Downregulation of ANG II receptor is associated with compensated pressure-overload hypertrophy in the young dog

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10.1152/ajpheart.00228.2001.—We studied the gradual onset of pressure overload (PO) induced by a mildly constricting aortic band in 8-wk-old puppies (n = 8) that increased to 98 ± 11 mmHg at 9 mo. Left ventricular (LV) weight/body weight was increased in PO versus sham-operated littermate controls [8.11 ± 0.60 (SE) vs. 4.46 ± 0.38 g/kg, P < 0.001]. LV end-diastolic pressure, diastolic pressure, and fractional shortening did not differ in PO versus control dogs. There were no inducible arrhythmias in response to an aggressive electrophysiological stimulation protocol in PO dogs. Furthermore, isolated cardiomyocyte function did not differ between control and PO dogs. LV angiotensin II (ANG II) levels were increased (68 ± 12 vs. 20 ± 5 pg/g, P < 0.01) as steady-state ANG II type 1 (AT1) receptor mRNA was decreased 40% and endothelial nitric oxide synthase mRNA levels were increased 2.5-fold in PO versus control dogs (P < 0.05). Total ANG II receptor binding sites of freshly prepared cardiac membranes demonstrated no difference in the dissociation constant, but there was a 60% decrease in maximum binding (Bmax) in PO versus control dogs (P < 0.01). LV ANG II levels correlated negatively with AT1 receptor mRNA levels (r = −0.75, P < 0.01) and total AT1 receptor Bmax (r = −0.77, P < 0.02). These results suggest that LV ANG II negatively regulates AT1 receptor expression and that this is an adaptive response to chronic PO before the onset of myocardial failure in the young dog.

angiotensin II; hypertension; angiotensin II receptors; arrhythmias

STUDIES have demonstrated that AT1 receptor levels are downregulated in failing human hearts and are unchanged in nonfailing hypertrophied ventricles (2, 14, 35, 42), suggesting that downregulation of the AT1 receptor represents a shift to end-stage heart failure (23). However, other studies in experimental animal models of hypertrophy and heart failure demonstrated a more varied response of the AT1 receptor in hemodynamic overload. AT1 receptor density was increased in the noninfarcted myocardium 1 wk after experimentally induced myocardial infarction in the rat (31, 33). In aortic coarctation in the rat, AT1 receptor density was increased at 3 days (11) and 2 wk (1) but normal (40) to decreased (28) after a more prolonged duration of pressure overload (PO) of 6 wk, without evidence of heart failure. In volume overload induced by aortocaval fistula in the rat (34) and mitral regurgitation in the dog (10), AT1 receptor transcript levels were decreased by 50% with uncompensated heart failure. In contrast, AT1 receptor density was increased in both spontaneously hypertensive rats (39) and cardiomyopathic hamsters (24) in the presence of cardiac hypertrophy but before the onset of heart failure. Taken together, these studies suggest that the AT1 receptor is increased early in the course of hemodynamic overload, whereas overt heart failure is associated with downregulation of the

tissues and that augmented formation of ANG II, acting through the ANG II type 1 (AT1) receptor on cardiomyocytes and fibroblasts, participates in the paracrine regulation of cardiac remodeling and ventricular function (36). Furthermore, prolonged elevation of ANG II has been associated with cardiac fibrosis (15, 16), exacerbation of impaired contractility and relaxation in the hypertrophied failing heart (7, 9, 27, 32), and arrhythmogenesis (9, 19). These structural and functional consequences of hemodynamic overload have also been associated with changes in AT1 receptor expression in the heart.

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AT$_1$ receptor, presumably due to prolonged elevation of left ventricular (LV) ANG II levels. Whether AT$_1$ receptor downregulation occurs as a consequence of heart failure or as an adaptive mechanism before the onset of failure in the overloaded heart is an open question.

In the current investigation, we evaluated a model of cardiac hypertrophy that produces substantial increases in LV mass but remains functionally normal at the LV chamber level (13). This model induces a gradual onset of PO by placing a mild aortic constriction in 8-wk-old puppies. As the animals grow, this constriction gradually increases in severity, and at 9 mo there is a 100% increase in LV mass compared with age-matched littermate animals. With the use of this model of compensated PO, we found an inverse relationship between elevated LV ANG II levels and AT$_1$ receptor transcript levels and ANG II receptor density in the setting of normal LV chamber function and hemodynamics, response to electrophysiological stimulation, interstitial collagen content, and isolated cardiomyocyte function.

**METHODS**

Creation of pressure overload. Creation of PO was performed in 8-wk-old puppies, and all followup care was performed at the Auburn University College of Veterinary Medicine. Each dog was screened to rule out *Ehrlichia canis et platsys* and *Dirofilaria immitis* before they were entered into the protocol. Six dogs underwent a control operation. To create PO, each dog was anesthetized with intravenous droperidol and fentanyl (1–1.5 ml), intubated, and maintained on a surgical plane of anesthesia with isoflurane (0.75–1.5%) and oxygen (2 l/min). Through a right third space intercostal incision, the ascending aorta was isolated and encircled with a 5-mm polyethylene band. While the LV and distal aortic pressures were measured, the band was tightened to produce a 20- to 30-mmHg peak systolic pressure gradient across the constriction. The chest was closed, the air in the chest removed, and the dog was allowed to recover. The dogs were placed on antibiotics (20 mg/kg amoxicillin), and their hemodynamic status was closely monitored for 24–36 h. Followup care included daily monitoring of the heart rate, respiratory rate, and temperature. The heart and lungs were auscultated on a daily basis. In each dog, serial chest X-rays were performed to identify the onset of pulmonary venous congestion, and furosemide was given at 2.2 mg/kg po bid if needed. Before death, echocardiography was performed in the conscious nonsedated state. This study was approved by the Institutional Animal Care and Use Committees of the University of Alabama at Birmingham (UAB) and Auburn University College of Veterinary Medicine.

Electrophysiological studies at UAB. Nine months after placement of the aortic band, six sham-operated and eight PO dogs were transported to the UAB, where the animals underwent programmed electrophysiological studies. Transseptal catheterization was performed to obtain LV systolic pressure. A quadripolar electrophysiology catheter was advanced from the femoral vein into the apex of the right ventricle (RV). Programmed stimulation was then performed with a S1 cycle length set at 10% less than the intrinsic heart rate. The stimulus strength was twice the diastolic threshold. After 10 extrastimuli, an S2 extrastimulus was introduced, starting at the S1-S1 cycle length and decremented by 10 ms until the heart was refractory to the S2 stimulus. The S1-S2 interval was increased by 20 ms, and a S3 stimulus was introduced in a similar fashion. Up to three extrastimuli were introduced. If no arrhythmias were induced, the electrophysiology catheter was moved to the RV outflow tract, and the pacing protocol was repeated.

Isolated cardiomyocyte function at Medical University of South Carolina. Four PO and six control sham-operated dogs were transported from the Auburn University College of Veterinary Medicine to the Medical University of South Carolina for studies of hemodynamics and isolated cardiomyocyte function. LV myocytes were harvested, isolated, and studied by high-speed videomicroscopy as previously reported by this laboratory (37). For these studies, the myocytes were maintained at 37°C and field stimulated at 1 Hz. Parameters computed from the digitized contraction profiles include percent shortening, velocity of shortening, velocity of relengthening, time to peak contraction, and time to 50% relaxation. After baseline measurements, contractile function was examined in myocytes after β-adrenergic receptor stimulation with 25 nM isoproterenol (Sigma; St. Louis, MO). This concentration of isoproterenol has been demonstrated previously to yield a near-maximal response in this isolated myocyte preparation (37).

Tissue preparation for biochemical and molecular biology assays. At the time of death, a deep surgical plane of anesthesia was induced with isoflurane inhalation, and a thoracotomy was performed after electrophysiological studies at the UAB. The heart was arrested with a lethal dose of KCl, removed from the chest, and rapidly cooled in ice-cold Krebs-Henseleit buffer. The coronary arteries were retrogradely perfused from the aorta with cold Krebs-Henseleit solution, and the heart was placed on a stainless steel tray on ice. After the atria and RV free wall were dissected from the interventricular septum and LV, the portions were weighed. Tissue samples were dissected from the LV anterior midwall from six sham-operated dogs and from the eight PO dogs euthanized at the UAB. The endo- and epimyocardium were carefully excised from the samples. Samples of 2 g were immediately frozen in liquid nitrogen and subsequently analyzed for ANG II content and angiotensin-converting enzyme (ACE), chymase, and AT$_1$ receptor mRNA levels.

ANG II receptor assay. Fresh cardiac tissue from the LV midwall was homogenized in ice-cold 0.25 M sucrose containing 0.03 M histidine, 1 mM EDTA, and 0.1 M phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 14,000 g for 20 min, and the pellet was discarded. Subsequently, the supernatant was centrifuged at 45,000 g for 40 min, and the resulting pellet was saved. This pellet was resuspended in 50 mM Tris-HCl (pH 7.4) containing 10 mM MgCl$_2$, 1 mM EDTA, and 0.1 M PMSF to give a protein concentration of 1–2 mg/ml. Membranes were stored at –80°C until use. Membrane protein (140–180 μg) was incubated in 50 mM Tris-HCl buffer (pH 7.4) containing 0.1 mM PMSF and 0.2% heat-inactivated bovine serum albumin, with increasing concentration of the $^{125}$I-radiolabeled ANG II antagonist [Sar$^1$,Ile$^8$]ANG II (125I-[Sar$^1$,Ile$^8$]ANG II). The reaction was initiated by the addition of membrane protein and continued for 60 min at 22°C. Total incubation volume was 200 μl. Nonspecific binding was measured in the presence of 1.0 μM unlabeled [Sar$^1$,Ile$^8$]ANG II. The binding assay was terminated by rapid vacuum filtration over glass filters. The filters were washed three times with 5 ml of 0.9% NaCl containing 0.02% bovine serum albumin. Saturation isotherms were performed with increasing concentrations of $^{125}$I-[Sar$^1$,Ile$^8$]ANG II between 0.01 and 2 nM. Binding assays were done in duplicate. AT$_1$ versus AT$_2$ receptor density

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Table 1. Hemodynamic and echo parameters for control and PO dogs

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th>Electrophysiological studies</th>
<th>Myocyte function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak systolic blood pressure, mmHg</td>
<td>99 ± 8</td>
<td>99 ± 7</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>LV peak systolic pressure, mmHg</td>
<td>116 ± 8</td>
<td>200 ± 11*</td>
<td>153 ± 16*</td>
</tr>
<tr>
<td>LV-aorta gradient, mmHg</td>
<td>17 ± 5</td>
<td>98 ± 12*</td>
<td>83 ± 11*</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure, mmHg</td>
<td>5.7 ± 1.2</td>
<td>4.8 ± 1.1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mmHg</td>
<td>7 ± 1</td>
<td>10 ± 2</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>LV posterior wall thickness, cm</td>
<td>0.9 ± 0.6</td>
<td>1.5 ± 0.3*</td>
<td>1.7 ± 0.2*</td>
</tr>
<tr>
<td>LV end-diastolic dimension, cm</td>
<td>3.8 ± 0.1</td>
<td>3.7 ± 0.4</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>LV end-diastolic dimension/wall thickness</td>
<td>3.6 ± 0.1</td>
<td>2.6 ± 0.4</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>LV fractional shortening, %</td>
<td>32 ± 6</td>
<td>32 ± 4</td>
<td>37 ± 9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 control dogs, 8 pressure-overloaded (PO) dogs in electrophysiological studies, and 4 PO dogs in studies of isolated cardiomyocyte function (myocyte function studies). LV, left ventricular. *P < 0.01 vs. control dogs.

Table 2. Morphometric parameters for control and PO dogs

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th>Electrophysiological studies</th>
<th>Myocyte function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>23.5 ± 2.0</td>
<td>23.4 ± 1.7</td>
<td>26.5 ± 3.1</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>104 ± 9</td>
<td>192 ± 19*</td>
<td>218 ± 44*</td>
</tr>
<tr>
<td>RV weight, g</td>
<td>44.1 ± 3</td>
<td>43.8 ± 4.3</td>
<td>51 ± 7</td>
</tr>
<tr>
<td>LV weight/body weight, g/kg</td>
<td>4.4 ± 0.35</td>
<td>8.1 ± 0.68*</td>
<td>8.0 ± 1.0*</td>
</tr>
<tr>
<td>RV weight/body weight, g/kg</td>
<td>1.87 ± 0.15</td>
<td>1.87 ± 0.01</td>
<td>2.0 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. RV, right ventricular. *P < 0.01 vs. control dogs.

was determined by inhibition studies using 2 nM 125I-[Sar1,Ile8]ANG II in the presence of increasing amounts (0.05–5 nM) of the AT2 receptor antagonist PD-123319.

RNA isolation and Northern blots. Heart tissue from the midwall of the LV was homogenized in guanidinum thiocyanate, and total RNA was extracted according to the method of Chomczynski and Sacchi (8) and as previously described in our laboratory (38). The results were expressed as the ratios of AT1, ACE, and chymase mRNA to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The probe specific for the AT1 receptor was provided by Burn and co-workers (4). The plasmid harboring AT1 receptor cDNA was cleaved with EcoRI to yield a 1.4-kb probe. The canine chymase was provided by Caughey and co-workers (6). The endothelial nitric oxide synthase (eNOS) probe was the 4.0-kb bovine eNOS cDNA probe obtained from Dr. Michael Fallon at the University of Pennsylvania (38). The ACE probe was generated by reverse transcription followed by DNA polymerase chain reaction using total RNA from the dog lung.

RNase protection assay. The RNase protection assay for ACE mRNA transcript levels was performed as previously described in our laboratory (38). The ACE probe was generated in our laboratory as previously described (38). The ACE probe was generated by reverse transcription followed by DNA polymerase chain reaction using total RNA from the dog lung.

Cardiac ANG II concentration. Cardiac ANG II concentrations were determined as previously described from our laboratory (10, 38). The results were expressed as the ratios of ACE mRNA to GAPDH mRNA.

Cardiac ACE activity using hippuryl histidyl leucine as substrate. Cardiac ACE activity was measured using an assay developed in our laboratory (10, 38). ACE is extracted from homogenized cardiac tissue with detergent, and the reaction product hippuric acid is isolated from the reaction mixture by reverse-phase HPLC, thus eliminating interference from the detergent, the ACE-specific substrate hippuril histidyl leucine, and unreacted reaction byproducts.

Chymase-like activity using ANG I as substrate. Chymase activity was measured as previously described in our laboratory (10, 38). Generated ANG II was quantitated using a reversed-phase Alltima 5 μm phenyl-HPLC column (Alltech; Deerfield, IL). The peak area corresponding to a synthetic ANG II standard was integrated to calculate ANG II formation with chymostatin and with chymostatin plus captopril. Chymostatin-inhibitable ANG II formation was considered to represent the chymase-like activity, and chymostatin-inhibitable plus captopril-inhibitable ANG II formation was considered to represent all other ANG II-forming pathways.

Collagen analysis. Morphological evaluation was performed on perfusion-fixed tissues from eight PO and four control dogs euthanized at the UAB. Intersitial collagen volume percent was quantitatively evaluated using the ×20 objective (×600 on the video screen) of an Olympus AH3 research microscope with a monochrome video CCD72 camera interfaced with a computer using an Image One (Universal Imaging; Westchester, PA) morphometry system. Endocardial and epicardial halves of the LV myocardium were examined with use of picrosirius red-stained sections and a 540-nm (green) filter to enhance contrast of the collagen with the background. With the use of a minimum of 20 randomly selected digitized images collected from the inner (endocardial) and outer (epicardial) halves of the LV and RV, mean volume percent intersitial collagen was determined for each region for each animal. Perivascular collagen of vessels >100 μm in diameter was ignored. All morphometric measurements were performed in a blinded manner. Animal regional mean values were used to calculate group means and statistical differences. Results are presented as means ± SE for each group.

Statistical analysis. All results are presented as means ± SE. A Student’s t-test was used to compare biochemical, molecular biological, and cardiomyocyte data in sham-operated controls versus PO LVs. A P value <0.05 was required for significance.
RESULTS

Morphometric and hemodynamic data. The pressure gradient across the aortic band at the time of death was 98 ± 11 mmHg (Tables 1 and 2). LV weight/body weight was increased in PO versus sham-operated control dogs (8.11 ± 0.60 vs. 4.46 ± 0.38 g/kg, P < 0.001). Mean pulmonary artery pressure and pulmonary capillary wedge pressure did not differ in PO versus control dogs. Echo-derived LV wall thickness was increased nearly twofold as LV end-diastolic dimension was unchanged in PO versus control dogs. Accordingly, mean LV end-diastolic diameter/wall thickness was decreased, but not significantly, in PO versus control dogs as fractional shortening did not differ in PO versus control dogs.

In the PO animals, there was a trend toward an increase in the LV endocardial volume percent interstitial collagen, which did not achieve statistical significance compared with sham-operated animals (Fig. 1). Furthermore, on gross inspection of histopathological sections of the heart, three of eight dogs had multifocal mild increases in interstitial collagen in the endocardial half of the LV (Fig. 2, A–C). There was no evidence of myocyte necrosis. Although not quantitatively measured, individual myocytes were markedly increased in cross-sectional area in the PO dogs compared with controls (Fig. 2, C and D), and there was a marked heterogeneity in the size of individual myocytes in PO dogs, whereas control dogs had a very homogeneous cross-sectional area (Fig. 2D).

Tissue renin-angiotensin system components. LV ACE and chymase mRNA levels increased twofold in PO versus control dogs (P < 0.05; Fig. 3). However, ACE activity (98 ± 22 vs. 87 ± 11 mU/g) and chymase activity (2.5 ± 0.7 vs. 2.2 ± 0.3 mU/g) did not differ in PO and sham-operated dogs. LV ANG II levels were increased greater than threefold (68 ± 12 vs. 20 ± 5 pg/g, P < 0.01; Fig. 4) as steady-state AT1 receptor mRNA levels were decreased 40% in PO versus control dogs (P < 0.05) and mRNA transcript levels of eNOS.
were increased twofold over those in sham-operated controls (P < 0.05; Fig. 3). Total ANG II receptor binding of freshly prepared cardiac membranes demonstrated no difference in the dissociation constant (Kd; 0.52 ± 0.20 vs. 0.68 ± 0.12), but maximum binding (Bmax) was decreased 60% in PO versus control dogs (P < 0.01; Fig. 5). Furthermore, there was a negative correlation between LV ANG II levels and AT1 receptor Bmax (r = −0.77, P < 0.02) and steady-state AT1 mRNA

Fig. 3. Angiotensin-converting enzyme (ACE; A), chymase (B), angiotensin II (ANG II) type 1 (AT1) receptor (C), and endothelial nitric oxide synthase (eNOS; D) steady-state mRNA levels in LVs in normal and aortic-banded (PO) dogs demonstrating increased expression of ACE, chymase, and eNOS and decreased AT1 in PO versus control dogs. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. *P < 0.05 vs. controls.

Fig. 4. LV ANG I (A) and ANG II (B) in the LV of normal control and aortic-banded (PO) dogs demonstrating a greater than threefold increase in PO versus control dogs. *P < 0.05 vs. controls.

Fig. 5. Representative results of Scatchard analysis (A) of 125I-labeled [Sar1,Ile8]-ANG II binding to heart membranes from normal control and aortic-banded (PO) dogs. a: PO dogs; b: control dogs. Mean maximum binding (Bmax, B) calculated from the results of 4 separate experiments was significantly reduced in PO dogs (29 ± 6 vs. 87 ± 16 fmol/mg protein, respectively, P < 0.01). Mean dissociation constant values calculated from the results of 4 separate experiments were not different between the two groups (0.68 ± 0.12 vs. 0.52 ± 0.20 nmol/l). Bound, radioligand bound; Free, free radioligand.
ANG II negatively regulates AT1 receptor expression and that this is an adaptive response to chronic PO before the onset of myocardial failure.

The findings of the current study are consistent with results in cultured neonatal cardiac myocytes and fibroblasts from rats. Incubation with ANG II resulted in a time- and dose-dependent decrease in AT1 mRNA levels in both cardiomyocytes and fibroblasts (36). Stretch alone of neonatal cardiomyocytes resulted in upregulation of renin, ACE, angiotensinogen, and AT1 receptor genes (29). Coadministration of ANG II with stretch did not abrogate increases in renin, ACE, and angiotensinogen transcripts but downregulated AT1 receptor genes, demonstrating a differential response of renin-angiotensin system genes to combined stretch and ANG II. In a similar fashion, the chronic hemodynamic stress of PO in the present study resulted in increased ACE and chymase but decreased AT1 receptor transcript levels.

ANG II has been shown to potentiate the impaired relaxation in isolated cardiomyocytes from rats with PO cardiac hypertrophy (27, 32) and to exacerbate a negative inotropic effect on myocardium from rats with congestive heart failure (5, 7). However, in this study, isolated cardiomyocyte function did not differ in PO and control dogs at rest or in response to isoproterenol despite a threefold increase in LV ANG II levels. These data have important functional significance for the following reasons. First, the differential effects of significant changes in LV mass and loading conditions in this model make assessment of intrinsic contractile function difficult. The isolated myocyte studies demonstrated that mechanical performance of the hypertrophied cardiocytes was normal. Second, the isoproterenol studies demonstrated an equivalent β-adrenergic response between the normal and hypertrophied cardiomyocytes. This suggests that the β-adrenergic receptor system may not be altered in cardiomyocytes in this model of LV hypertrophy despite compensatory changes in the ANG II receptor system.

In a previous study (3), PO in adult sheep was associated with depressed myocardial contractility, whereas normal contractility was observed in immaturation.

Table 3. Isolated myocyte contractile function in control and PO dogs

<table>
<thead>
<tr>
<th>Percent shortening</th>
<th>Baseline</th>
<th>Isoproterenol (25 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control dogs</td>
<td>5.5 ± 0.4</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>PO dogs</td>
<td>5.2 ± 0.8</td>
<td>9.1 ± 1.9</td>
</tr>
<tr>
<td>Shortening velocity, μm/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control dogs</td>
<td>77 ± 7</td>
<td>214 ± 14</td>
</tr>
<tr>
<td>PO dogs</td>
<td>88 ± 17</td>
<td>208 ± 55</td>
</tr>
<tr>
<td>Relengthening velocity, μm/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control dogs</td>
<td>91 ± 10</td>
<td>215 ± 21</td>
</tr>
<tr>
<td>PO dogs</td>
<td>106 ± 26</td>
<td>214 ± 56</td>
</tr>
<tr>
<td>Time to peak contraction, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control dogs</td>
<td>209 ± 11</td>
<td>169 ± 5</td>
</tr>
<tr>
<td>PO dogs</td>
<td>209 ± 17</td>
<td>177 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE.
ture lambs with gradual PO. Depressed contractility could not be explained by a greater pressure gradient, more severe hypertrophy, or longer duration of PO. Another study (13) demonstrated that capillary density and coronary flow reserve were reduced in adult sheep but normal in young lambs with LV hypertrophy. Our dogs showed no signs of heart failure or pulmonary congestion, nor did they manifest chamber or cardiomyocyte dysfunction after 9 mo of PO. However, Hittengen and co-workers (17) demonstrated that this same animal model developed congestive heart failure after 12 mo of PO. The onset of failure was associated with a significant increase in endo- and midmyocardial fibrosis and a decrease in endocardial flow reserve (17), both of which were normal in animals who did not develop heart failure. Thus, as the animals progressed to an adult stage, they developed abnormal coronary flow reserve and LV failure that did not relate to the extent of hypertrophy but did relate to the presence of interstitial fibrosis in the endo- and midmyocardial layers.

The presence of concentric cardiac hypertrophy has been associated with an increased incidence of ventricular arrhythmias (25, 30) and sudden cardiac death (22, 26) in patients with hypertension. There have been few animal models that systematically study the occurrence of provokable ventricular arrhythmias in the setting of PO cardiac hypertrophy. In the spontaneously hypertensive rat, however, the occurrence of ventricular fibrillation in response to programmed electrical stimulation correlated with LV weight and extensive myocardial fibrosis (33). In contrast, our dogs did not have extensive interstitial fibrosis despite a threefold elevation in LV ANG II levels. Taken together, these results suggest it is the chronic myocardial changes such as fibrosis and/or superimposed ischemia (9, 19) that are arrhythmogenic, not the isolated acute effects of ANG II itself.

It is of interest that the fibrosis response was minimal in the setting of a threefold increase in LV ANG II levels and marked cardiac hypertrophy. The effects of increased ANG II are counterbalanced by nitric oxide. Indeed, inhibition of nitric oxide synthase potentiated ANG II-induced cardiac fibrosis in the rat, suggesting an autocrine or paracrine role for nitric oxide on fibroblasts (18). Furthermore, nitric oxide and bradykinin decreased production of collagen in studies of isolated fibroblasts in vitro (21). There was a greater than twofold increase in steady-state mRNA levels of eNOS in PO dogs. Accordingly, this may explain the minimal fibrosis in these young animals, whereas 12 mo after induction of PO, adult animals do progress to mechanical failure and more extensive fibrosis (17). Another protective mechanism could be related to the marked downregulation in total ANG II receptor density. The highly significant reciprocal relationship of LV AT1 gene transcription and ANG II levels provides strong support for downregulation of the AT1 receptor by increased ANG II levels that could result in decreased AT1 receptor signaling and fibrosis.

The results of the current investigation suggest that ANG II concentrations negatively regulate the in vivo expression of the AT1 receptor in response to the gradual onset of PO. This may represent an adaptive/protective response in the young animal to chronic PO before the onset of extensive fibrosis and myocardial failure. Accordingly, this model may be of value in studying the transition to heart failure in chronic PO.

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