Enhanced Angiotensin II Activity in Heart Failure
Reevaluation of the Counterregulatory Hypothesis of Receptor Subtypes

Lionel H. Opie, Michael N. Sack

Abstract—There are strong data favoring the pathogenic role of angiotensin II type 1 receptor (AT₁) activation with subsequent promotion of myocyte growth and cardiac fibrosis in the development of cardiac hypertrophy and heart failure. An emerging hypothesis suggests that the activity of the angiotensin II type 2 receptor (AT₂) may counterregulate AT₁ receptor effects during cardiac development and during the evolution of cardiac hypertrophy and heart failure. In this review, we examine the potential role of AT₂ activity in the context of this hypothesis. In contrast to the counterregulatory hypothesis, studies in mice with an overabundance of, or a deficiency in, the AT₂ receptor do not suggest that AT₂ signaling is essential for cardiac development. Moreover, the proposed antigrowth effects of AT₂ receptor signaling in pathological cardiac hypertrophy could not be shown in two mice models both deficient in AT₂ receptors. The role of AT₂ receptor signaling in cardiac fibrosis is, however, still debatable because of conflicting data in the same two studies. In angiotensin II–evoked apoptosis in cardiomyocytes, the proposed proapoptotic role of AT₂ activity could not be confirmed. Furthermore, in the progression from the bench to bedside, the results of two large clinical trials in heart failure, namely ELITE II and Val-HeFT, can be explained without ascribing a major protective role to the unopposed activity of the AT₂ receptor in the failing myocardium. In this review, we conclude that the collective evidence does not strongly support a net beneficial effect of AT₂ stimulation in the diseased myocardium.

Key Words: angiotensin receptor subtypes n counterregulation n renin-angiotensin system n heart failure

Two of the most powerful cardiovascular regulators are the β-adrenergic and the renin-angiotensin system (RAS). The strength of the two systems is such that, between them, they virtually control cardiovascular physiology. The β-adrenergic system is concerned with the fight-or-flight reactions, achieving abrupt increases in heart rate and cardiac output, whereas in adult life, the RAS controls blood volume, peripheral vascular tone, and hence the blood pressure (Table 1). Both systems are subject to counterregulatory balances, postulated to be overridden by sustained activation in heart failure. Overactivity of the β-adrenergic system is self-limited by the uncoupling, internalization, and inactivation of the β-adrenergic receptor. In contrast, the mechanism of autoregulation of angiotensin stimulation in heart failure is less well-defined (Table 1). Recent reviews suggest that activity of the angiotensin II type 2 receptor (AT₂) may counterbalance the putative detrimental effects of angiotensin II type 1 receptor (AT₁) activation in heart failure via mediating opposite cellular functions.¹,²

To explore the putative counterregulatory role of AT₂ receptor signaling, we need to define the role of AT₁ signaling in the heart. It is already known that AT₁ stimulation leads to vasoconstriction, cell growth, positive inotropy, catecholamine release, and increased aldosterone secretion with fibrosis, all deemed to have a detrimental component in cardiac hypertrophy and heart failure. Thus, to confer counterregulatory effects, AT₂ receptor stimulation should oppose one or more of the phenotypic effects of AT₁ receptor stimulation. In this review, we examine the putative individual components of AT₂ receptor signaling in the heart, ie, in cardiac growth, apoptosis, and fibrosis. We also discuss the results of recent clinical trials aimed at specific inhibition of the RAS in heart failure. We conclude that these proposed counterregulatory effects may not be of major significance in the overall regulation of the RAS in cardiac hypertrophy and heart failure.

Signaling Pathways

Before describing the phenotypic response to the modulation of the AT₁ receptor system in the myocardium, we will briefly review AT₁ and AT₂ receptor subtype signaling. These data regarding signal transduction could provide insight into the counterregulatory hypothesis. In response to mechanical stretch of neonatal cardiomyocytes, both AT₁ and AT₂ receptors are upregulated.³ AT₁ receptor activates several well-defined paths, including vascular contraction mediated by...
TABLE 1. How Angiotensin II and β₁-Adrenergic Effects Interact and Contrast

<table>
<thead>
<tr>
<th>Site of Regulation</th>
<th>Angiotensin II</th>
<th>β₁-Adrenergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary</td>
<td>Ang II reinforces sympathetic stimulation</td>
<td>Start of adrenergic activation</td>
</tr>
<tr>
<td>Baroreflexes</td>
<td>Ang II facilitates ganglionic transmission</td>
<td>Prime site of regulation of BP</td>
</tr>
<tr>
<td>Terminal neurons</td>
<td>Ang II promotes NE release</td>
<td>NE synthesized, stored, and released</td>
</tr>
<tr>
<td>Receptor and postreceptor effects</td>
<td>Ang II AT₁ receptor</td>
<td>β₁-adrenergic receptor</td>
</tr>
<tr>
<td>G proteins and other signals</td>
<td>G proteins, linked to PKC, then to growth and PC; NF-κB activation</td>
<td>G proteins linked to PKA, calcium, pacemaker currents, and inotropy</td>
</tr>
<tr>
<td>Major organs regulated in adult life in physiological conditions</td>
<td>(1) VSM, degree of vasoconstriction; (2) kidney, inhibits renin release; (3) adrenal cortex, release of aldosterone.</td>
<td></td>
</tr>
<tr>
<td>Major effects in adult life</td>
<td>Control of blood volume and blood pressure</td>
<td>Control of heart rate and cardiac output</td>
</tr>
<tr>
<td>Counterregulation</td>
<td>Aldosterone—Na⁺ retention—renin release ↓ ; high BP—vagus</td>
<td>Vagal; β₁-ARK uncouples and internalizes β₁ receptor</td>
</tr>
<tr>
<td>Receptor subtypes</td>
<td>AT₁ may be opposed by AT₂ in disease, via kinins</td>
<td>β₁ may be supported by β₂ activity in health but opposed (controversial) in disease, via G₁b</td>
</tr>
</tbody>
</table>

Ang II indicates angiotensin II; PKC, protein kinase C; VSM, vascular smooth muscle; PC, preconditioning; BP, blood pressure; NE, norepinephrine; PKA, protein kinase A; and β₁-ARK, β₁-adrenergic receptor kinase, subtype 1.

intrinsic triplosphosphate (IP₃) and release of calcium from the sarcoplasmic reticulum, whereas myocardial growth is mediated by protein kinase C (PKC) activity and a multiplicity of paths that lead to mitogen-activated protein (MAP) kinase activity. Furthermore, through a path that involves oxygen radicals and ceramide, nuclear factor (NF)-κB may be activated. The latter is a pleiotropic nuclear regulatory peptide promoting both beneficial and adverse effects. Some pathways linked to stimulation of the AT₂ receptors are similar to AT₁ effects. NF-κB activation may be involved, especially in vascular smooth muscle, which in turn could lead to growth, fibrosis, or apoptosis, in a context-dependent manner. The AT₂ receptor may exert adverse effects during ischemia/reperfusion, mediated by IP₃ and PKC. There may also be a myocardial kinin protective path involving nitric oxide, bradykinin, and prostaglandins. A similar vascular kinin protective path is found in transgenic mice overexpressing the AT₂ receptor. However, once bradykinin is formed, it might inhibit the formation of angiotensin II to limit the proposed beneficial counterregulatory effects.

AT₂ Receptor in Cardiac Growth

In vascular smooth muscle cells, the AT₂ receptor exerts an antiproliferative effect whereas the AT₁ receptor promotes growth. In a variety of models of myocardial growth, both AT₁ and AT₂ receptors are upregulated. In neonatal rat cardiomyocytes, the antagonism of AT₁ receptor signaling in response to a hypertrophic stimulus suggests that the AT₂ receptor does mediate an antigrowth effect, as supported by an acute study of angiotensin-induced hypertrophic growth in rats. In contrast, when left ventricular hypertrophy (LVH) was induced by chronic infusion of angiotensin II in rats, losartan inhibited this process, showing the role of AT₁ receptors, whereas AT₂ receptor block neither changed the blood pressure nor the degree of ventricular hypertrophy. Finally, if the AT₂ receptor has antigrowth effects, then its deletion by genetic ablation should lead to LVH in response to a pressure load. Two recent studies using independently generated AT₂ receptor-null mice dispute this hypothesis (Table 2). In the first study, targeted deletion of the AT₂ receptor prevented LVH in response to aortic banding, and

TABLE 2. Effects on Cardiac Phenotype After the Genetic Manipulation of the AT₁ and AT₂ Receptors or of Cardiac RAS

<table>
<thead>
<tr>
<th>Method of Study and Reference</th>
<th>Experimental Preparation</th>
<th>Phenotypic Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ablation of AT₁ receptor</td>
<td>AT₁ receptor-null mice + aortic banding</td>
<td>No LVH, decreased fibrosis; AT₁ required for hypertrophy</td>
</tr>
<tr>
<td>As above</td>
<td>As above</td>
<td>Modest LVH as in wild type; increased perivascular fibrosis; no upregulation of AT₂ in wild type with LH</td>
</tr>
<tr>
<td>Overexpression of AT₂ receptor</td>
<td>Mice, hemodynamic measurements in vivo and in Langendorff hearts</td>
<td>AT₁-mediated pressor effects inhibited by AT₂ activity; no change in cardiac growth and development</td>
</tr>
<tr>
<td>Ablation of AT₁a receptor</td>
<td>AT₁a receptor-null mice + aortic banding</td>
<td>LVH still achieved as in wild-type mice; perivascular fibrosis equally severe as in wild type</td>
</tr>
<tr>
<td>Overexpression of cardiac angiotensinogen</td>
<td>Mice with LVH, testing ACE inhibition or AT₁ block in either prevention or regression of LVH</td>
<td>LVH despite normal BP, no fibrosis; both therapies effective in prevention and in regression; AT₁ block in wild type decreased tissue Ang II levels</td>
</tr>
<tr>
<td>Overexpression of renin, (mRen2)²⁷²⁴</td>
<td>High expression of murine transgene causes severe hypertension, LVH with fibrosis in rat</td>
<td>LVH and fibrosis both prevented by ramipril and amlodipine; progression of fibrosis not related to tissue renin-angiotensin but to blood pressure</td>
</tr>
</tbody>
</table>

LVH indicates left ventricular hypertrophy.
contractile function remained normal. In the second study, the heart-to-body weight ratio increased by ~15% in both deletion and wild-type mice in response to increased aortic pressure. Collectively, these mice deletion studies challenge the anti-growth effect of AT1 receptor signaling. The apparently conflicting data in the neonatal cardiomyocyte model and the acute infusion studies may be outweighed by data from these receptor deletion studies. Furthermore, the counterregulatory hypothesis is not supported by the fact that no obvious abnormal cardiac developmental phenotype is evident in multiple mice with genetic manipulations of the AT1 and AT2 receptor subtypes (Table 2).

**AT2 Receptor and Cardiac Apoptosis**

An alternative antigrowth effect could be in the promotion of programmed cell death (apoptosis). There are strong links between AT2 receptor activity and the augmentation of apoptosis in a variety of tissues, including vascular smooth muscle and endothelial cells, as well as fibroblasts, as outlined by Horiuchi et al. In contrast, proapoptotic effects of AT1 receptor signaling were not evident in two studies in cultured rat cardiomyocytes. In these studies, administration of angiotensin II provoked apoptosis that was inhibited by AT1 receptor blockade but not by the AT2 receptor blockade. Moreover, in mice with robust AT1 receptor expression, no evidence of augmented apoptosis was suggested. Hence, in the heart, the data do not support a counter-regulatory role of the AT1 receptor in mediating antigrowth effects. Perhaps the paradigm of context-specific regulation is relevant regarding the significance of AT1 receptor signaling and apoptosis.

**AT2 Receptor and Cardiac Fibrosis**

Excess AT1 stimulation leads to left ventricular fibrosis. To counterregulate this effect, AT2 receptor activation should attenuate myocardial fibrosis. There is an antifibrotic effect of AT2 receptor stimulation on fibroblasts extracted from cardiomyopathic hamsters. This concept is supported by a study in AT2 receptor–deficient mice exposed to aortic banding. In this study, perivascular fibrosis was attenuated in genetically ablated mice compared with wild-type control mice. In a preliminary report, cardiomyocyte fibrosis was attenuated in genetically ablated mice compared with wild-type control mice. In stark contrast, the AT2-deficient mice study by Senbonmatsu et al implies that AT2 receptor activity is important for the left ventricular interstitial fibrotic response to pressure overload. A third view is that the cardiac AT2 receptor stimulation has little or no influence on myocardial fibrosis and that fibrosis more likely is related to the prevailing blood pressure. In support of this view, the degree of postinfarct myocardial fibrosis in rats was attenuated by AT1 blockade without any significant effects of added AT2 blockade. In humans, myocardial fibrosis can be regressed by angiotensin-converting enzyme (ACE) inhibition that presumably reduces stimulation of both AT1 and AT2 receptors. Therefore, the relationship between AT2 receptor activity and fibrosis in the myocardium has not been definitively clarified. Moreover, an important recent point is that AT2 receptor stimulation can have bidirectional effects in different tissues, decreasing collagen synthesis in cultured fibroblasts but increasing synthesis in mesangial cells.

**From Bench to Bedside**

It is now firmly established that inhibition of ACE does have a markedly beneficial effect in the treatment of heart failure. ACE inhibitor therapy, however, does not completely block angiotensin II production, and in some patients, angiotensin II levels remain elevated, in part, because of the conversion of angiotensin I to angiotensin II by chymase activity. Thus, continued AT1 receptor stimulation could occur. This hypothesis is indirectly supported by the relative upregulation of the AT1 receptor in human heart failure, although this finding is controversial. Currently, no therapeutic agents that specifically act on the AT1 receptor are approved for clinical trials. However, numerous AT1 receptor blockers are available that could hypothetically shunt the activity of the cardiac RAS toward stimulation of the beneficial AT2 receptor.

Two separate clinical approaches evaluate this hypothesis. First, ACE inhibitor therapy was directly compared with AT1 receptor antagonist therapy in a randomized controlled clinical study, ELITE II, that was adequately powered in contrast to the underpowered first ELITE trial. In ELITE II, the ACE inhibitor captopril was compared with the AT1 blocker losartan in 3152 patients aged 60 years or older, with endpoints of all-cause mortality, sudden death, or resuscitated arrest. No significant differences were found, suggesting that the beneficial effect of these two classes of agents were both via the inhibition of AT1 receptor signaling and that beneficial AT2 signaling played no role. Second, the addition of the AT1 receptor blocker valsartan to preexisting therapy in the Val-HeFT study meant that the drug classes were effectively combined and compared with ACE inhibitor therapy alone. The preliminary report on 5009 patients with heart failure, mostly New York Heart Association classification grade II and grade III, showed unchanged rate of mortality but reduced rate of morbidity, including hospitalization. In other experimental and human heart failure studies, the combination of an AT1 blocker plus ACE inhibition resulted in a greater reduction in angiotensin II levels versus ACE inhibition therapy alone. Collectively, these data suggest that if combined RAS antagonist therapy is more successful in heart failure than individual antagonists alone, it is probably due to the combinatorial decrease in angiotensin II levels and less likely that increased stimulation of the AT1 receptor plays a major beneficial role.

**Conclusions**

Angiotensin II activation promotes adverse remodeling in experimental heart failure, and these effects can be attenuated by ACE inhibition and AT1 receptor blockade. This suggests major dependency on the AT1 receptor, without necessarily having to invoke any specific protective role of the AT2 receptor. We do, however, believe that potential counterregulation of the proposed AT1-induced perivascular fibrosis by AT2 activity deserves further investigation. Furthermore, different effects of increased AT1 activity in different tissues need to be considered. The overall data do not argue convincingly for a major compulsory protective role.
role of AT2 receptors in heart failure treated by AT1 blockade, despite the strong evidence in specific models.8,26 Rather, the totality of evidence, both clinical and laboratory, is that activity of the AT1 receptor is crucial in the progression of heart failure.

Moving from bench to bedside, there is no need to invoke the counterregulation hypothesis concerning the proposed benefits of increased AT2 activity to explain the results of these large clinical trials in heart failure. It appears that the concept of AT1 counterregulation by AT2 receptor activity is too simplistic to account for all the apparently conflicting data. We acknowledge that some downstream messengers of AT1 receptor activity unequivocally oppose those of the AT2 receptors, for example, AT1-induced vasoconstriction versus kinin-mediated vasodilation. Further work is now required to understand why paths communal to both AT1 and AT2 signaling, such as those involving NF-kB, may lead to either growth or apoptosis.

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References


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