudes of SBP in conscious, quiet SAD rats were more than 30 mmHg. Thus, enhanced V wave amplitude may be an indicator of successful baroreceptor denervation in SAD rats.

Vagus and sympathetic nerves play important roles in regulating BP and HP and alterations in their function can be analyzed with power spectrum analysis. HP spectral powers in the HF (1.04-1.13 Hz) range represent the function of vagus nerves and BP spectral powers in the LF range (0.38-0.45 Hz) are generally accepted as a marker of sympathetic modulation (Aubert *et al.* 1999, Pires *et al.* 2013, Cerutti *et al.* 1991, Howarth *et al.* 2005, Yeragani *et al.* 1998). The power spectrum was further analyzed to describe the production mechanism of V waves associated with autonomic nerve function. The present study indicated that, the SBP power in the LH range was reduced in the V waves of SAD rats whereas the HP power in the HF range showed no significant change. These results suggest that the sympathetic regulation of BP in V waves was depressed in SAD rats. Therefore, the dysfunction of sympathetic nerves might be one of the reasons for enhancement of V waves in conscious, quiet SAD rats.

In summary, these results indicate that enhancement of V waves may be a waveform characteristic of BP in conscious, quiet SAD rats. This enhancement may be related to depression of the sympathetic regulation of BP induced by sinoaortic denervation. The enhancement of V waves may add a new feature to SAD rats. Additionally, the V wave characteristics (an SBP amplitude of more than 30 mmHg) may be useful for evaluating the

success of sinoaortic denervation and simulation of high BPV. Future studies are needed to further investigate the mechanism(s) underlying the production of V waves.

## **Conflict of Interest**

The authors report no conflict of interest.

## **Acknowledgements**

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**Table 1. R-values for the correlation between 24-hour hemodynamic parameters and the amplitude, duration time and frequency of V waves.**

Variable	SBP	DBP	MBP	SBPV	DBPV	MBPV
Amplitude (SBP)	0.120	0.114	0.079	0.898**	0.828**	0.966**
Amplitude (DBP)	0.104	0.094	0.069	0.709**	0.735**	0.934**
Amplitude (MBP)	0.144	0.156	0.139	0.544**	0.781**	0.887**
Duration time	0.156	0.075	0.118	0.665**	0.658**	0.730**
Frequency	0.116	0.110	0.156	-0.801**	-0.796**	-0.597**

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; SBPV,

SBP variability; DBPV, DBP variability; MBPV, MBP variability. n = 26 (SAD + Sham). \*\*P < 0.01.

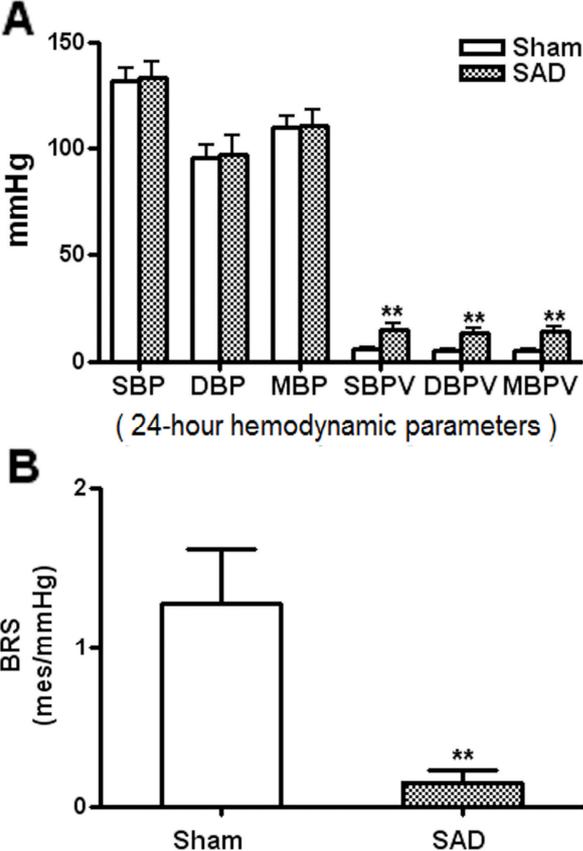
**Table 2.**

**R values for the correlation between the 24-hour BP and BPV and the corresponding hemodynamic parameters of V waves.**

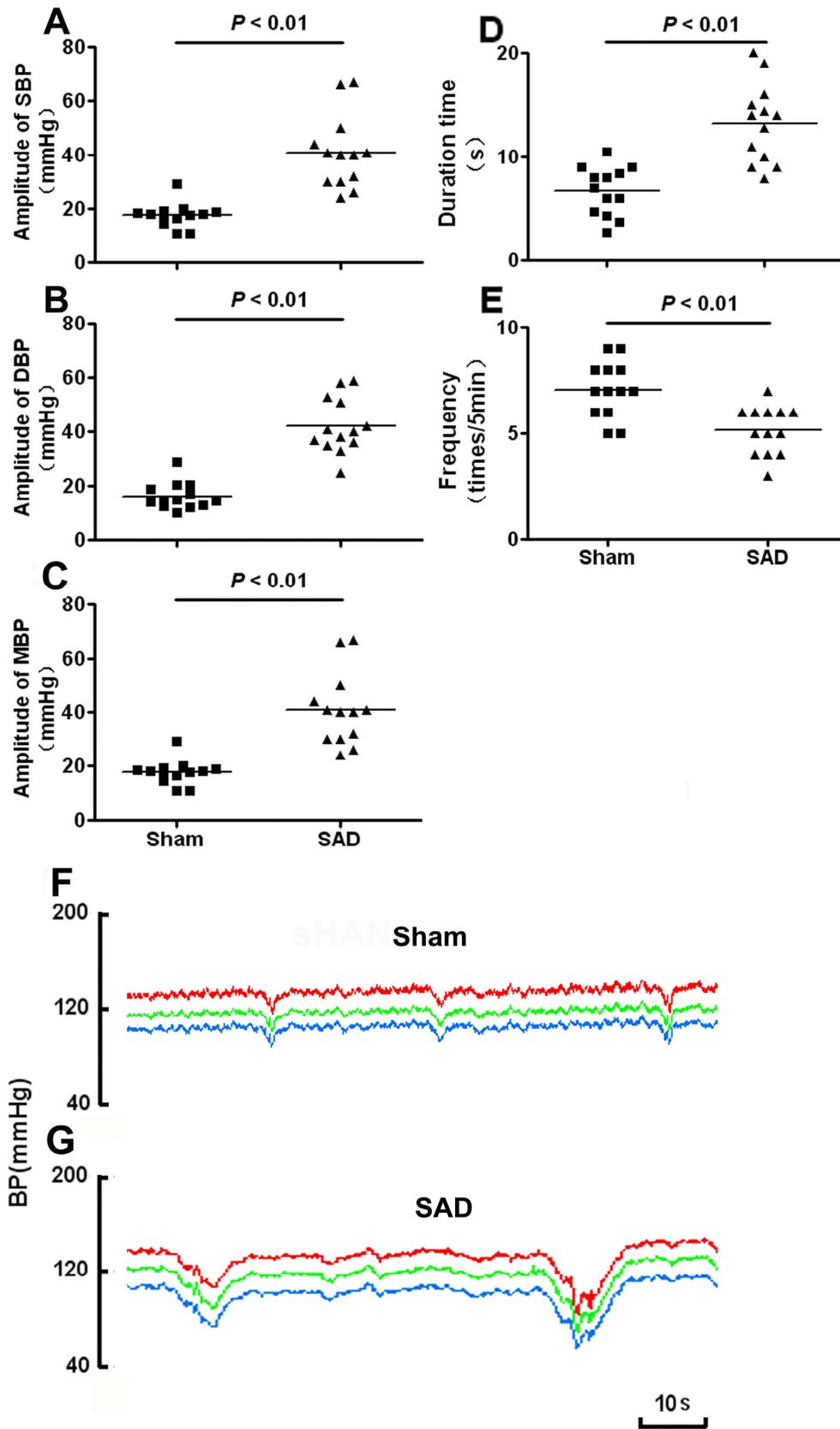
	R
V-SBP(mmHg)	-0.117
V-DBP(mmHg)	-0.166
V-MBP(mmHg)	-0.198
V-SBPV(mmHg)	0.847**
V-DBPV(mmHg)	0.929**
V-MBPV(mmHg)	0.957**

V-SBP indicates systolic BP of V waves; DBP, diastolic BP of V waves; MBP, mean BP of V waves; SBPV, SBP variability of V waves; DBPV, DBP variability of V waves; MBPV, MBP variability of V waves. n= 26 (SAD + Sham) . \*\*P < 0.01.

Fig.1



**Fig. 1.** Hemodynamic changes in SAD and Sham rats over 24 hours. (A) A comparison of BP and BPV in SAD and Sham rats. (B) A comparison of BRS in SAD and Sham rats. Values are means  $\pm$  SD (n = 13). \*\*P < 0.01 vs. Sham group.



**Fig. 2.** Amplitude (A, B, C), duration time (D), and frequency (E) of V waves and typical V

wave diagram in uncompressed state displayed with the MPA 2000 software in Sham (F) and SAD (G) rats. Red (top) represents SBP recording curve. Green (middle) represents MBP recording curve. Blue (bottom) represents DBP recording curve. Values are means  $\pm$  SD (n = 13). \*\*P < 0.01 vs. Sham group.

Fig.3

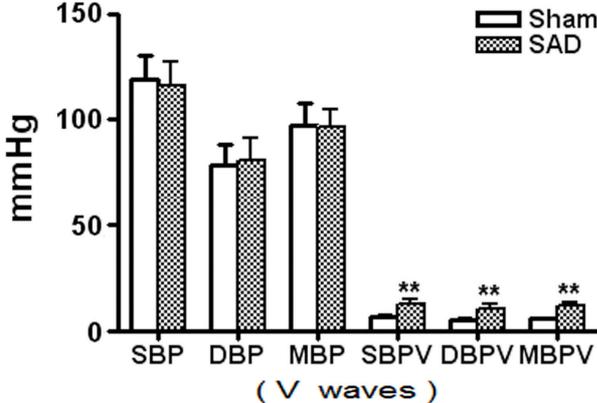


Fig. 3. Hemodynamic changes in V waves of SAD and Sham rats. Values are means  $\pm$  SD (n = 13). \*\*P < 0.01 vs. Sham group.

Fig.4

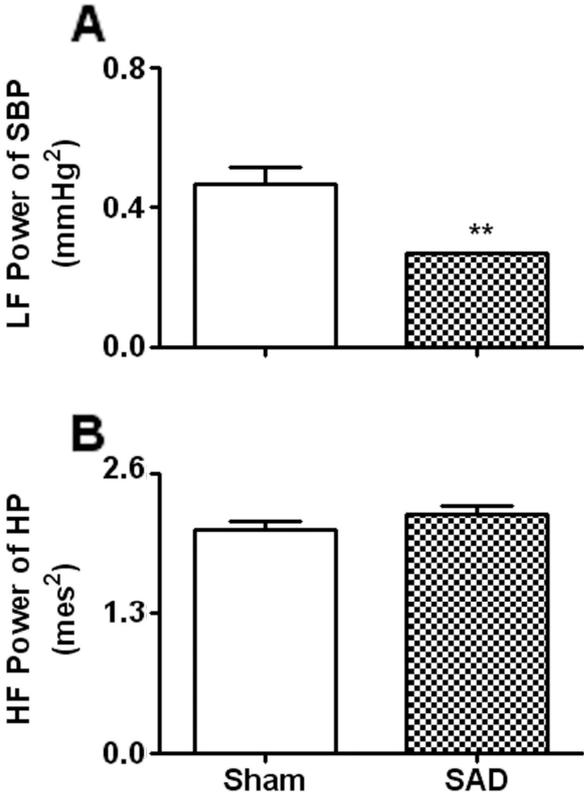


Fig. 4. Power spectrum changes in V waves of SAD and Sham rats. Values are means  $\pm$  SD (n = 13). \*\*P < 0.01 vs. Sham group.

Fig.5

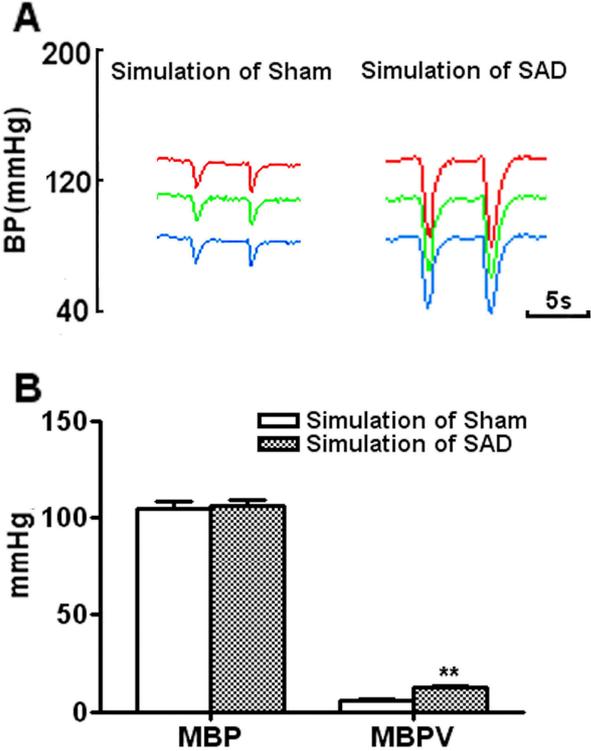


Fig. 5. Simulated hemodynamic changes. (A) Simulated V wave diagram for Sham and SAD rats in uncompressed state displayed with MPA 2000 software. (B) Simulated hemodynamic parameters for Sham and SAD rats. Values are means  $\pm$  SD (n = 4). \*\*P < 0.01 vs. Simulation of Sham group.