Brief Review

Is Angiotensin II a Direct Mediator of Left Ventricular Hypertrophy?
Time for Another Look

Timothy L. Reudelhuber, Kenneth E. Bernstein, Patrick Delafontaine

Hypertensive cardiac remodeling, characterized by left ventricular hypertrophy (LVH) and increased fibrosis, increases the workload of the heart and is a significant risk factor for cardiovascular morbidity and mortality.¹ There is a continuous relationship between left ventricular mass and the likelihood of cardiovascular events, including stroke, heart failure, and coronary artery disease, leading to myocardial infarction (MI).² Virtually all of the clinical trials have demonstrated that antihypertensive treatment achieves some reversal of LVH,³ and reduction of left ventricular mass during antihypertensive treatment has repeatedly emerged as a goal of paramount importance in reducing risk.⁴,⁵ Nevertheless, the interpretation of certain clinical trials has led to the widely held view that inhibition of the renin–angiotensin system (RAS) has favorable effects on cardiac remodeling that go beyond its blood pressure–lowering effects. This view has been supported by the experimental finding that cultured cardiomyocytes hypertrophy in response to exogenously added angiotensin II and has led to the model of the heart as a direct target of the RAS. In the current review, we critically analyze these arguments in light of recent clinical and experimental data with a particular emphasis on genetically modified animals designed to test for the role of a local cardiac RAS in the development of LVH.

From the Bedside

Although an extensive discussion of clinical trials that tested the role of the RAS in cardioprotection is beyond the scope of this review (the reader is referred to recent reviews in References 6 and 7), the results of most clinical studies suggest that the absolute reduction in blood pressure is the primary determinant of long-term outcome in patients. The Heart Outcomes Prevention Evaluation (HOPE) Trial⁸ initially demonstrated that an angiotensin-converting enzyme (ACE) inhibitor given to high-risk patients with vascular disease or diabetes reduced rates of death, MI, and stroke, and the authors concluded that the 22% relative risk reduction in events was not because of reduced blood pressure alone. However, these findings have been challenged on the basis that the reported modest reduction in blood pressure (≈3.3 mm Hg systolic) was likely an underestimate as a result of the study medication being taken at bed time and blood pressure being measured 12 to 18 hours later.⁹ Indeed, a subsequent substudy demonstrated that the evening dose of ACE inhibitor markedly reduced overnight blood pressure (decrease of 17/8 mm Hg).¹⁰ The landmark Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial (ALLHAT), comparing 4 antihypertensive agents (including an ACE inhibitor) showed that coronary heart disease risk (including fatal or nonfatal MI) and total mortality were similar for the 4 groups.¹¹,¹² Of note, however, the diuretic group had superior blood pressure control, and the secondary outcome of combined cardiovascular disease was lower in this group compared with the ACE inhibitor group. The conclusions of this study supporting the use of diuretics as preferred first-line therapy for hypertension have raised significant controversy and debate. For instance, there has been concern that diuretic use was associated with a higher risk of developing diabetes.¹³ The Valsartan Antihypertensive Long-term Use Evaluation (VALUE) tested the hypothesis that, for the same degree of blood pressure control, an angiotensin receptor antagonist would reduce cardiac morbidity and mortality more than a calcium channel antagonist in hypertensive patients at high cardiovascular risk. In fact, no significant difference was found between the 2 agents in the primary composite end point after a mean follow-up of 4.2 years.¹⁴ In contrast, the Losartan Intervention For End-point reduction in hypertension (LIFE) Trial, which studied whether selective blocking of angiotensin II (Ang II) action would improve LVH and consequent morbidity beyond simply reducing blood pressure, did show superiority of an angiotensin receptor antagonist versus a β-blocker in patients with essential hypertension and LVH.¹⁵-¹⁷ The Second Australian National Blood Pressure Trial compared outcomes in elderly patients with hypertension receiving ACE inhibition or diuretics.¹⁸ Although the primary outcome of cardiovascular events or all-cause mortality was marginally lower in the ACE inhibitor group, this study has been challenged on

Received January 26, 2007; first decision February 12, 2007; revision accepted March 31, 2007.

From the Laboratory of Molecular Biochemistry of Hypertension (T.L.R.), Clinical Research Institute of Montreal, Montreal, Quebec, Canada; the Department of Pathology and Laboratory Medicine (K.E.B.), Emory University, Atlanta, Ga; and the Department of Medicine (P.D.), Section of Cardiology, Tulane University Health Sciences Center, New Orleans, La.

Correspondence to Timothy L. Reudelhuber, Clinical Research Institute of Montreal, 110 Pine Ave West, Montreal, Quebec H2W 1R7, Canada. E-mail reudel@icrm.qc.ca

(Hypertension. 2007;49:1196-1201.)

© 2007 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/HYPERTENSIONAHA.106.075085

1196
methodologic grounds. Thus, clinical trials have not unambiguously resolved the question of whether Ang II promotes LVH by a direct effect on the heart, and the topic has justifiably been the subject of a great deal of experimental research.

Back to the Bench

Many laboratories have shown that Ang II has trophic effects on cultured cardiomyocytes, and these studies have led to important insights into the mechanisms of intracellular signaling by this hormone (reviewed in Reference 20). Nevertheless, because these studies are by necessity carried out in highly defined conditions that often include the lack of serum proteins and the absence of parallel stimulation by other hormones, the biological relevance of the hypertrophic effects of Ang II on cardiomyocytes in vitro has to be tested in whole animals. Making this leap has been difficult because of the compound effects of Ang II on vasoconstriction, aldosterone secretion, sodium reabsorption, and fluid volume, all of which can raise blood pressure and confound the differentiation of primary and secondary effects of Ang II on the heart. The various molecular strategies used by investigators to address this question in whole animals represent the epitome of experimental physiological sleuthing. Nevertheless, because of the pleiotropic effects of Ang II, they also require careful critical analysis.

One approach used by several groups is to treat animals with so-called “subpressor” doses of Ang II, and such studies have often reported a stimulation of cardiac hypertrophy and fibrosis that did not ensue from a detected increase in blood pressure. However, this approach cannot rule out systemic effects, and there is some debate as to whether they are truly subpressor, particularly during the night when rodents are most active. For this reason, they will not be dealt with further in this review. As an alternative, several groups have resorted to the unique ability to modify the genome of mice to inactivate genes by homologous recombination (knockout mice) and to target the tissue-specific overproduction of proteins using transgenesis. Numerous transgenic studies designed to test for the function of proteins in the heart have made use of the gene control regions of the mouse α-myosin heavy chain (α-MHC) gene (promoter) to target expression in cardiomyocytes. As with any transgene, understanding where and when the encoded protein will be expressed is important, because it affects the interpretation of the results, and this is particularly true for the α-MHC promoter. In mice, the α-MHC gene is first expressed in the developing cardiac tube as early as 7.5 days postcoitus. With the formation of distinct ventricular chambers between 8 and 9 days postcoitus, α-MHC gene expression gradually decreases in ventricular myocytes but continues to be expressed at high levels in atrial myocytes. By day 16 postcoitus, α-MHC gene expression reaches its lowest level in the ventricular cells but then increases and finally replaces β-MHC gene expression in ventricular muscle by 7 days after birth. α-MHC gene expression is maintained throughout adult life in the ventricular myocyte of the mouse. Expression of transgenes under control of the α-MHC promoter and enhancer closely mimics the expression pattern of the endogenous gene, with the exception of frequent reports of variable ectopic expression in the lung and testes. As a result, the encoded proteins are expressed at relatively high levels in the developing atria before being expressed in the ventricular myocytes.

In fact, the high level of activity of this promoter can generate high levels of protein production in the heart and may, in some cases, result in misleading artifacts. Buerger et al reported recently that driving high-level expression of Cre recombinase (a protein with no natural function in the mammalian heart) using the α-MHC promoter triggered a dilated cardiomyopathy. Cardiac defects were directly correlated with transgene expression levels and only became evident in mice older than 6 weeks of age. Surprisingly, survival of these mice could be improved with the ACE inhibitor Captopril, although the RAS was clearly not targeted by this transgenesis. Similar results have been reported with cardiac overexpression of green fluorescent protein and the yeast transcriptional activator Gal4. Although these proteins are not of mammalian origin, high-level expression of proteins that are normally found in the heart can also be deleterious. Liggett et al drove expression of the β2 adrenergic receptor at 60, 100, 150, and 350 times the normal level in the hearts of mice using the α-MHC promoter. Although all of the mouse lines exhibited the expected increase in basal adenylyl cyclase activity, all but the lowest expressing line developed fibrotic cardiomyopathy leading to premature death. Importantly, the severity of the symptoms was not proportional to the level of adenylyl cyclase activation. Taken together, these experiments underscore the difficulty in separating phenotypes because of the expressed protein from “noble” artifacts arising from the high level of cardiomyocyte expression driven with the α-MHC promoter and have driven investigators to develop attenuated and/or inducible promoters that allow more control over expression levels (see, eg, Reference 27). Nevertheless, the ability to target expression of proteins to cardiomyocytes with the α-MHC promoter has prompted its use by many laboratories. These studies can be roughly divided into 2 groups: those that sought to increase Ang II and those that targeted local increases in the Ang II AT1 receptor.

Making More Ang II

Mazzolai et al used the α-MHC promoter to express rat angiotensinogen in the cardiac myocytes of mice. Several lines of mice were obtained, but perhaps the most interesting one was a line (TG1306) for which the investigators reported no significant increase in plasma Ang II but almost twice the normal concentration of Ang II in the heart. This rather modest increase in cardiac Ang II nevertheless led to major physiological consequences: significant right and left ventricular cardiac hypertrophy that leads to an age-dependent decrease in cardiac function, development of dilated cardiomyopathy, and increased mortality. These physiological changes were mirrored by alterations in the cardiomyocytes themselves: cardiomyocytes isolated from TG1306 mice were longer and wider than those from controls and exhibited a 30% to 40% decrease in contraction rate. At face value, these results suggest that a modest increase (<2-fold) of Ang
II in the heart can have dramatic morphological and pathological effects. However, this approach may suffer from some limitations: the rat angiotensinogen used in this study is, surprisingly, a better substrate for mouse renin than the mouse angiotensinogen,\(^{31}\) and even a very small amount of rat angiotensinogen is sufficient to raise blood pressure in mice.\(^{32}\) Thus, angiotensinogen leaking out into the circulation might be producing Ang II elsewhere than the heart. Careful examination of the original publication\(^{28}\) suggests that this could indeed be the case: plasma renin content is \(\approx 4\) times lower in the TG 1306 mice than in controls. Because renin secretion from the kidney is repressed in response to increases in blood pressure or plasma Ang II, the low plasma renin levels seen in the TG1306 mice suggest that they are either more hypertensive than thought previously or that they release Ang II into the circulation. Either possibility would constitute a fatal flaw for a model of cardiac-specific effects of Ang II.

In a sophisticated variant of this approach, Xiao et al\(^{33}\) drove the cardiac overexpression of ACE under the control of the \(\alpha\)-MHC promoter by substituting this engineered gene for the normal ACE gene in mice (knock-in). As a result, the transgene simultaneously inactivated the normal ACE gene and drove ACE expression at \(\approx 100\) times the normal levels in atria and ventricles. These mice also had some expression of the transgene in the lung and testes, 2 commonly reported ectopic expression sites for transgenes driven by the \(\alpha\)-MHC promoter,\(^{22}\) suggesting that “knocking-in” the transgene to the ACE gene locus did not do much to alter the specificity of the \(\alpha\)-MHC promoter. In spite of the large increases in cardiac ACE expression achieved in the transgenic animals, the mice displayed only a 3- to 4-fold increase in cardiac Ang II content, showed marked enlargement of atria, and developed lethal heart block. This atrial enlargement was only evident in mice older than 3 days and gradually increased to result in atria that were 3 times larger than those of nontransgenic controls. The cause for the atrial enlargement (hypertrophy versus hyperplasia) was not reported. Importantly, this \(\alpha\)-MHC promoter-mediated overexpression of ACE, which resulted in local Ang II levels that were twice those reported by Mazzolai et al,\(^{28}\) did not result in any ventricular hypertrophy or fibrosis.

In a more direct approach, Van Kats et al\(^{34}\) used the \(\alpha\)-MHC promoter to target the cardiac expression of an engineered fusion protein designed to directly release Ang II in expressing tissues. Using this approach, transgenic mice were obtained that had cardiac levels of Ang II that were 20- to 50-fold greater than those seen in control littermates with no detectable increase in circulating Ang II. In spite of showing normal levels of cardiac Ang II receptors and a normal hypertrophic response to an exogenous Ang II challenge, the transgenic mice showed no evidence of cardiac hypertrophy at 3 months of age but did exhibit a slight increase in interstitial fibrosis. When the engineered fusion protein was altered to release a degradation-resistant form of Ang II, the levels of Ang II in the heart reached thousands of times the normal level and began to spill into the circulation. In spite of these high levels of Ang II in the heart, the animals did not develop LVH until their blood pressure began to rise in response to the increased circulating Ang II.

The lack of hypertrophy in the context of elevated cardiac Ang II has also more recently been reported by groups that engineered the inactivation of the ACE2 gene in mice.\(^{35\text{-}37}\) By removing a single amino acid from the carboxy-terminus of Ang II, ACE2 not only forms the Ang (1-7) peptide, but also acts as a clearance enzyme for Ang II. Indeed, mice in which the ACE2 gene has been inactivated have difficulty in clearing Ang II\(^{36,37}\) and have been reported by 1 group to have nearly twice the normal content of Ang II in the heart.\(^{35}\) Despite this increase in cardiac Ang II, none of the groups found evidence of ventricular hypertrophy in ACE2-deficient mice.

Two conclusions can be drawn from the studies published to date that targeted a cardiac-specific increase in Ang II. First, all but 1 of the studies found that there was no relationship between increased cardiac content of Ang II and hypertrophic remodeling, and the single differing study may have some technical limitations. The second conclusion that can be drawn from this series of studies is that if the RAS is involved in triggering cardiac hypertrophy directly, Ang II is either not the limiting factor, or its nefarious local effects are somehow counterbalanced in vivo. Could the determining factor be the Ang II AT\(_1\) receptor?

**Making More Ang II AT\(_1\) Receptor**

In the intact heart, the majority of Ang II receptors are not found on cardiomyocytes.\(^{38}\) In fact, freshly isolated cardiomyocytes express such low levels of angiotensin receptors that some investigators find it necessary to amplify their signaling by overexpressing exogenously added receptors for functional studies (see, eg, Reference 39). Similarly, a number of studies have addressed the role of a local RAS in LVH by using the targeted overexpression of the Ang II AT\(_1\) receptor in the cardiomyocytes of transgenic mice in an effort to amplify the resulting phenotype. Hein et al,\(^{40}\) using the \(\alpha\)-MHC promoter to increase the expression of the mouse AT\(_1\) receptor \(\approx 2\)-fold in the heart, reported high in utero death of transgenic mice, associated with grossly enlarged atria and heart block. The atrial enlargement in this case was attributed to atrial myocyte hyperplasia, and the phenotype of these animals resembles that of the cardiac ACE overexpression of Xiao et al\(^{33}\) described above. In contrast, using a very similar approach but expressing the human AT\(_1\) receptor to achieve a 200- to 400-fold increase in Ang II receptor the heart, Paradis et al\(^{41}\) reported ventricular hypertrophy that developed only in mice older than 1.5 months and that was clearly associated with ventricular myocyte enlargement. Once it appeared, the hypertrophy rapidly progressed to heart failure and death. The difference in phenotype in these 2 studies has never been satisfactorily resolved but could perhaps be attributed to the use of AT\(_1\) receptor coding sequences from 2 different species (mouse versus human).

More recent studies have revealed something interesting about the signaling mechanism that results from the overexpression of AT\(_1\) receptors in the heart: Wettschureck et al\(^{42}\) reported that mice deficient for \(G_{\alpha_\text{q}}/G_{\text{ox}1}\) (the G-protein complex that mediates the intracellular signaling of the AT\(_1\),
endothelin-1, and α-adrenergic receptors) specifically in cardiomyocytes failed to develop myocardial hypertrophy in response to pressure overload. In contrast, Zhai et al reported that overexpression (30 times normal) of an AT₁ receptor lacking the Gα₁₅-coupling intracellular loop resulted in greater atrial and ventricular enlargement than when equivalent levels of the nonmutated receptor were expressed in the heart. More recently, this same group demonstrated that driving a similar 30-fold increase in the expression of an AT₁ receptor that had lost the ability to transactivate the epidermal growth factor receptor did not lead to ventricular hypertrophy. Taken together, these studies suggest that different mechanisms exist for pressure-mediated and direct (Ang II-mediated?) effects through AT₁.

Three conclusions can be drawn from these experiments. First, overexpression of the Ang II AT₁ receptor in the heart can lead to remodeling. In all of the cases reported to date, AT₁ overexpression using the α-MHC promoter results in atrial enlargement, likely because of atrial myocyte hyperplasia, not hypertrophy. The biological relevance of this particular finding is still not clear, and it may represent one of the artifacts related to the high level of expression driven by this promoter in the atria of the fetuses, as discussed above. Nevertheless, 2 different groups have now shown that AT₁ overexpression can also result in some degree of ventricular myocyte hypertrophy. This is different from the experiments designed to increase cardiac Ang II and suggests that the AT₁ receptor could be the limiting factor in RAS-mediated direct effects on the heart. This also has important clinical implications, because it means that a local RAS in the heart may be most important in conditions that increase cardiac AT₁ receptor levels. An induction of AT₁ receptor has been reported at sites surrounding the infarction scar, as well as elsewhere in the hearts of rats 1 week after MI, and the functional significance of this increase will be interesting to test in some of the more recent genetic models described above. Second, whereas the Gα₁₅ protein is necessary for pressure-mediated cardiac remodeling, the Gα₁₅-binding domain of the AT₁ receptor is not required for mediating the atrial hyperplasia and ventricular hypertrophy resulting from the transgenic overexpression of the AT₁ receptor in the mouse heart. Thus, the hypertrophy because of AT₁ receptor overexpression in the heart is very likely not the same as that seen in hypertensive cardiac remodeling, and these experiments support the idea that not all of the AT₁ receptor signaling is Gα₁₅ dependent. Finally, like the experiments designed to increase cardiac Ang II, there is no simple and direct association between the overexpression of AT₁ in the cardiomyocyte and LVH. Although the time course of the development of ventricular myocyte hypertrophy is not reported in most of these studies, in the study by Paradis et al, the mice can tolerate several hundred-fold increases in cardiac AT₁ expression in utero (when the transgene promoter is first active) and for the first 1 to 2 months after birth with no obvious affect on the size of the ventricular myocytes. This result, alone, suggests that a piece of the puzzle is still missing.

Working Under Pressure

Although AT₁ overexpression in the heart appears to be more effective than local increases of Ang II concentration in triggering ventricular hypertrophy in these experimental conditions, the major question that remains is whether this is relevant to hypertensive cardiac remodeling. Recent results by Crowley et al cast some doubt. These investigators transplanted a single kidney taken from mice in which the Ang II AT₁ receptor (AT₁a) had been inactivated into normal recipient mice that had both kidneys removed and vice versa. In this way, they generated 4 groups of mice: normal mice with a kidney transplanted from a normal mouse, normal mice with a kidney transplanted from an AT₁ knockout mouse, and so forth. They then exposed each of the 4 groups to a sustained infusion of a massive dose of Ang II (1000 ng/kg per minute) for 4 weeks and measured both their blood pressure response and the resulting degree of hypertensive cardiac remodeling. Two important findings came from this study. First, both groups of mice with transplanted kidneys in which the AT₁ receptor had been inactivated failed to raise blood pressure in response to the Ang II infusion, whether or not they had AT₁ receptor in the rest of the body. This finding underscores the central role of the kidney in blood pressure regulation. Second (and more importantly for this review) cardiac remodeling correlated perfectly with increases in blood pressure. In other words, the absence of an increase in blood pressure, even the hearts that contained AT₁ receptor did not undergo remodeling when confronted with large increases in circulating Ang II for 4 weeks. These results confirm the lack of apparent local effects of increased cardiac Ang II seen in the experiments described above.

Conclusions

Largely because of the vast literature on the ability of Ang II to induce hypertrophy in cultured cardiomyocytes, it has become common to see statements in the introductory sections of articles indicating that “Ang II is a cardiotrophic hormone . . . .” However, the bulk of the evidence obtained in genetically modified experimental animals simply does not support a direct role of the cardiac RAS in LVH. It is clear from clinical and experimental studies (eg, Cre-overexpressing mice), however, that LVH can be regressed by RAS inhibition, even if the RAS activation is not the primary cause of the hypertrophy. It should also be noted that these results do not rule out a direct role for Ang II in cardiac remodeling when combined with other humoral, mechanical, or pathological stimuli, and the existing mouse models will be ideal for testing such interactions. However, because both the experimental data in mice and the results from several clinical trials suggest that the effects of Ang II on LVH are largely pressor dependent, it seems reasonable that effective and sustained blood pressure control should remain the primary target for LVH reduction.

Sources of Funding

This work was supported by Canadian Institutes of Health Research grant MOP13569 (T.L.R.) and National Institutes of Health grants DK039777 and DK051445 (K.E.B.) and HL70241 (P.D.).
Disclosures
None.

References
cardiac dysfunction by increasing local angiotensin II. *Hypertension*. 2006;47:718–726.


Is Angiotensin II a Direct Mediator of Left Ventricular Hypertrophy?: Time for Another Look

Timothy L. Reudelhuber, Kenneth E. Bernstein and Patrick Delafontaine

Hypertension. 2007;49:1196-1201; originally published online April 23, 2007;
doi: 10.1161/HYPERTENSIONAHA.106.075085

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/49/6/1196

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/