Prevention of concentric hypertrophy and diastolic impairment in aortic-banded rats treated with resveratrol

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HEART FAILURE is a primary cause of death in most parts of the world (12, 19). It is a multifactorial syndrome wherein the ability of the heart to pump sufficient amounts of blood to meet the metabolic demands of organs and tissues is compromised. Irrespective of the primary cause, the heart counters this insult with a compensatory hypertrophic response that includes activation of the sympathetic nervous system, the renin-angiotensin system, and various other mediators. As a consequence of this response, the heart undergoes remodeling by a series of changes in its structure, function, and phenotype (5, 21, 23). Although hypertrophy may be beneficial to the stressed myocardium, sustained hypertrophy leads to heart failure. Hypertrophy may be categorized into two types: that due to pressure overload (PO) or due to volume overload (VO) (5, 21, 23). Whereas PO results in concentric hypertrophy wherein the ventricular wall thickness increases without simultaneous chamber enlargement, VO results in eccentric hypertrophy wherein the chamber volume increases without any change in the ventricular wall thickness. The mechanisms underlying the development of hypertrophy and its transition to heart failure are poorly understood and are therefore a prime area of cardiovascular research.

Blockade of the β-adrenergic receptor signaling cascade as well as the renin-angiotensin-aldosterone system have been the major strategies used to combat cardiac hypertrophy. In the recent past, antioxidants have also been reported to render beneficial effects against the deleterious effects of cardiac hypertrophy in different experimental models (8, 10, 11, 20, 27). Furthermore, gene manipulation studies have also confirmed the role of antioxidants as potent antihypertrophic agents (25). In this regard, the French paradox stands out as an excellent example of reduced incidence of heart disease in the French population on account of regular wine consumption (6).

MATERIALS AND METHODS

Creation of animal model. The following experimental protocols were approved by the Animal Care Committee of the University of Manitoba and are in agreement with the Canadian Council on Animal Care Concerning the Care and Use of Experimental Animals (vol. 1, 2nd ed., 1993). Male Sprague-Dawley rats weighing 150–200 g were treated with resveratrol. Am J Physiol Heart Circ Physiol 292: H2138–H2143, 2007; doi:10.1152/ajpheart.00852.2006.—This study was designed to examine the effects of the antioxidant resveratrol on cardiac structure and function in pressure overload (PO)-induced cardiac hypertrophy. Male Sprague-Dawley rats were subjected to sham operation and the aortic banding procedure. A subgroup of sham control and aortic-banded rats were treated with resveratrol for 2 wk after surgery. Echocardiographic analysis of cardiac structure and function along with Western blot analysis of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), and redox factor-1 (ref-1) were performed in all groups after 4 wk of surgery. Banded rats showed significantly increased left ventricle-to-body weight ratio. Echocardiographic analysis showed that the interventricular septal wall thickness and left ventricular posterior wall thickness at systole and diastole were significantly increased in banded rats. Also, a significant increase in isovolumic relaxation time was observed in banded rats. Measured eNOS, iNOS, and ref-1 protein levels were significantly reduced in banded rats. Resveratrol treatment prevented the above changes in cardiac structure, function, and protein expression in banded rats. Aortic banding after 4 wk resulted in concentric remodeling and impaired contractile function due to PO on the heart. The 2-wk treatment with resveratrol was found to abolish PO-induced cardiac hypertrophy. Resveratrol may therefore be beneficial against PO-induced cardiac hypertrophy found in clinical settings of hypertension and aortic valve stenosis.

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subjected to the abdominal aortic banding procedure for induction of PO as described by us previously (4). Briefly, 5-wk-old rats were kept in a temperature- and humidity-controlled room with a 12-h light:12-h dark cycle for 1 wk before creation of the PO model. Standard rat chow and tap water were available ad libitum. All rats were anesthetized for surgery with 5% isoflurane carried by oxygen at a flow rate of 2 l/min. Rats were then maintained in surgical plane of anesthetic with 2% isoflurane. A midline laparotomy was performed, and the suprarenal abdominal aorta was exposed. A silk suture was used to tie off the vessel using a blunt 21-gauge needle as a guide. Successful bands were snug while blood flow to the kidneys and lower extremities was maintained. The abdominal musculature and the skin incisions were closed by standard techniques with absorbable suture and autoclips. Sham-operated rats served as controls and were subjected to the same surgeries except for the creation of the aortic band. At 2 wk after surgery, a subgroup of sham-operated and aortic-banded rats were administered resveratrol (2.5 mg·kg body wt$^{-1}$·day$^{-1}$) by oral gavage for a period of 2 wk. All the four groups (sham-operated, aortic-banded; resveratrol-treated, sham-operated; and resveratrol-treated, aortic banded) were maintained for a total of 4 wk.

Echocardiography. At 4-wk postsurgery, rats from all groups were weighed and anesthetized using isoflurane. Transthoracic two-dimensionally guided M-mode echocardiography and pulse-wave Doppler echocardiography were performed using a Sonos 5500 ultrasound system (Agilent Technologies, Andover, MA) equipped with a 12-MHz (s12) transducer as described by us earlier (4). The following parameters were measured: percentage of left ventricular (LV) fractional shortening (FS), LV ejection fraction (EF), cardiac output (CO), LV mass (LVM), heart rate (HR), LV internal dimensions at both diastole and systole (LVIDd and LVIDs, respectively), LV posterior wall dimensions at both diastole and systole (LVPWd and LVPWs, respectively), and interventricular septal dimensions at both diastole and systole (IVSd and IVSs, respectively).

The Doppler measurements using the apical five-chamber view was utilized to assess isovolumic relaxation time (IVRT).

Isolation of rat heart. After the 4-wk echocardiographic assessment, rats from all groups were weighed and anesthetized by using a
in vivo was carried out for all groups at 4 wk after surgery.

RESULTS

Preparation of the LV homogenate. LV tissue was pulverized and homogenized with a polytron homogenizer (Brinkmann, Westbury, NY) in a buffer containing 10 mM NaHCO₃, 5 mM NaN₃, and 15 mM Tris-HCl at pH 6.8 (10 ml/g tissue). This was aliquoted and frozen in liquid nitrogen before storage at −85°C until further experimentation.

Western blot analysis. The protein content of synthase eNOS, iNOS, and redox factor-1 (ref-1) from LV homogenates were determined by Western blot analysis as described previously (2, 7). Protein samples (20–25 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were probed with either rabbit polyclonal anti-eNOS antibody (1: 1,000 dilution) obtained from Cell Signaling Technology (Danvers, MA), mouse monoclonal anti-iNOS antibody (1:1,000 dilution) and rabbit polyclonal anti-ref-1 antibody (1:500 dilution), both obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Appropriate secondary antibodies were used, and the antibody-antigen complexes in all membranes were detected by the ECL PLUS kit (Amersham Life Science, Oakville, ON, Canada). An Imaging Densitometer model GS-800 (Bio-Rad, Hercules, CA) was used to scan the protein bands and quantified using the Quantity One 4.4.0 software from Bio-Rad.

Statistical analysis. Values are expressed as means ± SE. One-way analysis of variance was used to analyze variations between the means of groups. Significant values versus sham controls are defined as P < 0.05.

RESULTS

Echocardiography. Analysis of cardiac structure and function in vivo was carried out for all groups at 4 wk after surgery by echocardiography. Figure 1 shows the LV mass-to-body weight (BW) (LVm/BW) ratio, a general index for LV hypertrophy. The LVm/BW was ~40% greater in banded rats relative to sham control rats. The LVm/BW in band-treated rats was not significantly different when compared with that in sham-treated control rats; LVm/BW was decreased by ~21% in band-treated rats relative to banded rats (Fig. 1).

Cardiac structure. Figure 2A shows a typical representation of the echocardiogram showing cardiac dimension, whereas Fig. 2B illustrates representative echocardiograms from the four groups. Cardiac structure assessment involved measurements of LVID, LVPW, and IVS at both systole and diastole. LVIDs and LVIDd were unaltered in all groups (Fig. 3). LVPWs and LVPWd were significantly increased by about 21% and 17%, respectively, in banded rats relative to sham control rats (Fig. 4, A and B). As shown in Fig. 4, A and B, LVPWs and LVPWd were significantly decreased in band-treated rats when compared with banded rats (~17% for both). No significant difference was observed between band-treated rats and sham-treated control rats (Fig. 4, A and B). IVSs and IVSd were found to be significantly increased by about 17% and 53%, respectively, in banded rats compared with sham control rats (Fig. 5, A and B). IVSs and IVSd were also observed to be significantly decreased by about 22% and 27%, respectively, in band-treated rats relative to band-treated rats (Fig. 5, A and B). No significant difference was observed in IVSs between the band-treated rats and sham-treated control rats. However, resveratrol treatment induced a slight, but significant, increase in IVSd in sham-treated control rats compared with sham control rats, as shown in Fig. 5B.

**Fig. 3.** Two-dimensionally guided M-mode echocardiographic measurement of LVIDs (A) and LVIDd (B) in sham control and aortic-banded rats treated with and without resveratrol (means ± SE). n = 5–8 rats.

**Fig. 4.** Two-dimensionally guided M-mode echocardiographic measurement of LVPWs (A) and LVPWd (B) in sham control and aortic-banded rats treated with and without resveratrol (means ± SE). **P < 0.05 vs. sham; ##P < 0.05 vs. band; n = 4–8 rats.
Cardiac function. The cardiac parameters EF, CO, and HR were utilized for assessing systolic function, whereas IVRt was used for assessing diastolic function. The EF was slightly but significantly reduced by about 6% in both banded rats and sham-treated control rats relative to sham control rats (Fig. 6A). The EF was found to be unaltered in band-treated rats compared with sham-treated control rats (Fig. 6A). Furthermore, CO and HR were unaffected in all groups (Fig. 6, B and C, respectively). As shown in Fig. 7, IVRt was significantly elevated by ~33% in banded rats compared with sham control rats. Figure 7 also showed that IVRt was significantly lowered by ~18% in band-treated rats relative to banded rats. No significant alterations in IVRt were found in band-treated rats relative to sham-treated control rats (Fig. 7).

Western blot. The protein expression of eNOS, iNOS, and ref-1 was quantified by Western blot in all groups. The protein level of eNOS was significantly decreased by ~47% in banded rats versus sham control rats (Fig. 8A). The level of eNOS was significantly elevated by ~2.6-fold in band-treated rats when compared with banded rats. No significant difference was observed in eNOS levels between band-treated rats and sham-treated control rats (Fig. 8A). The level of iNOS protein was significantly decreased by about 41% in banded rats relative to sham control rats (Fig. 8B). The abundance of iNOS was found to be unaltered in band-treated rats versus sham-treated control rats but was significantly elevated by about 49% in banded rats versus banded rats (Fig. 8B). A significant reduction in the levels of ref-1 (~31%) was observed in banded rats relative to sham control rats (Fig. 8C). In addition, ref-1 was significantly elevated in band-treated rats compared with banded rats. The levels of ref-1 were unaltered in band-treated rats versus sham-treated control rats (Fig. 8C).

DISCUSSION

Four weeks of aortic banding resulted in significant hypertrophy in rats. This was evident from a significant increase in LVm/BW ratios. Furthermore, the hypertrophy was concentric in nature as evident from significant increases in the IVS and LVPW, an indication of increased septal and posterior wall thickness, respectively, at both systole and diastole. There was...
no change observed in LVID, an indication of increased chamber size, at systole and diastole. Results of the current study are consistent with results from our previous study (4). Treatment of aortic-banded rats with 2.5 mg·kg⁻¹·day⁻¹ resveratrol completely prevented development of hypertrophy because there was a normalization of the increase in left ventricle-to-body weight ratio. Resveratrol treatment also prevented concentric remodeling by normalizing IVS and LVPW at both systole and diastole. The beneficial effects of resveratrol in preventing PO-induced cardiac hypertrophy are consistent with another recent study, which showed beneficial effects of an analog of resveratrol (isorhapontigenin) in the aortic banding model (17). In that study, however, 3 wk of aortic banding resulted in no significant increase in LVPW, indicating no change in wall thickness. Furthermore, there was a significant increase in LV dimensions indicating LV dilation (17). Put together, these structural changes do not reflect concentric but eccentric remodeling (increased chamber size without change in wall thickness). In addition, no isorhapontigenin-treated sham-control group was included, a limitation of that study. In this regard, our previous study (4) has established that concentric remodeling occurs as early as 2 wk where LVPW as well IVS are increased significantly with no changes in LVID. Regardless of the type of remodeling, treatment with 50 μM isorhapontigenin prevented structural changes occurring in their aortic banding model (17). In another recent study, using a different model of hypertrophy (hypertension induced by partial nephrectomy), Liu et al. (20) showed that LV hypertrophy was reduced significantly upon treatment of partially nephrectomized rats with 50 mg trans-resveratrol.

In our study, 4 wk of aortic banding also resulted in significant changes in cardiac contractile function. With regard to systolic function, EF, the ability of the left ventricle to pump blood efficiently, was minimally reduced in aortic-banded rats, whereas CO and HR were not affected. With regard to diastolic function, IVRt, an indicator of ventricular relaxation time, was prolonged significantly in aortic-banded rats. The results of the current study are consistent with those of our previous study (4). Treatment of aortic-banded rats with resveratrol completely prevented diastolic dysfunction by normalizing IVRt. The beneficial effects of resveratrol in preventing PO-induced cardiac functional impairment are consistent with the above-mentioned recent study with isorhapontigenin treatment in the aortic banding model (17). Isorhapontigenin treatment completely prevented a reduction in fractional shortening, a measure of LV systolic function, in 3-wk aortic-banded rats.

Over the recent past there is growing consensus pointing toward NO as an endogenous antihypertrophic molecule (15), and exploiting its potential has been proposed to be an attractive strategy to combat cardiac hypertrophy (3). Our study showed that both isoforms of NOS (iNOS and eNOS), the enzyme responsible for NO production in the heart, are down-regulated by cardiac hypertrophy due to PO. Our results are consistent with a recent study showing a decrease in eNOS protein levels in aortic-banded mice (18) and downregulation of NO levels in partially nephrectomized rats (20). Recent studies showed that eNOS but not iNOS was antihypertrophic, because the extent of concentric hypertrophy in eNOS knockout mice was greater than that in wild-type mice subjected to aortic banding (24), whereas the extent of hypertrophy was not different between iNOS knockout mice and its matched wild-type subjected to deoxycorticosterone acetate salt treatment (26). Nevertheless, iNOS does play a significant role in preserving cardiac contractile function (26). Our results fit well with the antihypertrophic and cardioprotective roles of NO as we observe that resveratrol treatment, which increased iNOS and eNOS protein expression, completely abolished cardiac hypertrophy as well as prevented contractile dysfunction in aortic-banded rats. Our results with resveratrol are consistent with the study of Liu et al. (20), which reported a recovery of NO levels in trans-resveratrol-treated partially nephrectomized rats.

The results of the present study also demonstrated an increased expression of ref-1 in parallel with the upregulation of iNOS and eNOS protein expression in resveratrol-treated aortic-banded rats. This would tend to suggest an active DNA repair process is potentiated by resveratrol thereby preserving the genomic stability by repairing the apurinic/apyrimidinic sites. This would also tend to offer an explanation for the cardioprotective role of iNOS after resveratrol treatment. It is well known that iNOS may play a dual role in cellular defense system; it may potentiate cellular injury through reactive oxygen species production (14), or it can afford cellular protection as...
in the case of ischemic reperfusion or oxidative injury (16). The results of our study are consistent with the existing reports that local excessive NO generation can protect a tissue rather than becoming injurious to the cells. It is tempting to speculate, based on our results, that iNOS induced by resveratrol could promote DNA repair/survival machinery through the activation of ref-1. This also provides further support in favor of the hypothesis that resveratrol pharmacologically preconditions the heart, even a hypertensive heart as shown in the present study, against cellular injury through the induction of an intracellular defense mechanism by harnessing its own defensive machinery (9, 22).

In conclusion, the results of this study show that treatment of aortic-banded rats with resveratrol completely abolished the development of cardiac hypertrophy as well as prevented cardiac contractile dysfunction. The beneficial effects of resveratrol were in part mediated by induction of iNOS and eNOS and activation of ref-1.

GRANTS

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