Reviews

Translational Success Stories highlight how basic discoveries have led to clinical advances (such as the use of new drugs or diagnostic modalities in patients). This initiative reflects the renewed emphasis of our journal on translational research. It is hoped that these articles will stimulate efforts to translate basic insights into clinical practice.

Translational Success Stories: Angiotensin Receptor 1 Antagonists in Heart Failure

Louis J. Dell’Italia

Abstract: The title of the proposed series of reviews is Translational Success Stories. The definition of “translation” according to Webster is, “an act, process, or instance of translating as a rendering of one language into another.” In the context of this inaugural review, it is the translation of Tigerstedt’s and Bergman’s1 discovery in 1898 of the vasoconstrictive effects of an extract of rabbit kidney to the treatment of heart failure. As recounted by Marks and Maxwell,2 their discovery was heavily influenced by the original experiments of the French physiologist Brown-Séquard, who was the author of the doctrine that “many organs dispense substances into the blood which are not ordinary waste products, but have specific functions.” They were also influenced by Bright’s3 original observation that linked kidney disease with hypertension with the observation that patients dying with contracted kidneys often exhibited a hard, full pulse and cardiac hypertrophy. However, from Tigerstedt’s initial discovery, there was a long and arduous transformation of ideas and paradigms that eventually translated to clinical applications. Although the role of the renin-angiotensin system in the pathophysiology of hypertension and heart failure was suspected through the years, beneficial effects from its blockade were not realized until the early 1970s. Thus, this story starts with a short historical perspective that provides the reader some insight and appreciation into the long delay in translation. (Circ Res. 2011;109:437-452.)

Key Words: angiotensin II • heart failure • hypertension

Following the early work of Bright and Tigerstedt,1–3 it was not until the 1930s that Goldblatt4 and subsequently several other groups5–7 confirmed the pressor effects of ischemic kidney extracts, identified as a product of renin and at the time referred to “hypertensin” or “angiotonin.” Because this pressor substance was linked to an ischemic kidney, one can understand how an increase in blood pressure could be interpreted as a necessary adaptive response to maintain blood flow. However, even before the description of angiotensin, there was an important publication in 1952 from Skeggs et al7 that demonstrated increased “hypertensin” activity in arterial blood samples of hypertensive humans compared to normotensive subjects. The first definition of the amino acid structure of hypertensins I and II was reported by Skeggs et al in 1956,8,9 which was followed by a similar report by Page and Bumpus et al in 1957;10 soon after in a real gentlemen’s agreement among the research groups, there was consensus on the name, angiotensin (Ang). The evolution of the question to treat or not to treat hypertension was elegantly recounted in Daniel Levy’s book entitled A Change of Heart, which is a history of the Framingham Heart Study. This elegant work has an especially poignant first chapter regarding the medical history of President Franklin D. Roosevelt.11 For as late as 1967, Inglefinger, Relman, and Finland, in their Controversy in Internal Medicine, devoted an entire chapter to a defense of the position that the drug treatment of essential hypertension was of no benefit.12 Debates continued regarding the benefits of therapy in the 1960s even after publication of the findings of the first Veterans Administration study of severe hypertensive patients in 1967.13,14 Thus, ~70 years after discovery of the kidney extract, the clinical problem was identified. The remainder of this review addresses how Ang was connected to heart failure (Table 1).
Connection of Ang II to the Treatment of Heart Failure

In the early 1950s, studies by Sarnoff had shown that ganglionic blockade improved forward cardiac output in dogs with experimentally induced hypertension. In addition, there had long been the clinical observation that sublingual administration of nitroglycerin provided immediate relief in patients with dyspnea and paroxysmal nocturnal dyspnea. This knowledge most likely led to the first report of the central hemodynamic benefit of nitroglycerin in heart failure patients, which was reported in the New England Journal of Medicine in 1957 by Dr. John Beuregard Johnson from Howard University Freedmen’s Hospital. This study demonstrated that nitroglycerin improved shortness of breath and chest pain, along with reduction in pulmonary artery and systemic arterial pressures from indwelling catheters in 12 patients with hypertensive heart failure. As summarized in a review by Zelis, work by his group and others in the 1960s demonstrated increased sympathetically mediated vasoconstriction in heart failure patients. This information spawned the first studies by Franciosa and Cohn and Sanders et al in the early 1970s using nitroprusside or sublingual nitroglycerin in patients within the first 24 hours of acute myocardial infarction. Treatment resulted in improved congestive symptoms, left ventricular (LV) filling pressure, and cardiac output. Further studies demonstrated similar hemodynamic benefits of afterload reduction in patients with a chronic form of heart failure using phenolamine or nitroprusside. Thus, by 1973, the role of afterload reduction in the treatment of heart failure was firmly established in patients and the stage was set for the next series of clinical studies using renin-angiotensin aldosterone system (RAS) system blockade in patients with hypertensive heart failure.

Before 1970, it was well-appreciated that oversecretion of aldosterone and renin-angiotensin were responsible for end-organ damage in adrenal tumors and in malignant hypertension. In 1972, however, Brunner et al were the first to report that high renin levels were associated with heart attack, stroke, and LV hypertrophy in patients with essential hypertension. At the same time, Brunner et al reported striking reductions in blood pressure in 12 patients with advanced renovascular hypertension using the angiotensin peptide analog, saralasin. In 1974, Gavras et al reported the first successful application of saralasin in patients with heart failure secondary to severe hypertension. The angiotensin analog saralasin had to be administered intravenously and had a short 2-minute half-life. These peptide analogues of Ang II bound to the Ang II receptor and prevented Ang II binding but, in being structurally similar to Ang II, had agonist activity. However, even before the use of saralasin, in the 1950s Skeggs discovered the conversion of Ang I to the active form.

Table 1. Highlights of the Chronology of Key Discoveries in Angiotensin II and the Angiotensin II Receptor

<table>
<thead>
<tr>
<th>Year</th>
<th>Discovery</th>
<th>Reference</th>
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<tr>
<td>1898</td>
<td>Demonstration of pressor substance in renal extracts</td>
<td>Tigerstedt and Bergman1</td>
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<tr>
<td>1934</td>
<td>Renal artery constriction produces hypertension in dogs</td>
<td>Godblatt et al4</td>
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<tr>
<td>1940</td>
<td>Two groups independently demonstrate that renin acts on plasma substrate to produce pressor hormone</td>
<td>Braran-Mendez et al5, Page and Helmer6</td>
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<tr>
<td>1956</td>
<td>Two groups independently synthesize octapeptide later to be called Ang II</td>
<td>Skegs et al8,9, Bumpus et al10</td>
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<tr>
<td>1971</td>
<td>Saralasin introduced as first Ang II peptide antagonist</td>
<td>Pals et al195</td>
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<tr>
<td>1972</td>
<td>High renin levels associated with heart attack and stroke</td>
<td>Brunner et al24</td>
</tr>
<tr>
<td>1974</td>
<td>Demonstration of antihypertensive effects of saralasin in hypertensive heart failure</td>
<td>Gavras et al25,26</td>
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<td>1977</td>
<td>Captopril, first nonpeptide ACE inhibitor, described</td>
<td>Cushman</td>
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<tr>
<td>1982</td>
<td>Takeda compounds S-8307 and S-8308 have affinity for Ang II receptor but weak antihypertensive effect</td>
<td>Furukawa et al46</td>
</tr>
<tr>
<td>1988</td>
<td>Losartan, first orally active nonpeptide Ang receptor, described</td>
<td>Carini and Duncia</td>
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<td>1990</td>
<td>Identification of chymase in the human heart</td>
<td>Urata et al44</td>
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<tr>
<td>1991</td>
<td>Ang II AT1 receptor cloned</td>
<td>Rat vascular, Murphy et al130</td>
</tr>
<tr>
<td>1992</td>
<td>Ang II AT receptor subtypes cloned</td>
<td>Bovine adrenal, Sasaki et al131</td>
</tr>
<tr>
<td>1997</td>
<td>ELITE I: significant benefit on all-cause mortality in heart failure patients older than age 65 years when compared to captopril</td>
<td>Witt et al159</td>
</tr>
<tr>
<td>2002</td>
<td>ELITE II: ACE inhibitor and AT1, RB had equivalent mortality benefits with less side effects with AT1, RB</td>
<td>Witt et al160</td>
</tr>
<tr>
<td>2003</td>
<td>Val-HeFT Trial: combination therapy reduced incidence of combined end points driven by reduction in heart failure hospitalization</td>
<td>McMurray et al166</td>
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<tr>
<td>2003</td>
<td>CHARM-ADDED Trial: Combination therapy decreased cardiovascular death or heart failure hospital admissions</td>
<td>Pfeiffer et al163</td>
</tr>
<tr>
<td>2003</td>
<td>After myocardial infarction: combination therapy did not improve mortality</td>
<td>Konstam et al170</td>
</tr>
<tr>
<td>2009</td>
<td>Higher-dose losartan (150 mg daily) was associated with reduced death and hospital admissions</td>
<td>Konstam et al170</td>
</tr>
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ACE indicates angiotensin-converting enzyme; Ang, angiotensin; AT1, angiotensin II type 1; RB, receptor blocker.
Ang II, by angiotensin-converting enzyme (ACE) in horse plasma. Basic work in the 1960s and early 1970s by other pioneering investigators Ferreira, Vane, Erdos, and others fully characterized the enzyme ACE, a kininase II and Ang I convertase. This story was recounted by Erdos in *The ACE and I: How ACE inhibitors came to be* and by Vane in *The origin of captopril.* This work set the stage for the development of the first oral ACE inhibitor, captopril, which generated a great amount of interest and excitement because of the vast amount of basic science that had been performed on the ACE enzyme. Thus, before more work could be directed in development of Ang II receptor blockade, the late 1970s and early 1980s marked the emergence of many ACE inhibitor clinical trials. However, side effects of cough and alternative pathways for Ang II production were appreciated very early and became the impetus for development of Ang II receptor blocker.

Since the initial use of ACE inhibitors in the early 1980s, a number of clinical trials reported a relationship between ACE inhibitor therapy and cough. ACE is a zinc metalloenzyme dipeptidyl carboxypeptidase with active substrates that include not only Ang I but also bradykinin, enkephalins, chemotactic peptide, neurotensin, substance P, and leutenizing hormone-releasing hormone. These substrates are involved in bronchoconstriction and inflammation and their accumulation may contribute to cough or asthma-like symptoms or both. It is of interest that a recent report of the incidence of withdrawal attributable to ACE inhibitor-associated cough from nearly 200 000 randomized patients since 1990 was significantly greater than reported in the Physician Desk Reference/drug label.

In addition, even before the development of captopril, there was evidence for alternative pathways to ACE for formation of Ang II. In 1974, Boucher, Asselin, and Genest reported the first biochemical description of an alternative pathway to that of ACE for Ang II formation via tonin in the rat salivary gland. From 1976 to 1982, other reports of alternate Ang II forming enzymes included trypsin, kallikrein, and cathepsin-G. Tonin, tissue plasminogen activator, and elastase were reported to generate Ang II either from angiotensinogen or Ang I. Physiological evidence for the presence of alternative Ang II-forming mechanisms to ACE was first reported by Cornish, Joyner, and Gilmore in 1976. These investigators found that an extraluminal application of Ang I in situ to blood vessels of the hamster cheek pouch produced a vasoconstriction that was not inhibited by ACE inhibition but completely reversed by Ang II receptor antagonism. In 1981, Bolliaz and Gavras were the first to document a biphasic response in plasma Ang II levels with chronic ACE inhibitor treatment of hypertensive patients. The decrease in plasma Ang II and ACE levels during the first 24 to 48 hours was followed by the return to normal levels despite substantial ACE inhibition, subsequently referred to as the “ACE escape” phenomenon. It is important to note in Figure 1 that plasma aldosterone, also under the influence of Ang II, returned toward normal with chronic ACE inhibition.

In these early studies it was realized that the main effect of Ang I in the presence of ACE inhibition was attributable to a conversion to Ang II by one or more chymotrypsin-like enzymes. The potential pathophysiological significance of

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**Figure 1. Results of 6 months of treatment with angiotensin-converting enzyme (ACE) inhibitor in nine patients with hypertension.** Despite a remarkable decrease in blood pressure and plasma ACE activity, plasma angiotensin (Ang) II and aldosterone (Aldo) levels, which were initially significantly suppressed, increased back to normal levels over time. The lower number in the columns represents patients who were also treated with hydrochlorothiazide, 50 mg per day. *P<0.05, **P<0.01, ***P<0.001. Reproduced with permission J Cardiovasc Pharmacol.
alternative Ang II-forming pathways in human cardiovascular disease remained obscure until Urata and Husain reported that a chymotrypsin-like serine protease accounted for \( \approx 90\% \) of the Ang II-forming capacity in tissue extracts from human myocardium, suggesting that ACE was not the major Ang II-forming enzyme in the human left ventricle in vitro. To address the problems of ACE inhibitor-induced cough and ACE escape, DuPont (a pharmaceutical company in Wilmington, Delaware) set out to target the Ang II receptor. In the next section of this translational success story, it is fitting to provide a short tribute to those involved in the development of the first Ang II type 1 (AT\(_1\)) receptor antagonist, losartan.

**Development of the Ang II Receptor Blocker**

In 1982 leaders at DuPont, under the direction of Dr Robert I. Taber, hired two chemists, Dr John V. Duncia and Dr David J. Carini, who had just recently completed their PhD training and were assigned to synthesize an Ang II antagonist. Dr Andrew T. Chiu and Dr Pancras C. Wong, pharmacologists, were responsible for testing the synthesized compounds. However, the breakthrough came in 1982 with the publication of patents from Takeda of a nonpeptide imidazole-5-acetic acid derivative that antagonized Ang II vasoconstriction. The original Takeda compounds S-8307 and S-8308 had very weak antihypertensive effects but were selective for the Ang receptor. An insightful history entitled *How the antihypertensive losartan was discovered*, by Gaurab Bhardwaj, pointed out that DuPont had the benefit of a highly experienced head of research in Dr Taber and Dr Timmermans. However, the brunt of the synthesis and pharmacological work was performed by three recently graduated PhD bench scientists (Duncia, Carini, and Wong). Carini started with synthesizing variants of S-8307 without much success and moved to computer modeling of the Ang II structure and overlapping drug, with major assumptions (among many others) being made regarding the binding points of Ang II to its receptor that, through the previous work by Bumpus and others, being made regarding the binding points of Ang II to its receptor that, through the previous work by Bumpus and Husain, was demonstrated in the splanchic bed in 1968, in the uterus in 1970, and in the brain in 1971. The brain was of particular interest and generated most of the work over the next 10 years, because it was known that circulating components of the RAS could not cross the blood–brain barrier. In the late 1980s, studies reported beneficial effects of RAS inhibition in the heart in animal models of pressure overload that could not be explained on the basis of blood pressure reduction alone. This strengthened the concept of a local cardiac RAS and led to a number of studies in the late 1980s and early 1990s that demonstrated upregulation of ACE and Ang II formation in the pressure overloaded and myocardial infarction rat heart, in addition to angiotensinogen and AT\(_1\) receptor expression in the pressure-overloaded rat heart. The study by Hirsh and Dzau showed the relation between ACE expression and myocardial infarction size and LV dilatation, suggesting that higher LV wall stress determined local ACE expression.

These early animal studies were followed by the interesting results of the SAVE trial appearing in the *Lancet* in 1993, which reported significant reduction in mortality in chronic heart failure patients with ACE inhibitor therapy that could not be explained by reduction in blood pressure, and other cardioprotective effects of ACE inhibition were reported in the earlier publication. Studies over the past 20 years further supported local synthesis of most of the RAS components, particularly ACE and Ang II, that were also related to the severity of heart failure in the dog with volume overload, rats with pressure overload, and in patients with heart failure. The question whether renin was synthesized locally remained controversial; however, over the past 10 years there were major advances in knowledge of renin and prorenin and the prorenin/renin receptor.

The first important finding in the renin story was the identification of a human renin receptor that bound to renin in the mesangium of the kidney and to the subendothelium of the coronary artery. The location of a renin receptor on the cell surface combined with chymase in the interstitium provided a mechanism for Ang II formation on the cell surface in the heart and for substantial chymase-mediated-interstitial fluid Ang II levels. Further, it was found that binding of prorenin to the prorenin/renin receptor resulted in the activation of renin. Some data also suggested that binding of renin to the prorenin/renin receptor activated MAP kinases ERK1/2 and p38 pathways, leading to upregulation of profibrotic genes independent of Ang II generation. Thus, as depicted in Figure 2, it is now widely accepted that the majority of cardiac renin is taken from the circulation and that cardiac renin receptors may act on the cell surface to mediate interstitial Ang II formation. Most importantly, internalization of the renin receptor can direct intracellular signaling independent of Ang II formation that can result in organ damage even in the presence of AT\(_1\) receptor blockade.

In addition to uptake of renin from the circulation, Levi et al. reported that cardiac mast cells were an important source of renin in the heart. Mast cells are present in the mammalian heart at a concentration of \( \approx 50,000 \) mast cells/g in human heart tissue and are located in close proximity to vessels and nerves. Their density markedly increases in heart failure, ischemic cardiomyopathy, and experimental infarct models. The Levi group also showed that mast cell activation during cardiac ischemia-reperfusion caused the release of mast cell...
renin and that locally formed Ang II exacerbated norepinephrine release from cardiac sympathetic nerves and reperfusion arrhythmias.\(^\text{74}\) Taken together, the discovery of the renin receptor combined with the identification of mast cell renin underscores the potentially dynamic nature of the cardiac interstitium that participates in chymase-mediated Ang II formation on the cell surface in addition to prorenin/renin receptor-mediated intracellular signaling.

**Evidence for Intracellular Ang II Formation**

In addition to the process of prorenin/renin receptor internalization, recent studies demonstrated that under certain conditions intracellular formation of angiotensinogen and renin results in synthesis and retention of Ang II.\(^\text{75,76}\) Intracellular Ang II synthesis is important because such a mechanism would be resistant to AT\(_1\) receptor blockade. Cardiac myocytes, fibroblasts, renal mesangial, and vascular smooth muscle cells were shown to synthesize intracellular Ang II under hyperglycemic conditions.\(^\text{75-77}\) and chymase was up-regulated in coronary and renal arteries in patients with diabetes.\(^\text{78}\) However, Danser et al\(^\text{79}\) recently challenged the concept of intracellular Ang II formation. In studies of mice without AT\(_{1A}\), AT\(_{1B}\), and Ang II type 2 (AT\(_{2}\)) receptors, they reported that Ang II levels were extremely low, reflecting Ang peptide in blood or interstitial fluid of the tissue and suggesting that the only source of intracellular Ang II results from receptor internalization. Although this issue remains to be clarified, recent studies showed that high glucose can stimulate both smooth muscle cell chymase and cathepsin D, which can form intracellular Ang II from Ang I and angiotensinogen.\(^\text{77}\) Thus, the potential for intracellular Ang II synthesis may be dependent on the stimulating factor. At this time there may exist such a situation in patients with heart failure and diabetes.

Subsequent to synthesis, other studies demonstrated that intracellularly produced Ang II can act through AT\(_{1}\)-like receptors on the nuclear envelope of hepatocytes,\(^\text{80,81}\) direct chromatin binding,\(^\text{82}\) or through unidentified intracellular receptors. Ang II receptors were detected in the sarcolemma, T-tubules, and nuclei of rat cardiomyocytes.\(^\text{83}\) and intracellular Ang II increased angiotensinogen production in hepatocyte via AT\(_1\) nuclear receptors.\(^\text{84}\) Most recently, intracellular Ang II was shown to increase nitric oxide production in renal mesangial cell nuclei via AT\(_2\) nuclear receptors.\(^\text{85}\) Thus, this intracrine process can have subsequent effects on gene expression, cell growth and hypertrophy, and extracellular matrix production.\(^\text{86,87}\) Most importantly, because intracellular Ang II effects cannot be blocked by AT\(_1\) receptor blockers, future therapeutic targets that block intracellular Ang II synthesis may be necessary to fully interrupt the intracrine effects of Ang II.

**Ang-(1-7) and ACE2 and other Angiotensin Peptides in the Heart**

Work over the past 23 years generated a new paradigm of the RAS as a complex of alternate enzymatic pathways leading to the generation of separate peptides that have physiological effects at receptors other than the AT\(_1\) receptor subtype.\(^\text{88,89}\) One major finding was that the marked increases in Ang II during AT\(_1\) receptor blockade and Ang I during ACE inhibition were processed into Ang-(1-7) by a diversity of tissue endopeptidases.\(^\text{90}\) Ang-(1-7) was found to exert counter-regulatory effects of Ang II through a specific pathway involving a novel Ang-(1-7) receptor and was an endogenous inhibitor of the C-terminal active site of ACE.\(^\text{90,91}\) Studies in dogs using cardiac microdialysis demonstrated a substantial capacity for Ang-(1-7) formation from Ang I and Ang II in
the interstitial fluid space of the heart.\textsuperscript{92} The discovery of ACE2 identified a carboxypeptidase that converted Ang I to Ang-(1-9), but directly converted Ang II to Ang-(1-7).\textsuperscript{93} ACE2 was shown to have catalytic activity for Ang II 400-fold higher than for Ang I. Because blockade of AT\textsubscript{1} receptors or ACE inhibition increased cardiac ACE2 gene transcription, these findings led to the hypothesis that a part of their beneficial effects were mediated through the antitrophic and vasodilator actions of Ang-(1-7).\textsuperscript{94–97} Importantly, ACE2 was shown to play a crucial role in maintaining the balance of increased RAS expression in the pathophysiology of the cardiovascular, renal, pulmonary, and central nervous systems, as demonstrated in Figure 3.

In the failing human heart, there was an increase in ACE2 gene expression but it was also found that neutral endopeptidase played a significant role in the formation of Ang-(1-7).\textsuperscript{98} Other studies in animal models of heart failure demonstrated increased cardiomyocyte Ang-(1-7) in the border region of rats with coronary ligation.\textsuperscript{99} Lentiviral-mediated ACE2 overexpression reduced blood pressure and cardiac fibrosis in spontaneously hypertensive rats\textsuperscript{100} and in rats receiving Ang II infusion\textsuperscript{101} and had beneficial effects on cardiac ischemia-reperfusion injury.\textsuperscript{102} However, studies in transgenic mice with manipulations of the ACE2 gene produced conflicting results.\textsuperscript{103,104} A recent review of the collective data suggested that although ACE2 overexpression from birth may exert adverse effects, its overexpression after cardiac development protects the heart from pathophysiologic stress.\textsuperscript{105} Finally, Chappel et al\textsuperscript{106} recently identified an Ang-(1-7) receptor (AT7) and ACE2 in renal cortical nuclei of sheep. Further, these studies demonstrated that this intranuclear ACE2–Ang-(1-7)–AT(7) receptor pathway modulated Ang II-dependent reactive oxygen species formation within the nucleus, providing a unique protective mechanism against oxidative stress and cell damage. Recent reviews summarize drug development targeting ACE2 and the MAS receptor in cardiovascular diseases.\textsuperscript{96,107}

A newly described propeptide cleaved from angiotensinogen, Ang-(1-12), was isolated from rat tissue and recent studies suggested that Ang-(1-12) may be an alternate substrate for the formation of biologically active angiotensins.\textsuperscript{108} Ang-(1-12) was increased in cardiac myocytes of adult spontaneously hypertensive rats\textsuperscript{109} and production of angiotensin peptides from exogenous Ang-(1-12) was not decreased by the preadministration of a renin inhibitor in an isolated heart preparation.\textsuperscript{110} In the anephric rat, cardiac Ang-(1-12), Ang I, and Ang II increased while plasma levels decreased,\textsuperscript{111} suggesting a role of Ang-(1-12) as an intermediate substrate for cardiac Ang II formation. Ang-(1-12) added to the isolated rat heart resulted in increased Ang II production, coronary vasoconstriction, and impaired recovery from ischemia-reperfusion injury.\textsuperscript{112} Although the enzymatic mechanism responsible for cardiac Ang-(1-12) formation from angiotensinogen remains an open question, a recent study suggested a chymase-mediated mechanism in the ischemia-reperfusion rat heart.\textsuperscript{112} In keeping with these observations, studies from Ferrario’s\textsuperscript{113} laboratory showed that although both ACE and chymase form Ang II from Ang-(1-12), the latter assumed an even greater role for Ang II formation in spontaneously hypertensive rats. In summary, Ang-(1-12) is clearly present in the heart and represents an alternate substrate contributing to formation of Ang peptides by a nonrenin-dependent mechanism.

Ang III, also called Ang-(2-8), is generated from Ang II by aminopeptidase A, which cleaves the Asp\textsuperscript{1}–Arg\textsuperscript{2} bond in Ang II. The major physiological role of Ang III, which has an
affinity for both AT₁ and AT₂ receptors, is in the brain and kidney. Ang III is metabolized to Ang IV by aminopeptidase N and also has been largely associated with physiological functions in the central nervous system. In vascular smooth muscle cells, however, Ang IV has been shown to activate the proinflammatory transcription factor nuclear factor-κB, which regulates expression of several genes involved in atherogenesis and thrombosis.\(^\text{114}\)

## The Importance of Chymase in Heart Failure

Chymase is a family of chymotrypsin-like serine proteases stored in secretory granules of mast cells that was first described in the human heart in 1990.\(^\text{44,45}\) In addition to an Ang II-forming capacity that is 20-fold higher than ACE,\(^\text{44}\) chymase was reported to directly activate matrix metalloproteinases\(^\text{115,116}\) and degrade fibronectin,\(^\text{117}\) resulting in cell death through the loss of cell–extracellular matrix connections.\(^\text{118}\) In addition, chymase was shown to activate kalirein,\(^\text{119}\) transforming growth factor-β,\(^\text{120}\) and recently was reported to convert Ang-(1-12) to Ang II.\(^\text{112,113}\) In addition to other mast cell contents, the multiple actions of chymase on tissue remodeling were reported to be associated with the pathophysiology of plaque rupture and abdominal aortic aneurysm in humans.\(^\text{121}\) Based on amino acid sequence homology, chymase is divided into two groups, α and β.\(^\text{122}\) Only α-chymase has been identified in humans, dogs, and hamsters, whereas multiple isoforms of α-chymase and β-chymase are present in rodents.\(^\text{123}\) Chymase inhibition was reported to improve hemodynamics and prevent fibrosis in several dog and hamster models of heart failure due to mitral regurgitation, pacing tachycardia, and myocardial infarction.\(^\text{124–127}\)

Electron microscopy immunohistochemical studies demonstrated that the positively charged chymase molecule is stored in mast cells and tonically secreted into the interstitium and complexed to the extracellular matrix.\(^\text{128}\) In contrast, ACE is attached to endothelial cells, with the catalytic site exposed to the vascular lumen. Studies using in vivo cardiac microdialysis in the dog suggested that chymase was responsible for substantial Ang II formation in the interstitial fluid space of the heart that was several-fold higher than concentrations in the plasma.\(^\text{68}\) In the heart, the mRNA for chymase is many-fold lower than the protein levels. Recent studies demonstrated that mast cell-deficient Kit\(^\text{W/W}\)/Kit\(^\text{W/W}\) mice had no heart chymase activity, suggesting that chymase in the interstitium resulted from a tonic release from mast cells and represented the major source of interstitial fluid Ang II formation that was resistant to ACE inhibitor treatment.\(^\text{129}\)

Further, these studies demonstrated that chronic ACE inhibition caused a 14-fold increase in LV interstitial fluid chymase activity, which was abrogated by treatment with a bradykinin type 2 receptor blocker, an effect that was not observed in mast cell-deficient Kit\(^\text{W/W}\)/Kit\(^\text{W/W}\) mice. In chronic ACE inhibitor-treated mast cell-sufficient littermates, chymase inhibition decreased LV interstitial fluid Ang II by 16-fold, indicating the importance of mast cell chymase in regulating cardiac interstitial fluid Ang II. Further demonstration of bradykinin type 2 receptors on mast cells suggested that ACE inhibitor-mediated increase in interstitial bradykinin might play a part in the “ACE escape” by increasing chymase release from mast cells.

Finally, blockade of chymase has implications beyond the effects on Ang II formation because chymase activates matrix metalloproteinases and transforming growth factor-β, which promote tissue remodeling and apoptosis by disrupting intracellular connections. Recent studies also showed that the combination of ACE inhibitor and chymase inhibitor decreased infarct size and LV dilatation and improved LV function compared to ACE inhibitor alone in hamsters 1 month after coronary occlusion (Figure 4). These results suggested that chymase inhibitors, which target other enzymes in the pathophysiology of LV remodeling, can be a useful addition to ACE inhibition or AT₁ receptor blockade in the treatment of heart failure.

## Ang II Receptor Signaling

The classical actions of Ang II are mediated via specific, highly complex, intracellular signaling pathways that are stimulated after initial binding of the peptide to specific receptors, AT₁ and AT₂. In the early 1990s, multiple groups cloned both receptors, and by that time it was well-appreciated that their functional activity as well as tissue distribution were quite different.\(^\text{130–135}\) Numerous studies over the next 20 years have characterized the adverse effects of prolonged G-protein-coupled receptor (GPCR) signaling pathway for the AT₁ receptor in cardiovascular disease.\(^\text{136}\)

The first reports of the counter-regulatory physiological effects of the AT₂ receptor were reported in 1995,\(^\text{137,138}\) and it is now generally accepted that the AT₂ receptor mediates vasodilation, antagrowth, antihypertrophic, and cardioprotective effects. In addition, there has long been evidence that increased stimulation of the AT₂ receptor during AT₁ receptor blockade contributed to the beneficial effects of AT₁ receptor blockade.\(^\text{130}\) However, more recently, the pathophysiological role of the AT₂ receptor activation has been open to question.\(^\text{140,141}\) As with the ACE2 story, most of the controversy appears to occur with genetically altered animals.\(^\text{141}\) The use of recently developed, highly selective nonpeptide AT₂ receptor agonist, C21, demonstrated significant improvement in LV remodeling and function and a decrease in inflammation and scar size in rats treated with C21 starting 24 hours after coronary occlusion.\(^\text{142}\) Of interest, results were similar to that of AT₁ receptor blockade but not synergistic with AT₁ receptor blockade. C21 treatment was also shown to preserve renal structure by preventing inflammatory cell infiltration and collagen accumulation in the kidneys of spontaneously hypertensive stroke-prone rats.\(^\text{143}\) Applications to animal models of cardiovascular disease are ongoing to fully address the in vivo physiological and tissue benefits of the AT₂ receptor agonist.\(^\text{144}\)

Another exciting new area in AT₁ GPCR signaling are studies that challenge the traditional paradigm of “linear on–off switch” AT₁ GPCR signaling (Figure 5). In this mechanism, an agonist or antagonist for GPCR (such as AT₁ and β-adrenergic receptors) initiates or inhibits a signaling pathway before the process is terminated by G-protein-coupled receptor kinases or β-arrestins. Over the past 10 years there has been emerging evidence that a single ligand
(agonist or antagonist) can activate multiple signaling pathways with differing efficacies determined by induction of distinct conformational changes in the receptor. Thus, a given ligand may be an antagonist for one pathway while simultaneously activating other pathways through G-protein-dependent and G-protein-independent signaling, a condition called biased receptors. Much of this work has been performed in the context of recruitment of the multifunctional protein, arrestin, which to this point has been important in GPCR desensitization, internalization, and trafficking. More recent studies have demonstrated that arrestin recruitment to adrenergic or AT1 receptors initiate a second wave of signaling independent of G-protein activation, which now provides a new avenue for drug development. Specifically, there is increasing evidence that different ligands can induce unique conformational changes within the AT1 receptor that decrease GPPRC signaling while promoting selective G-protein-coupled receptor kinases and arrestin-mediated signaling.

Recently, Violin et al reported the efficacy of TRV120027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH), which competitively antagonizes Ang II-stimulated GPRC signaling and also stimulates arrestin recruitment and activates several kinase pathways via arrestin coupling. Compared to the standard AT1 receptor antagonists losartan and telmisartan, TRV120027 decreases blood pressure and increases opposed to attenuates cardiac performance. These striking differences in vivo between unbiased (losartan and telmisartan) and arrestin biased ligands (TRV120027) provide support for the use of biased ligands to selectively target specific receptor functions in drug discovery. Rockman et al have reported that cardiomyocyte cell stretch activates GPRC and arrestin signaling without G-proteins, resulting in an antiapoptotic phenotype. Interestingly, binding of the conventional AT1 antagonist prevents arrestin activation and increases apoptosis. Thus, the authors postulate that the stretch alone produces a conformational change in the AT1 receptor that induces prosurvival pathways and that a biased AT1 receptor blocker for arrestin may be the best choice to ameliorate the adverse effects of high diastolic load on the heart. In addition, naturally occurring mutations of these

Figure 4. Effect of chymase inhibition on left ventricular (LV) remodeling and function and mortality after coronary occlusion in the hamster. The combination of angiotensin-converting enzyme (ACE) inhibitor and chymase inhibitor produces the greatest improvement in LV remodeling, systolic function, and mortality. Graphs demonstrating LV ejection fraction (EF) (A), fractional shortening (FS) (B), average cardiomyocyte diameter (C), LV interstitial fibrosis (D), infarct area (E), LV end-diastolic dimension (F), and survival (G) are all significantly improved by ACE inhibitor plus chymase inhibitor in hamsters 1 month after total left anterior descending artery occlusion. Drugs were started within 24 hours after total occlusion. Reproduced with permission J Clin Invest.
receptors/kinases have been demonstrated to exhibit varying efficacy toward different pathways, particularly epidermal growth factor transactivation. The development of biased ligands has major implications for future targeted therapy directed at the adrenergic and angiotensin receptors. It appears at this time that AT2 receptor activation is not followed by interaction of the activated receptor with /H9252-arrestins and subsequent internalization, suggesting that classical /H9252-arrestin–mediated mechanisms do not participate in the homologous desensitization of the AT2 receptor, and that its regulation is different from the AT1 receptor and most other GPCRs.

**AT1 Receptor Blockade in Heart Failure**

Although the topic of this review is the translational story of AT1 receptor blockade in heart failure, a short mention of AT1 receptor blockade in hypertension is appropriate, because saralasin was first utilized in the treatment of hypertensive heart failure. By the 1970s, the value of lowering blood pressure was firmly established and there were increasing reports of the deleterious effects of Ang II on the cardiovascular/renal system. However, as late as 1997, JNC VI reported that there was no evidence that newer agents offered any advantage over thiazides and /H9252-blockers in reducing morbidity and mortality. The Losartan Intervention for Endpoint Reduction (LIFE) trial was the first to establish the beneficial effects of AT1 receptor blockade with losartan over /H9252 receptor blockade with atenolol in the reduction of combined end point of stroke, myocardial infarction, and cardiovascular death.

On the heart failure side, a comparison of an AT1 receptor blockade with an ACE inhibition in patients with heart failure was fueled by the side effect of cough and the clear evidence of chymase-mediated Ang II formation in the human heart. The first Evaluation of Losartan in the Elderly (ELITE) trial reported a significant benefit in all-cause mortality in heart failure in patients older than age 65 years when AT1 receptor blockade was compared to captopril. Although the larger-scale ELITE II clinical trial did not demonstrate a mortality benefit with losartan, the investigators concluded that ACE inhibitor and AT1 receptor blockade had equivalent mortality benefits with fewer side effects with AT1 receptor blockade. The Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity (CHARM) Alternative study demonstrated that the AT1 receptor blockade with candesartan significantly reduced cardiovascular deaths or hospital admissions for heart failure in patients with chronic heart failure intolerant of ACE inhibitors. Both the Optimal Trial in Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAL) and Valsartan in Acute Myocardial Infarction (VALIANT) trials, reported in 2002 and 2003, showed that ACE inhibitor and AT1 receptor blockade were equally effective in reducing mortality in patients after a myocardial infarction. However, in the VALIANT trial, combination ACE inhibitor and AT1 receptor blockade was associated with more adverse events without improvement in survival.

Over the past 10 years, clinical trials sought to achieve a more complete RAS blockade with maximum doses of ACE inhibitors and AT1 receptor blockers. This was based on the well-known fact that loss of negative feedback of renin release with RAS blockade led to an increase in renin release and downstream increase in RAS components, particularly Ang II stimulation of aldosterone release. The rationale for a more complete RAS blockade was further fueled when Rousseau et al showed that large subsets of patients had high plasma Ang II concentrations, despite enalapril treatment, and had a worse prognosis compared to those patients with suppressed Ang II. In support of this hypothesis was the additional observation that when added to full-dose enalapril
in the Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) pilot study, AT1 receptor blockade improved LV ejection fraction and reduced neurohumoral activation.165

The quest for more complete blockade of the RAS led to the studies combining maximum doses of ACE inhibitors and AT1 receptor blockers. The Val-HeFT and CHARM-Added trials represent the only two prospectively designed, adequately sized, and appropriately powered heart failure trials that tested AT1 receptor blockade to conventional therapy in heart failure.166,167 Both showed a statistically significant reduction in the primary or coprimary mortality morbidity composite outcomes despite increases in risk of renal dysfunction, hyperkalemia, and hypertension. These results led to strict guidelines for combined use of ACE inhibitor and AT1 receptor blockade in chronic systolic heart failure from multiple regulatory authorities (Table 2).168,169 In addition, the HEAAL trial firmly established the superiority of full (150 mg daily) over partial (50 mg daily) AT1 receptor blockade with losartan in decreasing mortality and hospitalizations in heart failure.170 In summary, these guidelines decreed that combined therapy of ACE inhibitor and AT1 receptor blocker should be considered in patients with advanced heart failure and repetitive cardiac decompensation that was presumably attributable to incomplete neuroendocrine blockade. The current evidence does not justify the use of an ACE inhibitor/AT1 receptor blocker combination in patients with heart failure attributable to LV dysfunction after myocardial infarction. However, adding an aldosterone blocker to an ACE inhibitor or AT1 receptor blocker in patients with heart failure and severe LV dysfunction271 or in patients with heart failure and LV dysfunction after myocardial infarction272 did reduce all-cause mortality and hospitalizations for heart failure.

Most recently, in pursuit of the goal for more complete RAS blockade, efforts have shifted to the addition of the renin inhibitor, aliskiren, to RAS blockade. In stable patients with hypertension, the combination of aliskiren and AT1 receptor blocker valsartan produced significantly greater blood pressure reductions than monotherapy with either agent alone, without an increase in side effects.173 Trials in heart failure to date, however, produced conflicting results. In a small study of 146 class III–IV heart failure patients, addition of aliskiren to an ACE inhibitor or AT1 receptor blocker and β-blocker resulted in a decrease in plasma NT-proBNP, without changes in blood pressure or indices of kidney function.174 However, consistent with the results of combination RAS blockade in postmyocardial infarction patients, addition of aliskiren to the standard therapy of RAS blockade in postmyocardial infarction patients resulted in a failure to attenuate LV remodeling, and a greater incidence of adverse effects with aliskiren, including hypotension, increases in creatinine and hyperkalemia.175 The Aliskiren and Aliskiren/Enalapril Combination on Morbidity in Patients with Chronic Heart Failure (ATMOSPHERE) study will attempt to definitively determine the role of a renin inhibition strategy additional to or as an alternative to conventional RAS blockade in patients with chronic systolic heart failure.176

**Table 2. Recommendations From Expert Committees on Combination Angiotensin-Converting Enzyme Inhibitor/Angiotensin II Type 1 Receptor Blockade in Heart Failure**

<table>
<thead>
<tr>
<th>Committee</th>
<th>Recommendation</th>
</tr>
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<tbody>
<tr>
<td>Canadian Cardiovascular Society Consensus 2009</td>
<td>AT1 receptor blockade should be added to ACE inhibitors for persistent heart failure symptoms despite optimal heart failure treatment</td>
</tr>
<tr>
<td>ACC/AHA Guidelines for Diagnosis and Management of Heart Failure in Adults 2009</td>
<td>Addition of AT1 receptor blockade to ACE inhibitor may be considered in patients with reduced LVEF already treated with conventional therapy</td>
</tr>
<tr>
<td>European Society of Cardiology 2008</td>
<td>Unless contraindicated or not tolerated, AT1 receptor blockade is recommended in patients with heart failure and LVEF &lt;40% who remain symptomatic on ACE inhibitor or beta blocker</td>
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**Heart Failure With Preserved LV Ejection Fraction**

The most challenging clinical problem in heart failure today is the treatment of heart failure with preserved LV ejection fraction, which comprises ~50% of heart failure cases. The characteristics of the LV in heart failure with preserved LV ejection fraction compared to systolic heart failure include normal to near-normal as opposed to elevated LV diastolic and end-systolic volumes, along with well-preserved to slightly increased LV wall thickness as opposed to LV wall thinning. Three completed trials evaluating the effect of AT1 receptor blockade (CHARM-Preserved,177 Irbesartan-Preserved178) or ACE inhibitor perindopril (PEP-HF179) demonstrated no effect on cardiovascular mortality; however, CHARM-Preserved did demonstrate a significant effect on hospitalizations. In recent studies, there was no additional beneficial effect of valsartan over conventional therapy on LV diastolic function with similar reductions in blood pressure.180 The lack of LV dilatation and wall thickening with heart failure with preserved LV ejection fraction is consistent with an underlying cause of hypertension that may be associated with fibrosis or cardiomyocyte calcium dysfunction; however, the exact myocardial mechanisms involved in the pathophysiology of heart failure with preserved LV ejection fraction are unknown.

After several weeks of ACE inhibition or AT1 receptor blockade, plasma aldosterone levels return to pretreatment levels in up to 30% to 40% of patients. Aldosterone and cortisol levels have been associated with a worse clinical prognosis in patients with heart failure.181 In 1990, Brilla and Weber first reported the effects of salt and aldosterone on myocardial fibrosis,182,183 and subsequently on oxidative stress and inflammation in the rat heart.184 There is now evidence in patients with hypertension that high dietary salt is also necessary for adverse myocardial effects manifested by LV hypertrophy.185 In a recent cross-sectional study conducted in patients with resistant hypertension, aldosterone excess and high dietary salt combined to sustain excessive urinary protein excretion,187 and salt restriction alone resulted in significant decreases in blood pressure in patients with resistant hypertension and hyperaldosteronism.188 Such studies generated a concept of “salt–aldosterone product” in the aldosterone-mediated inflammation that impairs both kidney and cardiac function. Finally, recent work has shed light on the
importance of cortisol in this aldosterone–salt axis in precipitating heart failure with preserved LV ejection fraction in a mouse model of pressure overload,189 as well as the complex interaction of parathyroid hormone, potassium, and trace elements in mediating the inflammatory state in heart failure,190,191 which was recently reviewed by Weber and outlined in Figure 6.192 All of these factors, in combination with high salt levels, may contribute to this therapeutic resistant state of heart failure with preserved LV ejection fraction, which if addressed at an early stage may prevent heart failure, thereby making this more of the “internists’ heart failure.” The results of these human and animal studies led to the ongoing multicenter randomized Trial of Aldosterone Antagonist Therapy in Adults with Preserved Ejection Fraction and Heart Failure (TOPCAT). In light of the foregoing discussion, it will be interesting to see whether aldosterone blockade alone will be sufficient in the treatment of heart failure with preserved LV ejection fraction.

Figure 6. Hypothesis for the clinical syndrome of heart failure with preserved left ventricular (LV) ejection fraction. Acute and chronic stressor states, including hemodynamic overload, activates the hypothalamic-pituitary-adrenal (HPA) axis. This includes the increased production of adrenocorticotropic hormone (ACTH) leading to an increased production of cortisol and aldosterone from the adrenal cortex and catecholamines from its medulla. Elevations in plasma aldosterone are inappropriate for normal or increased dietary sodium and are accompanied by increased urinary and fecal excretion of potassium, magnesium, calcium, and zinc, leading to hypokalemia, ionized hypomagnesemia and hypocalcemia, hypoazincemia, and hyposelenemia. In response, secondary hyperparathyroidism with bone resorption appears to restore extracellular calcium and magnesium homeostasis. The calcitropic parathyroid hormone (PTH) promotes intracellular calcium overloading of cardiomyocytes and their mitochondria. The accompanying hyperadrenergic state further aggravates this dyshomeostasis by promoting a shift of these cations from the vascular into the intracellular compartment. The cumulative excessive accumulation of intracellular calcium and associated mitochondrial calcium overloading contribute to the induction of oxidative stress by these organelles. The ensuing opening of their inner membrane permeability transition pore (mPTP) and consequent osmotic swelling lead to their degeneration and necrotic death of cardiomyocytes with a spillage of their contents and rise in serum troponins. Subsequent tissue repair accounts for a replacement fibrosis with increased myocardial stiffness. Repeated over time, there is a progressive myocardial fibrosis with the appearance of diastolic dysfunction while ejection fraction is preserved (heart failure with preserved LV ejection fraction). Adapted from Weber192 (Illustration credit: Cosmocyte/Ben Smith).

Perspectives
There are many future questions to be addressed by basic and clinical research. The RAS has many connections to many other neurohormonal systems, particularly the adrenergic nervous system. There are extensive numbers of AT1 receptors on neurons in the heart and in areas of the brain that regulate sympathetic outflow and oxidative stress in neurons.193 However, does full RAS blockade combined with β-receptor blockade withdraw important cardiovascular support in the class III–IV heart failure patient? Development of new AT1 receptor blocking drugs with biased signaling may alleviate this problem.149 New drug targets, such as chymase, which have direct actions on matrix cellular components while not affecting blood pressure may provide better targets, especially in patients after myocardial infarction and in patients with ischemic heart failure for whom maintenance of blood pressure is a major goal of therapy. The upcoming studies of the renin inhibitor in the ATMOSPHERE study will be of great interest. However, studies with currently available renin inhibitor aliskiren show
that blocking the active site of renin and prorenin does not alter their binding to the (pro)renin receptor or subsequent intracellular ERK1/2 activation.\textsuperscript{194} This may become especially important in diabetic individuals in whom there appears to be the most evidence for intracellular Ang II synthesis, which may mandate the development of drugs targeting intracellular synthesis. Finally, there is emerging evidence for the synergistic effects of salt with aldosterone and cortisol, as well as potential deficiencies of trace elements, that together may contribute to the heart failure with preserved LV ejection fraction. All of this knowledge now more than ever calls for the combined efforts of the basic, nutrition, and clinician scientists to achieve the next translational success stories in the treatment of heart failure.

Acknowledgments

This review is dedicated to the memory of Dr Robert A. O’Rourke. His devotion to translational research, teaching, and pursuit of cura personalis, care of the whole patient, was and continues to be an inspiration to his many students, trainees, and faculty. I am forever grateful to him for providing my start in academic medicine and for his continued mentorship and advice through the years.

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