Angiotensin Receptor Regulates Cardiac Hypertrophy and Transforming Growth Factor-β1 Expression

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Abstract The role of angiotensin II via the angiotensin type 1 or type 2 receptor in the development of cardiac hypertrophy was determined in adult male Sprague-Dawley rats subjected to coarctation of the abdominal aorta. Five groups of animals were studied: coarction, coarctation plus DuP 753, coarctaion plus PD 123319, sham plus DuP 753, or sham operation. Type 1 receptor blockade was accomplished with DuP 753 given in the drinking water and type 2 blockade with PD 123319 delivered by osmotic minipumps beginning with the day of surgery until 72 hours after aortic coarctation. Mean carotid blood pressures and the carotid-femoral artery blood pressure gradients were not different among coarctation, coarctation plus DuP 753, and coarctation plus PD 123319 animals. However, ratios of heart weight to body weight were higher in coarctation (4.95 ±0.8) or coarctation plus PD 123319 (4.52±0.5) than in sham animals (3.6±0.4; P < .005 and .05, respectively). In coarctation plus DuP 753–treated animals heart weight–body weight ratios were not different from sham or sham plus DuP 753 animals (3.9±0.4 versus 3.6±0.4 or 3.3±0.08, respectively). Type 1 receptor mRNA levels were significantly increased in the coarctation group, with the highest levels in the coarctation plus DuP 753 and sham plus DuP 753 groups. To determine whether growth factors were involved in the hypertrophic process, we measured transforming growth factor-β1 mRNA levels. Northern analysis demonstrated a twofold increase in coarctation animals compared with sham or coarctation plus DuP 753–treated animals. Therefore, cardiac hypertrophy induced by abdominal coarctation of the aorta is mediated by the angiotensin type 1 receptor and results in upregulation of the cardiac angiotensin type 1 and transforming growth factor-β1 genes. (Hypertension. 1994;33:587-592.)

Key Words • receptors, angiotensin • angiotensin II • growth substances • aortic coarctation • RNA, messenger

Cardiac hypertrophy is an important risk factor for sudden cardiac death in patients with cardiovascular disease. Therefore, the determination of the mechanisms leading to cardiac hypertrophy is of extreme importance. Cardiac hypertrophy is a physiological adaptation to an increase in workload. This extra mechanical load is often accompanied by increases in circulating catecholamines, angiotensin II (Ang II), and endothelin, which may play additional roles in mediating the hypertrophic process. Previous studies have implicated a potential role for Ang II by using converting enzyme inhibitors to prevent cardiac hypertrophy in rats after chronic abdominal coarctation. In humans, converting enzyme inhibitors have been successfully used to reduce left ventricular mass as a result of hypertension and increase survival after myocardial infarction. Although these studies suggest a role of the renin-angiotensin system, the potential participation of the kinin system could not be dismissed.

Cardiac hypertrophy results from coarctation of the abdominal aorta in prehypertensive rats. We examined the effect of AT1 receptor blockade with DuP 753 on cardiac growth and AT1 and TGF-β1 gene expression.
Methods

Abdominal Aortic Coarctation

Adult male Sprague-Dawley rats (250 to 300 g) (Hilltop Laboratory Animals) received pentobarbital anesthesia (5 mg/100 g IP) and underwent suprapancreal abdominal aortic coarctation via a left lateral flank incision (COARC group). The abdominal aorta was dissected free, a 22-gauge needle was placed adjacent to the aorta, and a ligature was placed around the blunt needle and aorta. The blunt needle was then removed, leaving the aorta constricted to the size of the needle. A second group of rats underwent coarctation of the aorta as described above but in addition was given DuP 753 in the drinking water (10.2±2.8 mg/kg per day) beginning the day of surgery (COARC+DuP 753 group). DuP 753 in the drinking water was changed daily and the fluid intake recorded. A third group of animals received a flank incision alone (SHAM), and a fourth group of SHAM animals received DuP 753 as above (SHAM+DuP 753). A fifth group of animals underwent coarctation of the aorta and placement of osmotic minipumps (model 1007D, Alzet Corp) in the intraperitoneal cavity containing the specific AT1 receptor antagonist PD 123319 (Parke-Davis) to deliver 30 mg/kg per day as recommended by the manufacturer.

On the third day after abdominal aortic coarctation the animals received pentobarbital anesthesia with subsequent placement of carotid and femoral arterial catheters. For determination of degree of aortic obstruction, carotid and femoral arterial blood pressure measurements were recorded on a multichannel physiograph (model 7754B, Hewlett-Packard). For assessment of AT1 receptor blockade, SHAM, DuP 753, and PD 123319 animals received a 0.1 mg/kg IV bolus of Ang II, and the pressor response was recorded. The animals were then killed by exsanguination, the hearts were removed and weighed, and the left ventricle was dissected free and immediately frozen in liquid nitrogen for RNA determinations.

RNA Determinations

Total RNA was extracted after the method of Chomczynski and Sacchi.18 Total left ventricle RNA from individual animals was electrophoresed under denaturing conditions, stained with ethidium bromide to prove RNA integrity, and transferred to nylon membranes (Zetaprobe GT, Bio-Rad Laboratories) for Northern analysis and/or diluted serially and transferred by vacuum to nylon membranes for dot blot analysis.19

RNA Hybridization

Membranes were hybridized after the method of Church and Gilbert20 with the use of a rat TGF-β1 cDNA (a generous gift of Su Wen Qian, National Cancer Institute, Bethesda, Md),21 a rat AT1 cDNA (a generous gift of J. Harrison and K. Lynch, University of Virginia) containing the entire coding region of the AT1 gene,22 a rat atrial natriuretic peptide (ANP) cDNA (Calbio), and, as a control for gene-specific expression and loading, a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.23 cDNAs were labeled by random priming (Random Priming Kit, Boehringer-Mannheim) to a specific activity equal to or higher than 1×10⁶ cpm/μg.24 mRNA levels were determined by autoradiography with multiple exposures within the linearity of the film (Kodak XAR) and measured with a laser densitometer (Ultrascan XL, Pharmacia LKB Biotechnology). The densitometric absorbance of the AT1 mRNA was corrected for GAPDH mRNA by dividing the respective densitometric absorbances. To confirm the reproducibility of results, we repeated each blot and hybridization at least twice.

Statistical Analysis

Hemodynamic, heart weight, and densitometric data were analyzed by one-way ANOVA or paired t test where appropriate. Statistical significance was defined as at a value of <.05.

Results

Hemodynamics

The hemodynamic profile of the animals after 3 days of abdominal coarctation is summarized in the Table. Coarctation of the abdominal aorta resulted in a significant difference in blood pressure between the carotid and femoral arteries (gradient, 41±12 mm Hg versus 0, COARC versus SHAM, P<.05). DuP 753 treatment did not alter the carotid-femoral artery gradient (COARC versus COARC+DuP 753, P=NS), consistent with a fixed aortic obstruction. In addition, DuP 753 treatment did not alter the carotid mean blood pressures, as blood pressures in all groups were not statistically different (Table). The pressor response to Ang II was clearly blunted in the COARC+DuP 753 and SHAM+DuP 753 groups compared with SHAM animals (20±2 versus 7±1 or 10±4 mm Hg, SHAM versus COARC+DuP 753 or SHAM+DuP 753, P<.05). AT1 receptor blockade with PD 123319 also did not affect the carotid blood pressure, and the pressor response to Ang II was similar to that in SHAM animals. Thus, the COARC, COARC+DuP 753, and COARC+PD 123319 animals were not hypertensive and had similar levels of aortic obstruction, with a demonstrable blockade of the AT1 receptor in the COARC+DuP 753 group.

Cardiac Hypertrophy

As a measure of cardiac hypertrophy, the heart weight–body weight ratio was determined. After 3 days of abdominal coarctation a significant increase in this ratio was observed (COARC versus SHAM groups, P<.005) (Fig 1). Treatment with DuP 753 prevented an
FIG 1. Bar graph shows heart weight-body weight ratios (milligrams per gram) after 72 hours of abdominal aortic coarctation and treatment with DuP 753 (DUP). Bars represent mean±SD of heart weight-body weight ratios of animals after abdominal coarctation (COARC, n=10), COARC plus DuP 753 (n=7), COARC plus PD 123319 (PD, n=4), SHAM plus DuP 753 (n=3), and sham (SHAM, n=5) controls. As shown, heart weight-body weight ratio significantly (P<.005) increased in COARC compared with SHAM, and DuP 753 treatment prevented development of cardiac hypertrophy (SHAM vs COARC+DUP, P=NS). Treatment with PD 123319 (COARC+PD) resulted in increased heart weight-body weight ratio similar to that in COARC animals. Statistical comparison by one-way ANOVA.

increase in the heart weight–body weight ratio in the COARC animals (COARC+DUP 753 versus SHAM, P=NS), without altering the ratio in sham animals (SHAM+DUP 753) (Fig 1). Treatment with PD 123319 did not prevent cardiac hypertrophy, with heart weight–body weight ratios similar to those of the COARC animals alone (COARC versus COARC+PD 123319, P=NS). Thus, cardiac hypertrophy resulting from aortic coarctation is mediated by Ang II via the AT₁ and not AT₂ receptor.

Expression of TGF-β₁ and ANP During Cardiac Hypertrophy

As coarctation of the abdominal aorta results in cardiac hypertrophy mediated by Ang II via the AT₁ receptor, the potential involvement of TGF-β₁ in this hypertrophic process was explored. TGF-β₁ gene expression in the hypertrophied heart was examined by Northern analysis as shown in Fig 2. TGF-β₁ gene expression was increased twofold in the hypertrophied left ventricle, whereas this increase was prevented by DuP 753 treatment (COARC, P<.05). Baseline TGF-β₁ levels were unchanged with DuP 753 treatment alone (SHAM+DuP 753, P=NS). Consistent with induction of the hypertrophic phenotype, ANP mRNA levels increased in the hypertrophied heart (COARC), and this phenotypic switch was blunted with DuP 753 treatment (COARC+DuP 753) (Fig 2). GAPDH gene expression and RNA loading were similar in all groups (Fig 2). Thus, cardiac hypertrophy after abdominal coarctation results in increased TGF-β₁ and ANP gene expression, and this increase in gene expression is prevented by AT₁ receptor blockade.

AT₁ Gene Expression During Cardiac Hypertrophy and DuP 753 Treatment

Accumulation of AT₁ mRNA during cardiac hypertrophy was determined by dot blot analysis with AT₁ mRNA levels corrected for GAPDH as shown in Fig 3. AT₁ mRNA levels increased significantly in the COARC group (COARC versus SHAM, P<.03) and further increased in the COARC+DuP 753 group (P<.03) compared with the SHAM or COARC groups. AT₁ mRNA levels were also significantly increased in
Coarctation of the abdominal aorta has been extensively studied as a model of cardiac hypertrophy, which results from pressure overload due to aortic constriction and leads to the eventual development of hypertension. The present study demonstrates that the cardiac hypertrophy produced in this study is primary and not due to changes in blood pressure. The development of cardiac hypertrophy can be prevented by specific AT₁ receptor blockade. This appears to be a specific effect of DuP 753 on the heart, as blood pressure or the degree of aortic obstruction was not altered in the COARC and COARC+DuP 753 animals. Induction of the hypertrophic response and its blunting with DuP 753 treatment in the COARC animals were confirmed by the analysis of ANP mRNA levels. Expression of the ANP in the ventricle serves as a sensitive marker for induction of the hypertrophic phenotype. In support of the role of the renin-angiotensin system in the regulation of cardiac hypertrophy in the coarctation model, two recent reports demonstrated that angiotensin-converting enzyme inhibitor treatment prevented the development of cardiac hypertrophy after abdominal coarctation, although possible involvement of the kinin system could not be dismissed in these studies. The present study confirms and extends those findings to demonstrate that the hypertrophic response appears to be mediated via the AT₁ receptor. Considering that cardiac hypertrophy with increased afterload is a necessary compensatory mechanism for decreasing wall stress and maintaining cardiac performance, Ang II via the AT₁ receptor may play an integral role in the regulation of cardiac growth and performance.

Although the mechanism by which Ang II produces cardiac hypertrophy is unknown, one potential mechanism is the induction of cardiac growth factors. In the heart, isolated cardiomyocytes demonstrate endogenous secretion of TGF-β, and the TGF-β gene is upregulated in the growing newborn heart and during cardiac hypertrophy. The present study confirms that TGF-β gene expression is increased in the hypertrophied left ventricle. In addition, we demonstrate the new finding that the increase in TGF-β expression can be inhibited with specific AT₁ receptor blockade. This appears to be a specific effect of DuP 753 treatment. The present study confirms that specific AT₁ receptor blockade. This appears to be a specific effect of DuP 753 treatment.
expression increased 3.3-fold in isolated cardiocytes but not in cardiac fibroblasts in a volume-overload model of hypertension and increased in vitro in neonatal cardiocytes treated with exogenous Ang II. Cardiac hypertrophy in the volume-overload model can also be prevented with DuP 753 treatment. Considering that cardiocytes can respond to exogenous TGF-β1, release TGF-β2 endogenously, and upregulate gene expression in one hypertrophy model, the cardiocyte appears to have the capacity to express and respond to TGF-β2 produced either intracellularly or in the local cellular environment. Although TGF-β1 is not been conclusively shown to stimulate growth of the heart in vivo, the demonstration of Ang II regulation of TGF-β1 gene expression via the AT1 receptor in the present study suggests that in this model Ang II regulates cardiac hypertrophy via the upregulation of TGF-β1 and possibly other growth factor genes. Taken together, the increased gene expression of TGF-β1 in the hypertrophied heart and its ability to alter cardiac gene expression suggest an important role in the regulation of cardiac growth and hypertrophy.

The adult heart is known to express the AT1 receptor gene although regulation of the cardiac AT1 gene is unknown. In the present study we demonstrated that AT1 mRNA accumulates in the left ventricle with pressure overload and with specific AT1 receptor blockade with DuP 753. The upregulation of receptor gene expression with receptor blockade is a hallmark feature of the G protein–coupled receptor family, of which the AT1 receptor is a member. If ligand binding were the only mechanism involved, it is unclear why AT1 mRNA accumulation was not decreased in the left ventricle of the COARC group as circulating levels of renin are increased in the first 48 hours of this model. As our COARC group was studied on day 3, when renin levels have in previous studies returned to baseline, AT1 mRNA levels could reflect new steady-state levels and thus not represent gene expression when renin levels were at their peak at 24 and 48 hours after coarctation. In addition to potential ligand effects, the left ventricle is exposed to elevated wall stress due to the aortic constriction. Mechanical stretch is a powerful stimulant of proto-oncogene expression and protein synthesis in the cardiocyte. The increase in proto-oncogenes could potentially induce transcription factors that alter the expression of many genes including the AT1. The mechanisms whereby Ang II regulates the expression of the cardiac AT1 gene are unknown and may include mechanical and/or ligand-receptor binding events, as demonstrated by the present data, and are currently under investigation in our laboratory.

We conclude that Ang II via the AT1 receptor is intimately involved in the regulation of cardiac hypertrophy after abdominal coarctation in the rat. Furthermore, we speculate that the Ang II–induced hypertrophic process is mediated by growth factors such as TGF-β1. Considering the nearly ubiquitous presence of the AT1 receptor in the animal tissues studied thus far, we speculate that Ang II may serve as a generalized regulator of cellular growth and hypertrophy.

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References


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