

Angiotensin II Type 2 Receptor Blockade Amplifies the Early Signals of Cardiac Growth Response to Angiotensin II in Hypertrophied Hearts

Jozef Bartunek, MD, PhD; Ellen O. Weinberg, PhD; Minoru Tajima, MD, PhD;
Susanne Rohrbach, BA; Beverly H. Lorell, MD

Background—We have previously shown that the acute molecular growth response of new protein synthesis and protein kinase C activation in response to angiotensin II (Ang II) is altered in left ventricular (LV) hypertrophy compared with normal hearts. We have also shown an upregulation of Ang II type 2 (AT₂) receptors in hypertrophied hearts relative to controls. Activation of AT₂ receptors is proposed to counteract growth effects of AT₁ receptor in response to Ang II. Thus, we tested the hypothesis that in hypertrophied hearts, the AT₂ receptor mediates inhibitory effects on the new cardiac protein synthesis in response to acute Ang II stimulation.

Methods and Results—Flaccid buffer-perfused adult normal and hypertrophied rat hearts were perfused with Ang II 10⁻⁸ mol/L plus prazosin 10⁻⁷ mol/L or Ang II plus the AT₂ blocker PD 123319 5×10⁻⁷ mol/L. New protein synthesis was measured by the rate of [³H]phenylalanine incorporation into the LV proteins. In normal hearts, Ang II (n=8) increased the rate of [³H]phenylalanine incorporation by 74±27% (*P*<0.05 versus no drug). Treatment with PD123319 (n=8) did not increase protein synthesis compared with Ang II alone (32±11% versus Ang II alone, *P*=NS). In hypertrophied hearts, Ang II alone (n=6) increased the rate of [³H]phenylalanine incorporation only by 23±13% (*P*=NS versus no drug). In contrast, treatment with PD123319 (n=7) induced a 76±21% increase in new LV protein synthesis compared with Ang II alone (*P*<0.05). AT₂ receptor blockade in Ang II-stimulated hypertrophied hearts was associated with enhanced membrane protein kinase C translocation and reduced LV cGMP content.

Conclusions—These data support the hypothesis that in adult hypertrophied rat hearts, inhibition of cardiac AT₂ receptors, which are upregulated in chronic LV hypertrophy, amplifies the immediate LV growth response to Ang II. This appears to be related to augmented Ang II-stimulated PKC activation and suppression of cGMP signaling. (*Circulation*. 1999;99:22-25.)

Key Words: angiotensin ■ hypertrophy ■ signal transduction

In neonatal cardiac myocytes¹ and intact adult hearts,^{2,3} angiotensin (Ang) II stimulates early signals of the cardiac growth, such as new cardiac protein synthesis, independently of the load. This response depends on translocation of protein kinase C (PKC)-ε from cytosol to membrane fraction and is blocked by the specific Ang II type 1 (AT₁) receptor antagonist losartan.¹⁻⁶ In contrast with neonatal myocytes, early signals of the cardiac growth response to Ang II in adult rat hearts do not involve the induction of proto-oncogenes, which occurs in response to load.¹⁻⁸ We have also shown that the acute cardiac growth response of new cardiac protein synthesis and PKC translocation in response to Ang II is blunted in hypertrophied hearts with aortic stenosis relative to normal hearts.^{2,3} Recent observations suggest that this may be mediated by the Ang II type 2 (AT₂) receptor. We⁹ and others¹⁰ have demonstrated an upregulation of LV AT₂ receptors in hypertrophied hearts. Similar upregulation of AT₂ receptors

was reported in human failing myocardium.^{11,12} Several studies have proposed that downstream signaling related to the AT₂ receptor differs strikingly from signaling of the AT₁ receptor and involves kinin/cGMP signaling rather than the PKC pathway.¹³⁻¹⁷ Transgenic experiments suggest that the AT₂ receptor signaling cascade has distinct biological roles compared with AT₁ receptor-mediated signaling.^{18,19} Consistent with this hypothesis, recent studies in vascular smooth muscle cells²⁰ and endothelial cells²¹ demonstrated that the AT₂ receptor exerts antiproliferative effects counteracting the growth-promoting effects of the AT₁ receptor. In contrast, the role of AT₂ receptor-mediated signaling in cardiac growth of the intact adult heart is not known. Thus, in the present study, we tested the hypothesis that in hypertrophied hearts, the AT₂ receptor mediates inhibitory effects on the new cardiac protein synthesis and PKC activation in response to acute Ang II stimulation.

Received July 31, 1998; revision received November 3, 1998; accepted November 5, 1998.

From the Cardiovascular Division, The Harvard Thorndike Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass. Correspondence to Beverly H. Lorell, MD, Cardiovascular Division, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215. E-mail blorell@bidmc.harvard.edu

© 1999 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

Weight and Hemodynamics of Isolated Hypertrophied and Normal Hearts

	Normal Hearts			LVH Hearts		
	No Drug (n=4)	Ang II (n=8)	Ang II+PD123319 (n=8)	No Drug (n=4)	Ang II (n=6)	Ang II+PD123319 (n=7)
Body weight, g	344±3	370±15	372±15	373±5	414±8	406±7
LV weight, g	0.85±0.03	0.89±0.03	0.91±0.03	1.41±0.09	1.66±0.08	1.56±0.12
LV/body weight, mg/g	2.46±0.09	2.39±0.05	2.43±0.04	3.53±0.11	4.02±0.23	3.85±0.27
Coronary flow, mL · min ⁻¹ · g ⁻¹	23.3±2.1	20.9±0.9	19.9±0.6	18.9±0.6	16.7±1.1	17.5±1.1

Methods

Weanling male Wistar rats (75 to 90 g, Charles River Breeding Laboratories, Wilmington, Del) underwent ascending aortic stenosis as previously described (n=44).^{2,3,9} Age-matched animals served as a control group (n=45). At 7 to 9 weeks after the banding, the isolated flaccid hearts were perfused by modified Krebs-Henseleit buffer at 37°C as previously described.^{2,3,7-9} For measurements of protein synthesis, the buffer contained 0.05% albumin and a mixture of amino acids as previously described.^{2,3,8,9}

To investigate the effects of AT₂ blockade on Ang II-induced new cardiac protein synthesis, normal and hypertrophied hearts were perfused with no drug (n=4), with Ang II 10⁻⁸ mol/L plus 10⁻⁷ mol/L prazosin (n=6 to 8), and with Ang II plus the selective AT₂ blocker PD 122319 (Parke-Davis, 5×10⁻⁷ mol/L, n=6 or 7). The α₁-blocker prazosin was used to prevent any indirect stimulation of protein synthesis via activation of the postsynaptic sympathetic system.^{3,4} The dose of PD 122319 was chosen on the basis of previous studies^{9,13,14} demonstrating a selective AT₂ receptor blockade in response to Ang II. After 60 minutes of perfusion, hearts were perfused for another 120 minutes with the same buffer, to which 0.5 mCi/L [³H]phenylalanine was added.^{2,3,8,22} The net LV protein synthesis during the 120 minutes of perfusion was assumed to be linear^{2,3,8,22} and was calculated as follows: phenylalanine incorporation (moles · g protein⁻¹ · h⁻¹) = phenylalanine (dpm · g protein⁻¹ · h⁻¹)/perfusate phenylalanine specific activity (dpm/mol).

To investigate whether AT₂ blockade modulates Ang II-stimulated PKC translocation, separate groups of normal and hypertrophied hearts were perfused for 15 minutes with no drug (n=4), with Ang II 10⁻⁸ mol/L plus 10⁻⁷ mol/L prazosin (n=5 or 6), and with Ang II plus the AT₂ blocker 5×10⁻⁷ mol/L (n=6 or 7). This time period was chosen on the basis of the time course of maximal activation of PKC by Ang II.² Cytosolic and membrane fractions from frozen LV tissue were prepared as described previously.^{2,23} Samples containing 50 μg protein were then separated on a 7.5% SDS-PAGE gel. Detection of PKC was carried out by use of anti-PKC-ε antibody (1:1000, Life Technologies) and an enzyme-linked chemiluminescence-Western blotting analysis system (Amersham International).

To investigate whether AT₂ blockade modifies LV cGMP content in Ang II-perfused hearts, additional groups of normal and hypertrophied hearts were subjected to Ang II 10⁻⁸ mol/L plus 10⁻⁷ mol/L prazosin (n=4 per group) and to Ang II plus the AT₂ blocker 5×10⁻⁷ mol/L (n=5 per group). LV cGMP levels were determined by enzymatic assay (Amersham, Life Science) and expressed as mol/mg protein.

All data are presented as mean±SEM. Two-way ANOVA followed by Fisher's exact test for post hoc analyses was used for multiple comparisons. Significance was accepted at *P*<0.05.

Results

Characteristics of Hypertrophied and Normal Hearts

There was an 80% to 90% increase in LV weight in aortic stenosis versus normal hearts (Table). By study design, coronary flow was adjusted at baseline to achieve a similar

coronary flow per gram in hypertrophied and normal hearts (Table).

Effects of AT₂ Blockade on Ang II-Induced Phenylalanine Incorporation

The rate of [³H]phenylalanine incorporation into proteins in the absence of drug was similar in normal and hypertrophied hearts (Figure 1). Corroborating our previous findings,² Ang II-induced phenylalanine incorporation increased in normal hearts but did not in hypertrophied hearts. In normal hearts stimulated with Ang II, the rate of phenylalanine incorporation did not increase further during AT₂ receptor blockade. In contrast, in hypertrophied hearts stimulated with Ang II, phenylalanine incorporation was increased by AT₂ receptor blockade.

Effects of AT₂ Blockade on Ang II-Induced PKC-ε Translocation in Adult Rat Hearts

In normal and hypertrophied hearts with no drug, the membrane fraction of PKC composed 41.6±2.7% and 46.3±1.3% of total PKC content (*P*=NS). Normal hearts stimulated with Ang II demonstrated a significant increase in membrane fraction of PKC (24.9±8.6% versus no drug, *P*<0.05) that was not further modified by AT₂ blockade (25.5±5.8% versus no drug, Figure 2, left). In hypertrophied hearts, Ang II alone did not cause a significant increase in membrane translocation of PKC (8.8±4.1% versus no drug, Figure 2,

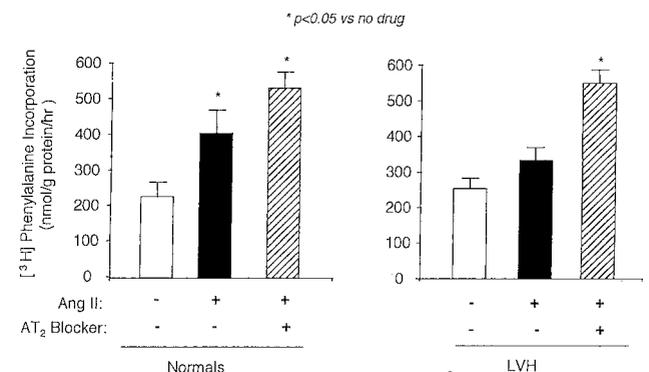


Figure 1. LV protein synthesis assessed by [³H]phenylalanine incorporation in normal and hypertrophied hearts. In normal hearts (left), Ang II induced a significant increase in rate of [³H]phenylalanine incorporation. In normal hearts, Ang II-induced cardiac protein synthesis did not significantly increase further during AT₂ blockade. In hypertrophied hearts (right), Ang II alone did not promote an increase in new LV protein synthesis compared with hearts perfused with no drug. In contrast, Ang II-stimulated LV protein synthesis was markedly amplified by selective AT₂ blockade in hypertrophied hearts.

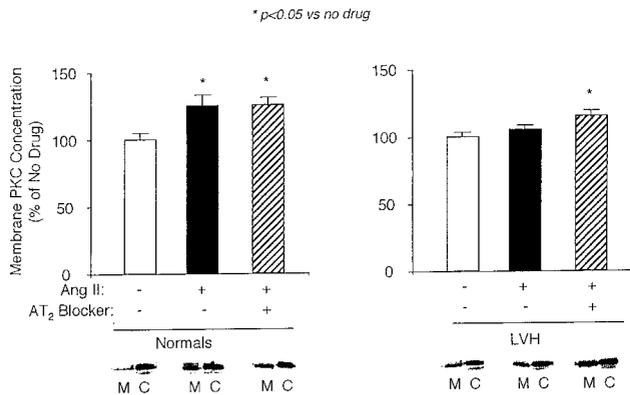


Figure 2. Membrane translocation of PKC- ϵ in normal and hypertrophied rat hearts. Left, Ang II induced a significant increase in membrane fraction of PKC compared with normal hearts with no drug. This increase in membrane fraction was not further modified by AT₂ blockade. Right, In hypertrophied hearts, Ang II alone did not cause a significant increase in membrane translocation of PKC compared with no drug. However, stimulation with Ang II in presence of AT₂ blockade significantly increased membrane fraction of PKC compared with no drug. M indicates membrane; C, cytosol.

right). In the presence of AT₂ blockade, however, Ang II significantly increased the membrane fraction of PKC compared with hypertrophied hearts with no drug ($15.4 \pm 4.7\%$ versus no drug, $P < 0.05$).

Effects of AT₂ Blockade on LV cGMP Content

In both normal and hypertrophied hearts stimulated with Ang II, selective AT₂ blockade significantly depressed LV cGMP levels (98.6 ± 7.6 versus 64.7 ± 6.1 fmol/mg protein, $P < 0.05$, and 75.5 ± 11.9 versus 45.8 ± 6.2 fmol/mg protein, $P < 0.05$, respectively).

Discussion

In the present study using a well-characterized intact buffer-perfused adult heart model, we directly examined the role of upregulated LV AT₂ receptors on new cardiac protein synthesis in response to Ang II in adult hypertrophied rat hearts. Consistent with previous observations,^{2,3} Ang II failed to cause an increase in new protein synthesis or PKC translocation in hypertrophied hearts. In normal hearts with a predominance of LV AT₁ receptors,^{9,10} Ang II-induced new cardiac protein synthesis did not increase further during selective AT₂ blockade. In contrast, in hypertrophied hearts, AT₂ receptor blockade increased Ang II-induced LV protein synthesis and PKC translocation. Thus, these data support the hypothesis that AT₂ receptor activation mediates inhibitory effects on acute growth response to Ang II in adult intact hearts with LV hypertrophy.

Recent studies suggested that AT₂ receptor-related biological effects are due to activation of kinins and intracellular cGMP signaling.^{13–17} Our data corroborate these findings by demonstrating that AT₂ blockade causes a decrease in LV cGMP content in intact hearts stimulated by Ang II. In addition, AT₂ blockade amplified PKC translocation in hypertrophied hearts stimulated with Ang II. Of note, this effect was absent in normal hearts. The presence of cross talk between PKC translocation and selective AT₂ blockade in

hypertrophied hearts with upregulated AT₂ receptors^{9–12} is consistent with a report of Yamada et al,²⁴ who demonstrated that AT₂ receptor activation inhibits mitogen-activated protein kinase. In addition, several authors proposed that AT₂ receptor activation may enhance apoptotic cell death.^{17,24} These hypotheses merit future studies in the present aortic stenosis model of chronic LV hypertrophy and failure.

The present study has several limitations. First, the isolated-perfused heart model does not allow investigation of later components of the growth response, namely myocyte hypertrophy. Second, it does not distinguish whether changes in protein synthesis or PKC translocation are localized predominantly to cardiac myocytes or also to matrix cells that express Ang II receptors.

In summary, the present study shows that AT₂ receptor blockade amplifies new cardiac protein synthesis in response to Ang II in hypertrophied hearts. This appears to be related to both augmented PKC activation by Ang II and suppression of cGMP signaling. Thus, these data suggest for the first time that the AT₂ receptor upregulation in chronic LV hypertrophy may blunt the acute LV growth response to Ang II. The beneficial or detrimental effects of chronic AT₂ receptor blockade on hypertrophic remodeling and the transition to failure in pressure-overload hypertrophy remain to be investigated.

Acknowledgments

This study was supported in part by National Heart, Lung, and Blood Institute grant HL-38189 (Drs Lorell and Weinberg) and by fellowship award F05 TW05261-01 from the Fogarty International Center, NIH (Dr Bartunek).

References

- Sadoshima J, Izumo S. Molecular characterization of angiotensin II-induced cardiac hypertrophy and hyperplasia of cardiac fibroblasts: critical role of AT₁ receptor subtype. *Circ Res*. 1993;73:413–423.
- Schunkert H, Sadoshima J, Cornelius T, Kagaya Y, Weinberg EO, Izumo S, Riegger G, Lorell BH. Angiotensin II-induced growth responses in isolated hearts: evidence for load-independent induction of cardiac protein synthesis by angiotensin II. *Circ Res*. 1995;76:489–497.
- Schunkert H, Weinberg EO, Bruckschlegel G, Rigger AJG, Lorell BH. Alteration of growth responses in established cardiac pressure overload hypertrophy in rats with aortic banding. *J Clin Invest*. 1995;96:2768–2774.
- Zou Y, Komuro I, Yamazaki T, Aikawa R, Kudoh S, Shiojima I, Hiroi Y, Mizuno T, Yazaki Y. Protein kinase C, but not tyrosine kinase or Ras, plays a critical role in angiotensin II-induced activation of Raf-1 kinase and extracellular signal-regulated protein kinases in cardiac myocytes. *J Biol Chem*. 1996;271:33592–33597.
- Paul K, Ball NA, Dorn GW II, Walsh RA. Left ventricular stretch stimulates angiotensin II-mediated phosphatidylinositol hydrolysis and protein kinase C ϵ isoform translocation in adult guinea pig hearts. *Circ Res*. 1997;81:643–650.
- Moalic JM, Bauters C, Himbert D, Bercovici J, Mouas C, Guicheney P, Baudoin-Legrois M, Rappaport L, Emanoil-Ravier R, Mezger V, Swynghedauw B. Phenylephrine, vasopressin and angiotensin II as determinants of proto-oncogene and heat-shock protein gene expression in adult rat heart and aorta. *J Hypertens*. 1989;7:195–201.
- Schunkert H, Jahn L, Izumo S, Apstein CS, Lorell BH. Localization and regulation of *c-fos* and *c-jun* protooncogene induction by systolic wall stress in normal and hypertrophied rat hearts. *Proc Natl Acad Sci U S A*. 1991;88:11480–11484.
- Thienelt CD, Weinberg EO, Bartunek J, Lorell BH. Load-induced growth responses in isolated rat hearts: role of the AT₁ receptor. *Circulation*. 1997;95:2677–2683.
- Lopez JJ, Lorell BH, Ingelfinger JR, Weinberg EO, Schunkert H, Diamant D, Tang SS. Distribution and function of cardiac angiotensin AT₁ and AT₂ receptor subtypes in hypertrophied rat hearts. *Am J Physiol*. 1994;267:H844–H852.

10. Suzuki J, Matsubara H, Urakami M, Inada M. Rat angiotensin II (type IA) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. *Circ Res.* 1993;73:439–447.
11. Asano K, Dutcher DL, Port JD, Minobe WA, Tremmel KD, Roden RL, Bohlmeier T, Bush EW, Jenkin MJ, Abraham WT, Reynolds MV, Zisman LS, Perryman MB, Bristow MR. Selective downregulation of the angiotensin II AT₁-receptor subtype in failing human myocardium. *Circulation.* 1997;95:1193–2000.
12. Haywood GA, Gullestad L, Katsuya T, Hutchinson HG, Pratt RE, Horiuchi M, Fowler MB. AT₁ and AT₂ angiotensin receptor gene expression in human heart failure. *Circulation.* 1997;95:1201–1206.
13. Siragy HM, Carey RM. The subtype-2 (AT₂) angiotensin receptor regulates renal cyclic guanosine 3',5'-monophosphate and AT₁ receptor-mediated prostaglandin E₂ production in conscious rats. *J Clin Invest.* 1996;97:1978–1982.
14. Siragy HM, Carey RM. The subtype 2 (AT₂) angiotensin receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest.* 1997;100:264–269.
15. Liu YH, Yang XP, Sharov VG, Nass O, Sabbah HN, Peterson E, Carretero OA. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure: role of kinins and angiotensin II type 2 receptors. *J Clin Invest.* 1997;99:1926–1935.
16. Gohlke P, Pees CH, Unger T. AT₂ receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. *Hypertension.* 1998;31(pt 2):II-349–II-355.
17. Kurabayashi M, Yazaki Y. Downregulation of angiotensin II receptor type 1 in heart failure: a process of adaptation or deterioration? *Circulation.* 1997;95:1104–1107.
18. Hein L, Barsh GS, Pratt RE, Dzau VJ, Kobilka BK. Behavioral and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice. *Nature.* 1995;377:744–747.
19. Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BLM, Inagami T. Effects on blood pressure and exploratory behavior of mice lacking angiotensin II type-2 receptor. *Nature.* 1995;377:748–750.
20. Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE, Dzau VJ. The angiotensin II type 2 (AT₂) receptor antagonizes the growth effects of the AT₁ receptor: gain-of-function study using gene transfer. *Proc Natl Acad Sci U S A.* 1995;92:10663–10667.
21. Stoll M, Steckelings U, Paul M, Bottari SP, Metzger R, Unger T. The angiotensin AT₂-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J Clin Invest.* 1995;95:651–657.
22. Kent RL, Hooper JK, Cooper G IV. Load responsiveness of protein synthesis in adult mammalian myocardium: role of cardiac deformation linked to sodium influx. *Circ Res.* 1989;64:74–85.
23. Yuan S, Sunahara FA, Sen AK. Tumor promoting phorbol ester inhibits cardiac function and induces redistribution of protein kinase C in perfused beating hearts. *Circ Res.* 1987;61:372–378.
24. Yamada T, Horiuchi M, Dzau VJ. Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci U S A.* 1996;93:156–160.

Angiotensin II Type 2 Receptor Blockade Amplifies the Early Signals of Cardiac Growth Response to Angiotensin II in Hypertrophied Hearts

Jozef Bartunek, Ellen O. Weinberg, Minori Tajima, Susanne Rohrbach and Beverly H. Lorell

Circulation. 1999;99:22-25

doi: 10.1161/01.CIR.99.1.22

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1999 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circ.ahajournals.org/content/99/1/22>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation* is online at:
<http://circ.ahajournals.org/subscriptions/>