Angiotensin Receptor Regulates Cardiac Hypertrophy and Transforming Growth Factor-\(\beta_1\) 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: coarctation, coarctation plus DuP 753, coarctation 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 given 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-\(\beta_1\) 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-\(\beta_1\) 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.\(^1\,2\) 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,\(^3\,4\) 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.\(^4\) In humans, converting enzyme inhibitors have been used to successfully reduce left ventricular mass as a result of hypertension\(^5\) and increase survival after myocardial infarction.\(^6\,7\) Although these studies suggest a role of the renin-angiotensin system, the potential participation of the kinin system could not be dismissed.

Cardiocytes are known to bind Ang II with high affinity.\(^8\) Cardiac Ang II receptors have been divided into subtypes by their affinity for nonpeptide inhibitors into angiotensin type 1 (AT\(_1\)) sensitive to DuP 753 and type 2 (AT\(_2\); sensitive to PD 123319). Recently, the AT\(_1\) receptor was cloned from rat vascular smooth muscle,\(^9\) the bovine adrenal,\(^10\) and human lymphocytes.\(^11\) The AT\(_1\) gene encodes for a 2.3-kb transcript present in the adult heart, although at a relatively lower copy than in the kidney or liver.\(^9\) The cardiac action of Ang II mediated by these membrane receptors includes stimulation of cardiac contractility and acceleration of protein synthesis.\(^12\) The AT\(_1\) receptor is principally responsible for the Ang II–induced cardiovascular, renal, and central nervous system actions\(^13\) studied thus far. The functional role of the AT\(_2\) receptor in the heart is unknown, but it may play a role in smooth muscle cell proliferation after balloon injury.\(^14\) The role of the vascular AT\(_2\) receptor in mediating cardiac hypertrophy in vivo has not been clarified.

An active area of research is focused on the role of growth factors in cardiac hypertrophy.\(^14\) The normal heart expresses the genes and proteins for the \(\beta\)-type transforming growth factors (TGF-\(\beta\)).\(^15\) The TGF-\(\beta_1\) gene is upregulated in the growing newborn heart\(^15\) in cardiocytes surrounding an area of infarction\(^16\) and during cardiac hypertrophy,\(^17\) suggesting a potential role in cardiac growth and repair. Whether Ang II via the AT\(_1\) receptor is involved in the regulation of the TGF-\(\beta_1\) gene during cardiac hypertrophy has not been explored.

The following study was designed to determine the role of Ang II in the development of cardiac hypertrophy resulting from coarctation of the abdominal aorta in prehypertensive rats. We examined the effect of AT\(_1\) receptor blockade with DuP 753 on cardiac growth and AT\(_1\) and TGF-\(\beta_1\) 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 suprapennial abdominal coarctation via a left lateral flank incision (COARC group). The abdominal aorta was dissected free, a blunt 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 the 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 μg/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. 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.21

RNA Hybridization

Membranes were hybridized after the method of Church and Gilbert with the use of a rat TGF-β1 cDNA (a generous gift of Su Wen Qian, National Cancer Institute, Bethesda, Md),24 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 × 10⁹ 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 P < 0.05.

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 < 0.05). 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 < 0.05). 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 < 0.005) (Fig 1). Treatment with DuP 753 prevented an
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 (COARC+DuP 753) (Fig 3). AT₁ mRNA levels were also significantly increased in COARC animals alone (COARC versus COARC+DuP 753, \(P=NS\)). Thus, cardiac hypertrophy resulting from aortic
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.\textsuperscript{4-27} The present study demonstrates that the cardiac hypertrophy produced in this study when hypertensive phase of this model can be prevented by specific AT\(_1\) receptor blockade. This indicates that pressure overload due to aortic constriction and leads to the eventual development of hypertension.\textsuperscript{4-27} The present study demonstrates that the cardiac hypertrophy produced in this study when hypertensive phase of this model can be prevented by specific AT\(_1\) 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 was 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.\textsuperscript{28} 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,\textsuperscript{5,29} 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 AT\(_1\) 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 AT\(_1\) 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-\(\beta\),\textsuperscript{30} and the TGF-\(\beta\) gene is upregulated in the growing newborn heart\textsuperscript{15} and during cardiac hypertrophy.\textsuperscript{17} The present study confirms that TGF-\(\beta\) gene expression is increased in the hypertrophied left ventricle. In addition, we demonstrate the new finding that the increase in TGF-\(\beta\) expression can be inhibited with specific AT\(_1\) receptor blockade. Considering that the mean carotid and femoral artery blood pressures were unchanged in the COARC and COARC+DuP 753 groups, the present data would suggest that Ang II plays a specific role in the regulation of the TGF-\(\beta\) gene. However, it is not clear from this study whether the increase in TGF-\(\beta\) mRNA is localized to ventricular myocytes versus nonmyocytes. In support, TGF-\(\beta\) gene

![Figure 3. Dot blot and bar graph show left ventricle angiotensin type 1 receptor (AT\(_1\)) mRNA levels corrected for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 72 hours after abdominal aortic coarctation and treatment with DuP 753 (DUP753 in figure). A. Dot blots for AT\(_1\) mRNA in which total RNA (10 to 0.25 \(\mu\)g) was serially diluted and loaded in each well. Groups are sham controls (SHAM, \(n=6\)), coarctation (COARC, \(n=5\)), and COARC+DuP 753-treated (\(n=5\)) animals. B. 2.5-\(\mu\)g dilution of same membrane probed for the control gene GAPDH. C. UV transillumination of 2.5-\(\mu\)g dilution as a demonstration of equivalent loading. D. Bars represent densitometric absorbance of the 2.5-\(\mu\)g dilution of AT\(_1\) autoradiograph corrected for GAPDH mRNA levels (mean\(\pm\)SEM). As shown, AT\(_1\) mRNA levels are significantly elevated in the COARC left ventricle and even further after AT\(_1\) receptor blockade with DuP 753. *\(P<0.03\), COARC vs SHAM; **\(P=0.08\), COARC+DuP 753 vs SHAM; \(\triangle\)\(P<0.03\), COARC vs COARC+DuP 753, determined by one-way ANOVA.

![Figure 4. Dot blot and bar graph show angiotensin type 1 receptor (AT\(_1\)) mRNA levels in hearts from sham-operated rats after 72 hours of DuP 753 (DUP753 in figure) treatment. A. Dot blot for AT\(_1\) mRNA with total RNA serially diluted (10 to 0.25 \(\mu\)g) and loaded in each well. Groups are sham animals (SHAM, \(n=3\)) and SHAM+DuP 753-treated (\(n=3\)) animals. B. Same membrane hybridized to a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA to confirm gene-specific expression and RNA loading. C. UV transillumination of membrane in the prehypertensive equivalent RNA loading. D. Bar graph summarizes AT\(_1\) densitometric data corrected for GAPDH (mean\(\pm\)SEM). Statistical analysis by paired \(t\) test. AT\(_1\) mRNA levels are significantly increased in the left ventricle of SHAM+DuP 753-treated animals. Autoradiographic exposure, 72 hours for AT\(_1\) and 20 hours for GAPDH.
expression increased 3.3-fold in isolated cardiocytes but not in cardiac fibroblasts in a volume-overload model of hypertension\(^1\) and increased in vitro in neonatal cardiocytes treated with exogenous Ang II.\(^2\) Cardiac hypertrophy in the volume-overload model can also be prevented with DuP 753 treatment.\(^3\) Considering that cardiocytes can respond to exogenous TGF-\(\beta\),\(^4\) release TGF-\(\beta\) endogenously,\(^5\) and upregulate gene expression in one hypertension model,\(^6\) the cardiocyte appears to have the capacity to express and respond to TGF-\(\beta\) produced either intracellularly or in the local cellular environment. Although TGF-\(\beta\) is not been conclusively shown to stimulate growth of the heart in vivo, the demonstration of Ang II regulation of TGF-\(\beta\) gene expression via the AT\(_1\) receptor in the present study suggests that in this model Ang II regulates cardiac hypertrophy via the upregulation of TGF-\(\beta\) and possibly other growth factor genes. Taken together, the increased gene expression of TGF-\(\beta\) 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 AT\(_1\) receptor gene\(^7\) although regulation of the cardiac AT\(_1\) gene is unknown. In the present study we demonstrated that AT\(_1\) mRNA accumulates in the left ventricle with pressure overload and with specific AT\(_1\) 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 AT\(_1\) receptor is a member.\(^8\)\(^,\)\(^9\)\(^,\)\(^5\)\(^,\)\(^6\) If ligand binding were the only mechanism involved, it is unclear why AT\(_1\) 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.\(^4\) As our COARC group was studied on day 3, when renin levels have in previous studies returned to baseline,\(^4\) AT\(_1\) 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\(^2\)\(^,\)\(^3\)\(^,\)\(^7\) in the cardiocyte. The increase in proto-oncogenes could potentially induce transcription factors that alter the expression of many genes including the AT\(_1\) receptors. The mechanisms whereby Ang II regulates the expression of the cardiac AT\(_1\) 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 AT\(_1\) 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-\(\beta\). Considering the nearly ubiquitous presence of the AT\(_1\) receptor in the animal tissues studied thus far, we speculate that Ang II may serve as a generalized regulator of cellular growth and hypertrophy.

**Acknowledgments**

Dr Everett was supported by University of Virginia Child Health Research Center grant NIH IP30HD2881001. Dr Tufo-McReddie was the recipient of a Fellowship Award from the American Heart Association, Virginia Affiliate (VA-91-62). Dr Gomez was supported by National Heart, Lung, and Blood Institute grant HL-41899; a Research Career Development Award (HL-02307), and the Center of Excellence in Pediatric Nephrology and Urology (DK-44756).

**References**


Angiotensin receptor regulates cardiac hypertrophy and transforming growth factor-beta 1 expression.
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Hypertension. 1994;23:587-592
doi: 10.1161/01.HYP.23.5.587

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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