All-trans retinoic acid prevents development of cardiac remodeling in aortic banded rats by inhibiting the renin-angiotensin system

Rashmi Choudhary, Ants Palm-Leis, Robert C. Scott III, Rakeshwar S. Guleria, Eric Rachut, Kenneth M. Baker, and Jing Pan

1Department of Renal Medicine, University of Colorado Health Sciences Center, Denver, Colorado; and 2Department of Medicine, Scott and White Hospital, 3Cardiovascular Research Institute, Division of Molecular Cardiology, College of Medicine, Texas A&M University System Health Science Center, and 4Division of Pathology, Central Texas Veterans Health Care System, Temple, Texas

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Choudhary R, Palm-Leis A, Scott RC III, Guleria RS, Rachut E, Baker KM, Pan J. All-trans retinoic acid prevents development of cardiac remodeling in aortic banded rats by inhibiting the renin-angiotensin system. Am J Physiol Heart Circ Physiol 294: H633–H644, 2008. First published December 21, 2007; doi:10.1152/ajpheart.01301.2007.—This study was designed to determine the effect of all-trans retinoic acid (RA) on the development of cardiac remodeling in a pressure overload rat model. Male Sprague-Dawley rats were subjected to sham operation and the aortic constriction procedure. A subgroup of sham control and aortic constricted rats were treated with RA for 5 mo after surgery. Pressure-overloaded rats showed significantly increased interstitial and perivascular fibrosis, heart weight-to-body weight ratio, and gene expression of atrial natriuretic peptide and brain natriuretic peptide. Echocardiographic analysis showed that pressure overload induced systolic and diastolic dysfunction, as evidenced by decreased fractional shortening, ejection fraction, stroke volume, and increased E-to-Ea ratio and isovolumic relaxation time. RA treatment prevented the above changes in cardiac structure and function and hypertrophic gene expression in pressure-overloaded rats. RA restored the ratio of Bcl-2 to Bax, inhibited cleavage of caspase-3 and -9, and prevented the decreases in the levels of SOD-1 and SOD-2. Pressure overload-induced phosphorylation of ERK1/2, JNK, and p38 was inhibited by RA, via upregulation of mitogen-activated protein kinase phosphatase (MKP)-1 and MKP-2. The pressure overload-induced production of angiotensin II was inhibited by RA via upregulation of expression of angiotensin-converting enzyme (ACE)2 and through inhibition of the expression of cardiac and renal renin, angiotensinogen, ACE, and angiotensin type 1 receptor. Similar results were observed in cultured neonatal cardiomyocytes in response to static stretch. These results demonstrate that RA suppresses myocardial cell hypertrophy in response to mechanical stretch, ANG II, endothelin-1, and phenylephrine. Recent in vivo studies have shown that RA prevents ventricular fibrosis and remodeling during the development of hypertension in spontaneously hypertensive rats (SHR) (25) and after myocardial infarction (29). We recently demonstrated (10) that RA inhibits ANG II- and stretch-induced cell apoptosis and production of intracellular reactive oxygen species (ROS) in neonatal cardiomyocytes, indicating that RA signaling may have an important role in preventing cardiac remodeling and the transition from adaptive cardiac hypertrophy to heart failure.

In the present study using the suprarenal aortic constriction-induced pressure overload model, we present the first in vivo evidence that RA prevents the development of cardiac remodeling, by inhibiting LV hypertrophy, fibrosis, and apoptosis, via regulating the expression of RAS components.

MATERIALS AND METHODS

Animals and surgical procedures. All protocols were approved by the institutional Animal Care and Use Committee and conform with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Pub. No. 85-23, revised 1996). Male Sprague-Dawley rats (200–250 g, Harlan, Indianapolis, IN) were randomized into four groups (n = 10): 1) sham operated, 2) RA-treated sham operated (RA; 30 mg·kg body wt⁻¹·day⁻¹), 3) aortic constriction (AC), and 4) AC with RA treatment (AC+RA). RA was administrated orally every 24 h for 3 days before operation.

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Address for reprint requests and other correspondence: J. Pan, Cardiovascular Research Institute, College of Medicine, Texas A&M Univ. System Health Sciences Center, 1901 South 1st St., Bldg. 205, Temple, TX 76504 (e-mail: jpan@medicine.tamhsc.edu).

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RA was mixed with vehicle (corn oil) and gavaged at the indicated dose throughout the study. An equal volume of vehicle was given to sham-operated and AC rats. On the fourth day, pressure overload was induced by suprarenal aortic constriction, as described previously (37). Briefly, rats were kept in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle for 1 wk before creation of the pressure overload model. 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 a surgical plane with 2% isoflurane. A midline laparotomy was performed, and the suprarenal abdominal aorta was tied off, with a blunt 21-gauge needle as a guide. After the needle was removed, the abdominal musculature and skin incisions were closed by standard techniques with absorbable suture. Sham-operated rats served as controls and were subjected to the same surgery except for the creation of the aortic constriction. All four groups were maintained for a total of 22 wk.

Echocardiographic measurements. Rats from all groups were weighed and anesthetized with isoflurane. Echocardiographic analysis was performed monthly (for 5 mo), with an HP sonos 5500 (Hewlett-Packard) with a 12-MHz imaging transducer. LV mass, relative wall thickness, LV fractional shortening (FS), LV ejection fraction (EF), cardiac output (CO), heart rate (HR), LV internal dimension at both diastole and systole (LVIDd and LVIDs, respectively), velocity of circumferential fiber shortening (VCF), peak velocity of early (Evel) and late (A) filling waves, and isovolumic relaxation time (IVRT) were measured from the mitral inflow recording, as previously described (44). Peak early diastolic velocity (Ee) was measured from the Doppler tissue image (DTI) recording. We also measured the ratio of E to Ee, which is a noninvasive indicator of LV end-diastolic pressure (LVEDP) (28).

In vivo hemodynamic measurements. Hemodynamic parameters were measured 22 wk after aortic constriction. Rats were anesthetized, and the right carotid artery was cannulated with a micromanometer-tipped catheter for recording of blood pressure (BP) with a blood pressure analyzer (BPA 400, Micro-Med, Louisville, KY). After the catheter was advanced into the LV, LVEDP, maximal rate of pressure rise (+dP/dtmax) and decrease (−dP/dtmax), time constant of LV relaxation (τ), and duration of relaxation (DREL) were measured directly with a heart performance analyzer (HPA 410, Micro-Med). After hemodynamic measurements, rats from all groups were weighed and anesthetized with a cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg) before being euthanized. Hearts, lungs, and kidneys were removed, weighed, flash-frozen in liquid nitrogen, and subsequently stored at −80°C until further experimentation.

Fig. 1. Effect of retinoic acid (RA) on pressure overload-induced cardiac hypertrophy and fibrosis. A: echocardiographic measurement of thickness of end-systolic interventricular septum (IVSs) and left ventricular (LV) posterior wall (LV PWs) and LV mass index at 1, 3, and 5 mo after suprarenal aortic constriction. LV mass index is expressed as the LV mass-to-body weight ratio. LV mass = (1.04[LVIDd + LV PWd + IVSd]3 − LVIDd3) × 0.8 + 0.6 (44), where LVIDd, LV PWd, and IVSd are end-diastolic LV internal dimension, LV PW, and IVS. Data are expressed as means ± SE. Sham-operated (Sham) and RA-treated (RA) groups: n = 6; aortic constriction (AC) and RA+AC groups: n = 10. *P < 0.05 vs. Sham group; #P < 0.05 vs. AC group. B: hematoxylin and eosin-stained transverse sections of the LV myocardium after 5 mo of pressure overload. C: representative photographs of Masson trichrome staining, magnification ×40. D and E: real-time RT-PCR analysis of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) gene expression in the heart after 5 mo of pressure overload. F and G: plasma level of ANP and BNP at 1, 3, 7, and 154 days of aortic constriction. Data are expressed as means ± SE (Sham and RA groups: n = 6; AC and RA+AC groups: n = 10). *P < 0.001 vs. Sham group; #P < 0.001 vs. AC group.
were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with antibodies against ACE, ACE2, Bcl-2, Bax, Caspase 9, Caspase 3, SOD-1, SOD-2, mitogen-activated protein kinase phosphatase (MKP)-1, MKP-2, actin (Santa Cruz Biotechnology, Santa Cruz, CA), renin, and Ao (RDI Research Diagnostics). Specific phospho-ERK1/2, JNK, and p38 antibodies were from Cell Signaling Technology (Beverly, MA). Binding of primary antibody was detected with horseradish peroxidase-conjugated goat anti-mouse or goat anti-rabbit secondary antibody and visualized with an enhanced chemiluminescence detection kit (PerkinElmer Life Sciences, Boston, MA). Membranes were reprobed with β-actin to confirm equal loading.

Cultured neonatal rat cardiomyocytes. Primary cultures of neonatal cardiomyocytes were prepared from the ventricles of 1- to 2-day old Sprague-Dawley rat pups as previously described (30). Cells were cultured onto laminin-coated six-well BioFlex culture plates (Flexcell International). For the stretch model, serum-starved cells were subjected to static stretch with the Flexcell 3000 Strain Unit. The vacuum produced a 19% elongation on the flexible bottom membranes. Control plates were not subjected to stretch.

ANG II, ANP, and BNP measurement. The levels of ANG II, ANP, and BNP in plasma from rats after 1, 3, 7, and 154 days of aortic constriction and in conditioned medium from cultured neonatal cardiomyocytes were measured. Samples were purified with a C18 Sep-Pak column (Waters Associates, Milford, MA), and the levels of ANG II, ANP, and BNP were determined by ELISA (Peninsula Laboratories, Belmont, CA).

Statistical analysis. All data are expressed as means ± SE. Multiple comparisons were performed by one-way ANOVA and Tukey-Kramer exact probability test (GraphPad, San Diego, CA). A value of \( P < 0.05 \) was considered statistically significant.

RESULTS

Effect of RA on pressure overload-induced cardiac hypertrophy and fibrosis. Echocardiographic analysis showed that pressure overload induced myocardial hypertrophy in rats after 1, 3, and 5 mo of aortic constriction. The thickness of the end-systolic LV posterior wall (LVPWs) and end-systolic interventricular septum (IVSs) and LV mass index increased significantly in aortic constricted rats, compared with sham-operated rats (Fig. 1A). As shown in Table 1, heart weight (HW) and HW-to-body weight ratio (HW/BW) were significantly increased after 5 mo of pressure overload, consistent with the echocardiographic results. RA significantly inhibited the increased thickness of LVPWs and IVSs and HW in AC rats (\( P < 0.05 \), compared with AC group). We observed lower
HW/BW and LV mass index in the RA+AC group compared with AC rats; however, the difference did not reach statistical significance ($P > 0.05$). A modest decrease in body weight was observed in both the RA and RA+AC groups relative to non-RA-treated rats (Table 1). RA did not lower the increased systolic BP. However, RA treatment prevented the significant increase in HR and average lung weight seen in the 22-wk untreated AC group relative to control, indicating RA-mediated prevention of pulmonary edema (Table 1).

Microscopic observations of hematoxylin and eosin-stained transverse sections of the LV myocardium revealed that the hearts of AC rats displayed marked structural abnormalities, with significant increases in LV free wall and septum thickness (thickness was measured in 5 sections of each sample, 6 samples/group; data not shown). RA inhibited the increased thickness of the LV free wall and septum (Fig. 1B), consistent with the echocardiographic analysis. A diffuse interstitial and perivascular fibrosis was evident in AC pressure-overloaded rats. RA treatment ameliorated the severe fibrosis, both in the interstitial and perivascular areas (Fig. 1C). These results indicate that RA inhibits pressure overload-induced concentric cardiac hypertrophy and fibrosis.

Fig. 2. Effect of RA on pressure overload-induced systolic dysfunction. A–G: heart systolic function was evaluated echocardiographically after 1, 3, and 5 mo of aortic constriction. EF, ejection fraction; SV, stroke volume; FS, fractional shortening; CO, cardiac output; LVIDs, LVIDd, LV internal dimension at systole and diastole; VCF, velocity of circumferential fiber shortening. H: hemodynamic studies were performed after 5 mo of aortic constriction. $+dP/dt$, rate of pressure rise. Data are expressed as means ± SE (Sham and RA groups: $n = 6$; AC and RA+AC groups: $n = 10$). *$P < 0.05$ vs. Sham group; # $P < 0.05$ vs. AC group.
Effect of RA on pressure overload-induced expression of ANP and BNP. The enhanced production of ANP and BNP is a reliable marker for cardiac hypertrophy (49). Using quantitative real-time RT-PCR, we demonstrated that RA inhibited pressure overload-induced gene expression of ANP and BNP after 5 mo of aortic constriction (Fig. 1, D and E). We further elucidated the effect of RA on the plasma level of ANP and BNP (Fig. 1, F and G). Consistent with the cardiac gene expression, the plasma level of ANP and BNP increased rapidly on day 1 after AC and remained elevated up to 5 mo. The increased ANP and BNP levels were inhibited by RA after 7 days of pressure overload, and this effect was maintained for the duration of the study.

Effect of RA on pressure overload-induced LV systolic dysfunction. As shown in Fig. 2, after 1 mo of pressure overload there was a tendency toward increased LV contractility in AC rats, as assessed by stroke volume (SV; 80% increase compared with sham), CO (60% increase), and VCF (58% increase), indicating compensatory adaptation. Overall contractility and cardiac systolic function were much worse than those in sham-operated rats after 3–5 mo of mechanical load and continued to deteriorate, as demonstrated by a progressive decline in %EF, %FS, SV, CO, VCF, and +dP/dt. This was also consistent with progressively increased LVIM and LVIDd (Fig. 2) and an increase in HR (Table 1). LV contractility and systolic function were normal in RA-treated rats at all time points, suggesting that RA prevented pressure overload-induced systolic dysfunction during the development of cardiac remodeling.

Effect of RA on pressure overload-induced LV diastolic dysfunction. Pressure overload-induced diastolic dysfunction was demonstrated by decreased DTI Ea and increased E/normal.
E/Ea, IVRT, \( -dP/dt \), LVEDP, \( \tau \), and DREL after 3 and 5 mo of aortic constriction (Fig. 3). Normal diastolic function was demonstrated in each group after 1 mo of aortic constriction. These data indicate that pressure overload-induced compensated cardiac hypertrophy began to decompensate after 1 mo of pressure overload. RA prevented the progressive decline in diastolic heart function. Thus RA has an important role in preventing the development of cardiac remodeling and the transition from adaptive cardiac hypertrophy to maladaptive heart failure.

**Effect of RA on pressure overload-induced apoptosis.** Studies have suggested that cardiac myocyte apoptosis is an important component, responsible for the transition from hypertrophy to heart failure (35). To determine whether inhibition of cell apoptosis is involved in the protective effect of RA, we evaluated the effect of RA on pressure overload-induced changes in expression of the Bcl-2 family of proteins. As shown in Fig. 4A, a relative decrease in Bcl-2 and an increase in Bax expression were observed in AC rats. RA increased the expression of Bcl-2 and decreased expression of Bax, thus restoring or increasing the Bcl-2-to-Bax ratio (Fig. 4B), after 5 mo of aortic constriction. Caspase-9 is a key protein involved in the mitochondrial apoptosis pathway. The cleaved form of the enzyme (38 kDa) increased in intensity in pressure-overloaded LV. RA prevented the cleavage of caspase-9 (Fig. 4A and D). Myocardial caspase-3 activation is a final common step in caspase-dependent apoptosis. Caspase-3 normally exists as a 35-kDa inactive precursor that is cleaved proteolytically to an active p17 subunit when cells are induced to undergo apoptosis. As shown in Fig. 4A, the intensity of the cleaved enzyme (17 kDa) increased significantly after 5 mo of pressure overload. RA inhibited the increase in cleaved caspase-3 to a degree comparable to that in sham-operated rats (Fig. 4C). These results suggest that RA prevents pressure overload-stimulated myocardial apoptosis, by inhibiting proapoptotic and promoting antiapoptotic signaling. SOD is a potent antioxidant enzyme that scavenges ROS. To determine whether the protective effect of RA is due to regulation of SOD and inhibition of ROS production, we evaluated the effect of RA on SOD-1 and SOD-2 protein expression. A significant decrease in the expression of SOD-1 and SOD-2 was observed in AC rats (Fig. 4, A, E, and F). RA treatment prevented pressure overload-induced downregulation of SOD-1 and SOD-2, indicating that RA may have beneficial effects in reducing intracellular oxidative stress via increasing the antioxidant defense system.

**Effect of RA on pressure overload-induced activation of MAP kinase cascade.** We previously reported (30) that the antihypertrophic effect of RA in neonatal cardiomyocytes is mediated by upregulation of MKPs and inhibition of the activation of MAP kinases. Thus we determined whether the inhibitory effect of RA on pressure-overloaded heart is regulated through similar signaling mechanisms. The phosphorylation of ERK1/2, JNK, and p38 and expression of MKP-1 and MKP-2 were determined by Western blot analysis. As shown in Fig. 5, phosphorylation of ERK1/2, JNK, and p38 was observed after 5 mo of aortic constriction. RA treatment significantly inhibited the increase in cleaved caspase-3 to a degree comparable to that in sham-operated rats (Fig. 4C). We further determined the effect of RA on expression of MKPs. Downregulation of MKP-1 and MKP-2

![Figure 4](http://ajpheart.physiology.org/)

Fig. 4. Effect of RA on pressure overload-induced apoptosis. A: after 5 mo of pressure overload, LV protein was extracted and analyzed by Western blotting with antibodies against Bcl-2, Bax, caspase-3, caspase-9, SOD-1, and SOD-2. Sample loading was controlled by using antibody against actin. Representative experiments are shown. B–D: intensity of the bands (total of 3 rats/group) was measured by densitometry, and relative intensity was calculated after subtraction of the actin level in each sample. The ratio of Bcl-2 to Bax (B), activation of caspase-3 (C) and caspase-9 (D), and expression level of SOD-1 (E) and SOD-2 (F) are expressed as means ± SE (n = 3). *P < 0.05 vs. Sham group; #P < 0.05, ##P < 0.01 vs. AC group.
was observed in AC rats, and RA treatment upregulated the expression of MKP-1 and MKP-2 (Fig. 5, A, E, and F). These results are consistent with our in vitro studies, indicating that inhibition of the MAP kinase cascade is also involved in the antihypertrophic effect of RA in the pressure overload model.

**RA inhibits pressure overload-induced expression/activation of RAS components.** To determine whether the inhibitory effect of RA on cardiac remodeling is mediated by regulating the expression/activation of the RAS, we used real-time quantitative RT-PCR to assess gene expression of cardiac and renal RAS components. As shown in Fig. 6, A–E, pressure overload induced upregulation of cardiac renin, Ao, ACE, and AT1 genes, all of which were downregulated by RA. Similar results were observed in renal tissue (data not shown). The expression of cardiac AT2 was upregulated by RA (Fig. 6E). Similar results were observed in the protein expression of RAS components (Fig. 6F), suggesting that changes in protein levels are primarily due to changes in gene expression. The expression of ACE2 was upregulated in RA-treated samples. Pressure overload induced a significant increase in plasma levels of ANG II after the first day of aortic constriction, which was maintained at an elevated level up to 5 mo (Fig. 6G). RA inhibited this increase in ANG II after 7 days of treatment. These results suggest that the protective effect of RA may be through inhibition of production of ANG II and reduction in AT1-mediated signaling events.

**RA inhibits mechanical stretch-induced expression of RAS components in vitro in cardiomyocytes.** To further determine whether the inhibitory effect of RA on cardiac remodeling is mediated by regulation of the expression of RAS components, we evaluated the effect of RA on expression of RAS components after 12 h of mechanical stretch in neonatal cultured cardiomyocytes. Mechanical stretch induced upregulation of renin, Ao, ACE, and AT1 gene expression, which was significantly inhibited by RA (Fig. 7, A–D). The gene expression of AT2 is downregulated in stretched cardiomyocytes, and RA reversed the decreased expression of AT2 (Fig. 7E). RA also inhibited the stretch-induced release of ANG II in cell culture medium (Fig. 7F). These in vitro data are consistent with the in vivo results, further demonstrating that regulation of the RAS by RA has an important role in RA-mediated protective effects on pressure overload-induced development of cardiac remodeling.

**DISCUSSION**

In the present study, we have demonstrated that RA prevents the development of cardiac remodeling induced by pressure overload in the pressure overload model. RA inhibits pressure overload-induced expression/activation of RAS components. RA also inhibits stretch-induced expression of RAS components in vitro. These findings suggest that RA may be a promising therapeutic agent for the prevention of cardiac remodeling in hypertension.
overload. RA preserved cardiac function by inhibiting hypertrophic growth, fibrosis, and apoptosis. RA-induced upregulation of MKP-1 and MKP-2 resulted in dephosphorylation and inactivation of MAP kinases. In addition, we demonstrated for the first time that RA significantly inhibits pressure overload-induced production of ANG II, via upregulation of ACE2 expression and through inhibition of cardiac and renal RAS component expression.

We and others (43, 47, 53) have shown that RA has a potent inhibitory effect on hypertrophic stimulus-induced cell growth in cultured cardiomyocytes, indicating that RA-mediated signaling may have an important role in preventing the development of cardiac remodeling. Using the pressure overload-induced hypertrophic model, we demonstrated that RA treatment attenuated the increased HW and thickness of the LVPW and IVS after 1, 3, and 5 mo of pressure overload. RA also inhibited both ANP and BNP gene expression, as well as secretion during the early phase of cardiac hypertrophy, suggesting that RA-mediated signaling inhibits pressure overload-induced cardiac hypertrophy. Previous studies have shown that administration of RA leads to remodeling of white adipose tissues, resulting in a reduction of adiposity and body weight (8, 26). We also observed a moderate weight loss in RA-treated rats, which contributes to the higher HW/BW and LV mass index in RA-treated animals. Using the SHR model, Lu and colleagues (25) showed that RA had no significant inhibitory effect on the increased LVPW, LV weight (LVW), and LVW/BW, which may be due to the model or the lower dosage of RA (5–10 mg·kg body wt⁻¹·day⁻¹). In the present study, we chose a higher dose (30 mg·kg body wt⁻¹·day⁻¹) to ensure the efficacy of RA, according to previous studies (27).

Although chronic pressure overload-induced LV hypertrophy can be viewed as a compensatory mechanism to maintain cardiac output and normalize wall stress, LV hypertrophy in
the long term is an independent risk factor for a range of adverse consequences, such as myocardial ischemia, systolic and diastolic dysfunction, arrhythmias, and increased cardiac mortality. Thus prevention or regression of LV remodeling provides a potentially important therapeutic target. That treatment with RA inhibits the development of LV hypertrophy and prevents the transition from hypertrophy to decompensation suggests mechanistic involvement of this signaling pathway. Pressure overload induced a compensated LV hypertrophy with normal or increased systolic function in the early phase. However, despite the increase in LV mass, LV function declined after 1 mo of aortic constriction. Deterioration of both systolic and diastolic function was observed after 3 and 5 mo of pressure overload. Importantly, prevention of the hypertrophic response by RA ameliorates the systolic and diastolic dysfunction, preventing progression from hypertrophy to heart failure. It is well known that diminished contractility of the hypertrophic cardiac myocyte is the main factor of ventricular dysfunction in the failing heart. A key feature of the failing heart is the impairment in Ca\(^{2+}\) handling, which affects both the systolic and diastolic phases of the cardiac cycle (5, 50). Previous studies showed that RA inhibits ANG II-induced increases in the intracellular Ca\(^{2+}\) concentration (43) and increases sarc(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) in cardiac myocytes (34). RA also increases expression of the Na\(^+/\)Ca\(^{2+}\) exchanger, an important Ca\(^{2+}\) transport system, which regulates intracellular Ca\(^{2+}\) homeostasis (19). These data suggest that inhibition of fibrosis by RA contributes to the improved systolic and diastolic heart function in RA-treated animals.

Recent studies have suggested that cardiomyocyte apoptosis is an important component in heart failure (35). Importantly, the apoptotic signals are those that have largely been demonstrated to induce cardiomyocyte hypertrophy. It has been proposed that persistent growth stimuli can drive hypertrophied cardiomyocytes to lose intracellular survival signals that normally suppress the development of the apoptotic process, and as a consequence, growth factors become apoptotic factors (6). Increased cardiomyocyte apoptosis has been demonstrated in pressure overload models (41). Caspases are central to the related signaling is involved in RA-mediated protective effects on cardiac remodeling.

The present study provides evidence that the mechanisms whereby RA prevents the development of LV remodeling involve inhibition of fibrosis, cardiomyocyte apoptosis, and the expression of RAS components. Myocardial fibrosis is one of the histological hallmarks of myocardial remodeling and increased extracellular matrix content, results in exaggerated mechanical stiffness, and contributes to both systolic and diastolic heart failure (45). Perivascular fibrosis surrounding intracoronary arterioles impairs myocyte oxygen availability, reducing coronary reserve and exacerbating myocardial ischemia. We have observed that RA inhibits pressure overload-induced interstitial and perivascular fibrosis, which is consistent with previous studies that have shown that RA inhibits ANG II-induced cell proliferation and collagen secretion in neonatal cardiac fibroblasts (17) and prevents ventricular fibrosis in SHR (25) and in rats after myocardial infarction (29). These data suggest that inhibition of fibrosis by RA contributes to the improved systolic and diastolic heart function in RA-treated animals.
apoptotic cascade, since they mediate both the mitochondrial and death receptor apoptotic pathways (14). We (10) and others (20) have demonstrated that stretching of cardiomyocytes and increasing LV wall stress can trigger both the mitochondrial and death receptor pathways. The death receptor- and mitochondrion-mediated apoptotic pathways, through their initiators, caspase-8 and -9, respectively, trigger the self-amplifying caspase cascade, resulting in activation of the downstream effector caspase-3 (36). In the present study, both caspase-9 and caspase-3 activity were significantly increased in pressure-overloaded LV. This increase was accompanied by a significant deterioration in myocardial contractility and ventricular function. It is possible that the apoptotic process is initiated in the transition to heart failure, because of the altered balance between proapoptotic and antiapoptotic factors induced by pressure overload. Studies have shown that the Bcl-2 family of proteins has an important role in the regulation of cardiomyocyte apoptosis (7). Bcl-2 family members consist of two functional categories: those that inhibit apoptosis (Bcl-2 and Bcl-xL) and those that induce apoptosis (Bax, Bad). The overall effect of the Bcl-2 family depends on the relative levels of death-inhibiting versus death-promoting family members. In the present study, the expression of the proapoptotic factor Bax was enhanced and the antiapoptotic factor Bcl-2 decreased throughout the remodeling process. The decreased Bcl-2-to-Bax ratio likely contributes to the activation of caspase-9 and caspase-3 and the initiation of apoptosis in AC rats. A significant increase in the Bcl-2-to-Bax ratio and decreased caspase-9 and caspase-3 cleavage were observed in RA-treated rats, which were accompanied by an observed increase in myocardial contractility and improved heart function. These results indicate that apoptosis has an important role in the transition from hypertrophy to heart failure.

Oxidative stress induces cardiac myocyte apoptosis in vitro (3, 42) and may be a major contributing factor in heart failure. We have demonstrated (10) that oxidative stress has an important role in mechanical stretch- and ANG II-induced apoptosis in neonatal cardiomyocytes. Pressure overload induced downregulation of SOD-1 and SOD-2, which resulted in accumulation of ROS, thus contributing to the decreased Bcl-2-to-Bax ratio and subsequent cell apoptosis. RA has been shown to exert antiapoptotic activity in some cell populations, such as embryonic neurons and mesangial cells (1, 2, 48). We recently demonstrated (10) that RA prevents mechanical stretch- and ANG II-induced cell apoptosis by promoting the antioxidant defense system, reducing intracellular oxidative stress, and restoring mitochondrial function, in neonatal cardiomycocytes. Our present data demonstrated that RA prevented the decrease in the levels of SOD-1 and SOD-2, indicating that RA, by increasing the antioxidant defense system, results in inhibition of oxidative stress and an increased Bcl-2-to-Bax ratio, likely contributing to the improved heart function in RA-treated animals.

We previously showed (30) that the inhibitory effect of RA on stretch-induced cardiomyocyte hypertrophy is mediated by upregulation of MKPs and inhibition of MAP kinases in neonatal cardiomyocytes. In rats, we observed downregulated expression of MKP-1 and MKP-2 and activation of MAP kinases after 5 mo of pressure overload. RA significantly upregulated the expression of MKP-1 and MKP-2, which contributed to the inhibitory effect of RA on activation of MAP kinases. We (30) and others (16, 23, 39) have previously demonstrated that MAP kinases are involved in numerous pathological mediators (neurohormones, cytokines, mechanical stretch)-induced cardiac hypertrophy. Activation of ERKs is linked to cell survival and hypertrophy, whereas p38 activation is believed to accelerate the death pathways and p38 activation may have an antiapoptotic effect. The activation of JNK is likely also linked to the apoptotic response (33). Thus the protective effect of RA may be partially mediated by the MAP kinase cascade.

There is increasing evidence that RA regulates gene expression of RAS components, including renin, ACE, ACE2, and AT1. Renal expression of Ao, renin, and the AT1 receptor in the kidney was reduced by RA in anti-Thy1.1 nephritic rats (12). It has also been shown that RA downregulates the expression level of AT1 and upregulates expression of ACE2 in the heart of SHR, which was accompanied by a decrease in blood pressure (51, 52), indicating that RA-mediated signaling is involved in regulating RAS components during the development of hypertension. However, the effects of RA on the circulating and/or local RAS during the process of cardiac remodeling remain unclear. In the present study, pressure overload significantly increased expression of Ao, renin, and ACE, at both the mRNA and protein levels, in heart and kidney, which resulted in an increased plasma level of ANG II. The gene expression of AT1 was also increased in the pressure overload model in both heart and kidney, but the AT1 mRNA level was not altered by pressure overload. RA treatment significantly reduced the expression of cardiac Ao, renin, ACE, and AT1, followed by a decrease in the level of ANG II, both in the in vivo pressure-overloaded heart and in vitro with

![Fig. 8. Putative model depicting the mechanism of RA signaling in pressure overload-induced cardiac remodeling. Pressure overload stimulates production of ANG II, which acts through AT1, promoting intracellular reactive oxygen species (ROS) generation and stimulation of signaling pathways leading to activation of MAP kinase cascades, resulting in cardiac remodeling. RA, binding with the retinoic acid receptor (RAR)/retinoid X receptor (RXR) heterodimer, upregulates the expression of MKPs, which results in dephosphorylation and inactivation of MAP kinases, and RA inhibits oxidative stress by increasing the antioxidant defense system (SOD-1 and SOD-2), resulting in the increase in Bcl-2-to-Bax ratio. Pressure overload-induced production of ANG II is suppressed by RA, via inhibition of the expression of RAS components and through upregulation of ACE2, which converts ANG II to ANG-(1–7).](http://ajpheart.physiology.org/10.1152/ajpheart.00036.2008)
stretched cardiomyocytes. We previously demonstrated (10, 30) that ANG II-induced cardiac hypertrophy and cardiomyocyte apoptosis are inhibited by RA. In the present study, we demonstrated that pressure overload-stimulated production of ANG II was inhibited by RA from the early phase of cardiac remodeling. We also showed an increased protein level of ACE2 in RA-treated heart, which is consistent with a previous study (52). ACE2 has been implicated in heart function, hypertension, renal disease, and diabetes, with effects being mediated, in part, through the ability to convert ANG II into ANG-(1–7). Studies have shown that ANG-(1–7) acts as an endogenous inhibitor of ANG II, providing a negative feedback mechanism for the regulation of the actions of ANG II (21). Increased ACE2 expression may contribute to the inhibitory effect of RA on production of ANG II. Our data suggest that inhibition of production of ANG II and reduction in AT1-mediated signaling events may have a major role in RA-mediated protective effects on pressure overload-induced development of cardiac remodeling.

The regulatory mechanisms of RA on the RAS are not well elucidated. RA functions by binding to two classes of nuclear receptors, the RA receptor (RAR) family and the retinoid X receptor (RXR) family (9, 18). On ligand binding, RAR/RXR heterodimers bind to specific genomic DNA sequences, designated as retinoic acid response elements, in the promoters of numerous target genes, resulting in transcriptional stimulation or repression. It has been shown that RA inhibits the transcriptional activity of activator protein-1 (AP-1) and NF-κB (4, 15). The promoter region of the renin gene contains putative AP-1 elements (40) and NF-κB, which have been implicated in the regulation of the expression of RAS components (24, 38). These studies suggest a possible mechanism by which RA regulates expression of the RAS. RA-induced activation of RAR/RXR leads to transcriptional inhibition of AP-1 or NF-κB, resulting in inhibition of expression of RAS components. Additional studies will be necessary to fully elucidate the RA-mediated regulatory mechanisms of the RAS.

In conclusion, this study presents the novel finding that RA improves cardiac function and protects against the development of cardiac remodeling by inhibiting pressure overload-induced cardiac hypertrophy, fibrosis, and apoptosis. The protective effect of RA is mediated, in part, by inhibiting the activation of the MAP kinase cascade and expression of RAS components (Fig. 8).

GRANTS

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