Increased Systolic Performance With Diastolic Dysfunction in Adult Spontaneously Hypertensive Rats

Oscar H. Cingolani, Xiao-Ping Yang, Maria A. Cavasin, Oscar A. Carretero

Abstract—Hypertensive heart disease is characterized by early development of hypertrophy and fibrosis that leads to heart failure (HF). HF develops in spontaneously hypertensive rats (SHR) after 18 months; however, it is not clear whether hypertrophy leads to altered cardiac performance at an earlier age in these rats. We studied cardiac performance in 10- to 11-month-old SHR and age-matched Wistar-Kyoto rats (WKY), using pressure-volume (PV) conductance catheter system to evaluate systolic and diastolic function in vivo at different preloads, including preload recruitable stroke work (PRSW), +dP/dt, and its relation to end-diastolic volume (+dP/dt–EDV) and preload-adjusted maximal power (PWRmax–EDV²) as well as the time constant of left ventricular pressure decay, τ (τ), as an index of relaxation. The slope of the end-diastolic pressure-volume relation (EDPVR) and the ex vivo PV relation, both indexes of stiffness, were also calculated for each heart, and the Doppler E/A ratio was determined. In addition, plasma samples were obtained to assess B-type natriuretic peptide levels (BNP). We found that PRSW was higher in SHR than in WKY (174.5 ± 15.6 versus 92.6 ± 18.9 mm Hg; P < 0.01). +dP/dt and +dP/dt–EDV were also enhanced in SHR versus WKY (9125 ± 662 versus 6633 ± 592 mm Hg/sec, P < 0.01, and 28.14 ± 4.35 versus 17.2 ± 2.8 mm Hg/s per μL, P < 0.02). In addition, PWR–EDV² was elevated in SHR (7.3 ± 1.5 versus 3.1 ± 0.6 mW/μL²). τ was prolonged in SHR (14.5 ± 1 ms versus 10.8 ± 0.8 for WKY, P < 0.02) and EDPVR was significantly greater in SHR than in WKY (0.01 ± 0.005 versus 0.004 ± 0.001, P < 0.05). The ex vivo pressure-volume relation was also steeper for SHR and the E/A ratio was 2.53 ± 0.15 for SHR versus 1.67 ± 0.08 for WKY (P < 0.02). BNP was 45 ± 2.5 pg/mL for SHR and 33.3 ± 1.8 pg/mL for WKY (P < 0.02). Taken together, these data suggest that at 10 to 11 months of age, before HF develops, SHR have increased systolic performance accompanied by delayed relaxation and increased diastolic stiffness. (Hypertension. 2003;41:249-254.)

Key Words: rats, spontaneously hypertensive ■ hypertrophy ■ systole ■ diastole ■ fibrosis

The heart is one of the main target organs in hypertension. Changes in myocyte expression of both contractile and noncontractile proteins and increased interstitial fibrosis are typically found during the natural course of hypertensive heart disease and result in progressive pathological hypertrophy, which may lead to heart failure (HF). However, it is not clear whether hypertrophy leads to altered cardiac performance at an earlier age in these rats. We studied cardiac performance in 10- to 11-month-old SHR and age-matched Wistar-Kyoto rats (WKY), using pressure-volume (PV) conductance catheter system to evaluate systolic and diastolic function in vivo at different preloads, including preload recruitable stroke work (PRSW), +dP/dt, and its relation to end-diastolic volume (+dP/dt–EDV) and preload-adjusted maximal power (PWRmax–EDV²) as well as the time constant of left ventricular pressure decay, τ (τ), as an index of relaxation. The slope of the end-diastolic pressure-volume relation (EDPVR) and the ex vivo PV relation, both indexes of stiffness, were also calculated for each heart, and the Doppler E/A ratio was determined. In addition, plasma samples were obtained to assess B-type natriuretic peptide levels (BNP). We found that PRSW was higher in SHR than in WKY (174.5 ± 15.6 versus 92.6 ± 18.9 mm Hg; P < 0.01). +dP/dt and +dP/dt–EDV were also enhanced in SHR versus WKY (9125 ± 662 versus 6633 ± 592 mm Hg/sec, P < 0.01, and 28.14 ± 4.35 versus 17.2 ± 2.8 mm Hg/s per μL, P < 0.02). In addition, PWR–EDV² was elevated in SHR (7.3 ± 1.5 versus 3.1 ± 0.6 mW/μL²). τ was prolonged in SHR (14.5 ± 1 ms versus 10.8 ± 0.8 for WKY, P < 0.02) and EDPVR was significantly greater in SHR than in WKY (0.01 ± 0.005 versus 0.004 ± 0.001, P < 0.05). The ex vivo pressure-volume relation was also steeper for SHR and the E/A ratio was 2.53 ± 0.15 for SHR versus 1.67 ± 0.08 for WKY (P < 0.02). BNP was 45 ± 2.5 pg/mL for SHR and 33.3 ± 1.8 pg/mL for WKY (P < 0.02). Taken together, these data suggest that at 10 to 11 months of age, before HF develops, SHR have increased systolic performance accompanied by delayed relaxation and increased diastolic stiffness. (Hypertension. 2003;41:249-254.)

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a micromanometer-conductance catheter system at different preloads to incorporate volume measurement in the assessment of cardiac performance and thereby eliminate load dependency. We wanted to test the hypothesis that in these adult hypertensive rats, enhanced cardiac mass leads to increased systolic performance in the presence of diastolic dysfunction. We also wanted to determine whether diastolic dysfunction is due to alterations in relaxation, stiffness, or both. To this end, we calculated preload-recruitable stroke work (PRSW), maximum dP/dt—end-diastolic volume relation (+dP/dt—EDV), and preload adjusted maximal power (PWR_{max}/EDV^2) to assess systolic performance and the time constant of left ventricular (LV) pressure decay, τ (τ) as a parameter of relaxation. The slope of the end-diastolic pressure-volume relation (EDPVR), an indicator of end-diastolic stiffness and Doppler early to late transmitral peak flow velocity ratio (E/A) were determined at the same time to evaluate diastolic function. We also obtained ex vivo pressure-volume (PV) curves after arresting the heart with KCl.

Methods

Animals and Procedures

Nine- to ten-month-old SHR and WKY from Taconic Farms were housed and maintained at a constant room temperature (22°C) and 12-hour light/dark cycle. They were fed standard rat chow and water ad libitum. After remaining in the animal facility for 1 week, blood pressure was measured once a week for 3 weeks by the tail-cuff method. By the fourth week, when they were 10 to 11 months old, rats were anesthetized with the long-lasting agent thiobutabarbital (Inactin, 100 mg/kg IP). Additional boluses of 10 mg/kg were given if necessary to stabilize anesthesia. The abdomen, chest, and neck were shaved, and the following procedures were performed: (1) echocardiography and Doppler; (2) in vivo cardiac PV loops; (3) ex vivo PV relationships.

Blood samples were obtained from the inferior vena cava for BNP determination at the end of the in vivo experiments, and the animals were euthanized.

Echocardiography and Doppler

Echocardiography and Doppler (Acuson, Sequoia C 256 with 15-MHz transducer) were performed while simultaneously recording ECG.

M-mode echocardiography was performed in the anterior short-axis view. LV posterior wall thickness was determined at the end of both diastole and systole. Pulsed-wave Doppler early to late transmural peak diastolic flow velocity ratio (E/A) was measured to assess diastolic function as described previously. Aortic systolic velocity-time integral (VTI) and aortic root diameter were determined and stroke volume calculated according to this formula:

\[ \text{Stroke volume} = \left( \frac{\text{VTI}}{\tau} \right), \]

where \( \tau \) is aortic diameter/2.

All Doppler spectra were recorded for 5 to 7 cardiac cycles at a sweep speed of 200 mm/s.

In Vivo PV Loops

Once the echocardiographic measurements were obtained, animals were placed on a warm pad (37°C). The right jugular vein was cannulated for fluid administration, and a 14-gauge catheter was placed in the trachea. They were ventilated with a positive-pressure ventilator (Harvard, model 680) with an FiO₂ of 0.21. Hexitel (2%) in 0.9% NaCl was infused intravenously during the procedure (200 μL/kg per minute for 10 minutes; then 20 μL/kg per minute throughout the experiment). The abdomen was opened and the diaphragm incised to reach the heart. A small stab was made with a 26-gauge needle at the LV apex, and a 2F miniaturized combined conductance catheter-micromanometer (model SPR-819, Millar Instruments) connected to a pressure-conductance unit (MPCU-200).

End-Systolic Stress and Mean Velocity of Circumferential Fiber Shortening Calculations

The LV end-systolic and end-diastolic radii were calculated from the respective volumes, assuming that the heart has a spherical shape with radius R. Midwall circumferential end-systolic stress (σ) was calculated according to the formula σ = (PR/h)/(2R + h), where P is end-systolic pressure, R is chamber radius (both calculated from the conductance catheter), and h is wall thickness, determined by echocardiography.

The ejection time of each heart was calculated from the duration of the VTI and corrected for HR. The mean velocity of circumferential fiber shortening (Vcf) was determined by dividing the shortening fraction by the rate-corrected ejection time according to the formula Vcf = (EDD — ESD)/EDD · ET**, where EDD = end-diastolic dimension, ESD = end-systolic dimension, and ET = rate-corrected ejection time.

Brain Natriuretic Peptide Determination

Before ending each experiment, 1 mL of blood was obtained from the inferior vena cava to measure plasma B-type natriuretic factor levels (BNP) (RIA kit, Peninsula Laboratory).
Statistical Analysis

Results were expressed as mean±SEM. Differences between groups were compared by Student t test. A value of P<0.05 was used as a criterion for statistical significance.

Results

Systolic blood pressure, heart rate, body weight, and LV weight are shown in the Table. SHR were smaller and had a faster heart rate than WKY. LVW/BW was significantly higher in SHR. Also, the ratio of LV radius to posterior wall thickness (R/H) was reduced by 37% in SHR (1.68±0.01 versus 2.31±0.07 in WKY), indicating increased concentricity in the hypertensive animals.

Figure 1 shows that EDV and SV were slightly higher in WKY, although the difference was not significant. EF was normal and similar in both strains, whereas EDP tended to be higher in the hypertensive rats (9.6±1.6 mm Hg versus 5.9±0.7 for WKY; P=0.08).

Plasma BNP, a marker of pressure-overloaded, was significantly increased in SHR (45±2.5 pg/mL versus 33.3±1.8 for WKY; P<0.05).

Figure 2 shows typical PV loops obtained after inferior vena cava occlusion in both strains, and overall results of EDPVR and ESPVR are shown in Figure 3. EDPVR differed between strains (0.01±0.005 versus 0.004±0.001 mm Hg/μL for SHR and WKY, respectively; P<0.02), suggesting that end-diastolic stiffness is increased in SHR. ESPVR was 1.14±0.24 mm Hg/μL for SHR versus 0.36±0.11 for WKY (P<0.02), suggesting increased systolic performance in the hypertensive rats.

In addition to the above parameters, PV loops recorded at different preloads can be used to derive other useful systolic function indexes that may be influenced less by loading conditions and cardiac mass. One of them is PRSW. This index is a modification of Sarnoff’s curve and represents the slope of the relation between stroke work and EDV. It has been described as independent of chamber size and mass and is sensitive to changes in contractile function. 28 Figure 4A shows PRSW for one SHR and its normotensive control. The slope is steeper in SHR than in WKY, indicating increased systolic performance. The figure also shows that overall PRSW was 174.5±15.6 mm Hg for SHR versus 92.6±18.9 for WKY (P<0.01). We also determined the relation between +dP/dt and EDV. +dP/dt is a classic parameter that is quite sensitive to changes in contractility but dependent on changes in preload. Analysis of the +dP/dt end-diastolic volume relation allowed us to compare +dP/dt of SHR and WKY at a given end-diastolic volume. Figure 4B shows that the slope of this relation was steeper in SHR (28.4±4.35 versus 12.7±2.8 mm Hg/s per μL for WKY; P<0.02), again indicating enhanced contractility.

Another parameter that may be useful for assessing changes in systolic performance is preload-adjusted maximal ventricular power (PWRmax/EDV). 28 Maximal power is the instantaneous peak product of pressure and flow and is highly dependent on preload. Dividing maximal power by the square of the end-diastolic volume (PWRmax/EDV2) yields an index that is minimally influenced by vascular loading. Changes in this parameter between SHR and WKY (7.3±1.5 versus 3.1±0.6 W/μL2, respectively; P<0.05) also suggest increased systolic performance in these hypertensive animals. Figure 5 shows that although +dP/dt was augmented in SHR (9125±662 versus 6633±392 mm Hg/s for WKY; P<0.01),

![Figure 1](image1.png)  
*Figure 1. End-diastolic volume (EDV), stroke volume (SV), ejection fraction (EF), and end-diastolic pressure (EDP) of SHR (black bars) and WKY (white bars).*

![Figure 2](image2.png)  
*Figure 2. In vivo PV loops obtained with a micromanometer conductance catheter system at different preloads, showing differences in ESPVR and EDPVR between WKY and SHR. Note that figures are drawn to a different scale.*
−dP/dt (the maximal velocity of the drop in pressure) was not significantly different. According to this, the ratio between both maximal velocities increased, suggesting a negative lusitropic pattern in the hypertrophic heart of SHR. The time constant of isovolumic pressure decay increased by ≈40% in these animals (14.5±1 versus 10.8±0.4 ms for SHR and WKY, respectively; P<0.05), likewise indicating impaired relaxation; however, the Doppler E/A ratio indicated a predominant “restrictive pattern.” The increased EDPVR observed in SHR as well as the increased E/A ratio all suggest that the predominant manifestation of dysfunction is increased end-diastolic stiffness. However, the delayed relaxation assessed by the ratio of +dP/dt/−dP/dt and the prolonged τ make it difficult to rule out an incomplete relaxation state that could have influenced EDPVR. Nevertheless, in the ex vivo experiments (Figure 6), in which the hearts were quiescent and therefore the delayed relaxation observed in vivo could not account for the shift in the PV relation, the curve was steeper in SHR, confirming the increased stiffness found in the beating heart with the pressure-conductance catheter.

Discussion
To our knowledge, this is the first study comparing the contractile function in SHR with its normotensive control WKY using PV methodology. In addition, we assessed diastolic function in these animals by using this technique and two others. PV analysis is a useful approach for examining the intact chamber function independently of load.

Diastolic dysfunction was expected in the SHR, based on the increased wall thickness and fibrosis and the impaired relaxation and augmented chamber stiffness reported by others using this model.1,4,29 These alterations were detected in vivo in our study, based on the increased EDPVR, the restrictive pattern of the Doppler E/A ratio, and the leftward shift in the PV relation observed in the ex vivo experiments. Impaired relaxation was also detected in our hypertensive

Figure 3. Overall values (slopes) of ESPVR and EDPVR. Black bars represent SHR; white bars, WKY. *P<0.02.

Figure 4. A, Relation between stroke work (SW) and EDV for one SHR (重庆) and one WKY (•). Inset depicts overall slope values. B, Same for +dP/dt–EDV. Note that for both relationships, slope values are higher for SHR (black bars) than for WKY (white bars), suggesting that systolic performance is increased in hypertensive rats. *P<0.02.

Figure 5. Basal hemodynamic parameters: maximal velocity of rise in pressure (+dP/dt), maximal velocity of fall in pressure (−dP/dt), +dP/dt/−dP/dt ratio, time constant of pressure decay (τ), and transmitral diastolic Doppler E/A ratio. Black bars represent SHR; white bars, WKY. *P<0.05.
animals, as reflected by the prolonged \( \tau \) and the fact that for a given maximal velocity of contraction \( (+\,dP/dt) \), maximal velocity of relaxation \( (-dP/dt) \) was decreased. Relaxation, an active process, depends mostly on calcium uptake by the sarcoplasmic reticulum during diastole, and under normal conditions usually takes place during the first third of diastole. End-diastolic stiffness, although it can be influenced by different factors, is affected more by myocardial structural components. We observed both increased stiffness and delayed relaxation in SHR. The fact that chamber compliance was also reduced in these rats in the ex vivo PV experiments suggests the important role played by the passive structural components of the ventricle. The Doppler E/A ratio also supported these findings. Whereas this ratio has been shown in different studies to detect alterations in relaxation early in hypertension (“inverted” E/A ratio), it can also show decreased compliance, usually at a later stage, when the ventricle stiffens. This was the case in our hypertrophic rats, in which according to our findings, stiffness seemed to predominate at this age.

Plasma BNP, a recently accepted indicator of HF, was significantly elevated in the rats with hypertrophy, clearly showing the value of this peptide as an early marker of diastolic dysfunction. Other investigators have observed elevated BNP in patients with isolated diastolic HF.

The EF values did not differ between SHR and WKY. EF is known to be influenced by both preload and afterload and therefore cannot reliably be used to assess contractile function in models where both preload and afterload are altered. Instead, all indexes derived from the PV loops showed enhanced contractile pump function in the hypertensive rats. Historically, ESPVR (Ees or E max) was proposed as a fairly load-insensitive index of contractility. ESPVR was increased in SHR; however, as this relation can be altered not only by changes in inotropic state but also by changes in chamber geometry and other diastolic factors, we decided to calculate other parameters as well. PRSW, the linear relation between SW and EDV, may be sensitive to changes in systolic function. It has the advantage of being stable throughout hearts of different sizes; and although it integrates data from the entire cardiac cycle, it is influenced most of all by systole. PRSW was augmented by \( \approx 80\% \) in SHR compared with WKY.

\[ +dP/dt – EDV \]

another PV-derived index known to be sensitive to contractile changes and insensitive to preload, also increased by \( \approx 100\% \) in SHR. When maximal power normalized for preload \( (PWR_{max}/EDV^2) \) was determined for both strains, a similar increase was again detected in SHR.

Taking all our data together, we could conclude that the SHR heart has enhanced pump function. According to these results, and considering that most of these adult hypertensive animals that already have diastolic dysfunction will have HF in the next few months, we questioned whether the enhanced contractile function could be the result of an exuberant hypertrophic response leading to an excess of parallel sarcomeres, working each of them with subnormal function. The end-systolic stress–rate corrected mean velocity of circumferential fiber shortening (Vcf) relation, which is known to be independent of heart rate and afterload, can be used to evaluate contractility at the myocardial level (contractile state corrected by chamber radius/wall thickness ratio). By plotting \( \rho \) against Vcf, as seen in Figure 7, we observed that both SHR and WKY showed a similar inverse linear correlation, as described for hearts with similar degrees of inotropism. If sarcomeres were increased in number and inotropism depressed, we would expect to see the same slope for SHR, but with a lower ordinate intercept (ie, lower Vcf for a
given level of stress). On the contrary, our findings indicated that although systolic performance of the SHR ventricle is enhanced, this is due to the adequate hypertrophic response observed in this model that compensates for the increased wall stress these myocytes are subject to, which at this point, seems to have preserved inotropism. Increased systolic function with normal inotropic state has been previously described in dogs in a model of short-term hypertrophy. However, we would like to emphasize that the data reported herein cannot necessarily be extrapolated to other models.

In conclusion, compared with WKY, the adult SHR has increased systolic performance with diastolic dysfunction. The supernormal systolic function is due to a compensated hypertrophic response with preserved inotropism. Alterations in both active relaxation and passive compliance characterize the diastolic dysfunction observed in these adult hypertensive animals.

References

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Hypertension. 2003;41:249-254; originally published online February 3, 2003;
doi: 10.1161/01.HYP.0000052832.96564.0B
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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