Chapter 2
Newer Insights into the Biochemical Physiology of the Renin–Angiotensin System: Role of Angiotensin-(1-7), Angiotensin Converting Enzyme 2, and Angiotensin-(1-12)

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Abstract Knowledge of the mechanisms by which the rennin–angiotensin system contributes to cardiovascular pathology continues to advance at a rapid pace as newer methods and therapies uncover the nature of this complex system and its fundamental role in the regulation of blood pressure and tissue function. The characterization of the biochemical pathways and functions mediated by angiotensin-(1-7) [Ang-(1-7)], angiotensin converting enzyme 2 (ACE2), and the mas receptor has revealed a vasodepressor and antiproliferative axis that within the rennin–angiotensin system opposes the biological actions of angiotensin II (Ang II). In addition, new research expands on this knowledge by demonstrating additional mechanisms for the formation of Ang II and Ang-(1-7) through the existence of an alternate form of the angiotensinogen substrate [angiotensin-(1-12)] which generates Ang II and even Ang-(1-7) through a non-renin dependent action. Altogether, this research paves the way for a better understanding of the intracellular mechanisms involved in the synthesis of angiotensin peptides and its consequences in terms of cell function in both physiology and pathology.

Introduction

Knowledge of the mechanisms contributing to the pathogenesis of cardiovascular disease is today at a crossroad, possibly one of its most important stages, due to rapid advances in genetics, cellular signaling mechanisms, and the addition of new therapies. Concepts, often heavily weighted by a reductionist approach to accepting the multi-faceted nature of the mechanisms contributing to organ changes in the evolution of chronic disease processes, have been confronted by new discoveries

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E.D. Frohlich, R.N. Re (eds.), *The Local Cardiac Renin–Angiotensin Aldosterone System*, DOI 10.1007/978-1-4419-0528-4_2,

that do not match previous tenets[1,2]. Advances in molecular biology are bringing to the physician new and sometimes bewildering views, which he has to learn and judge in relation to clinical facts and to pressures derived from the new problems generated by advancing technology, earlier diagnosis, and longer survival. The evolution of knowledge on the contribution that the renin–angiotensin system has in the regulation of tissue perfusion in both health and disease is a good example. The past decade brought to the forefront the complexity of the renin-angiotensin system, which is more elaborate than originally accepted. The basic research knowledge of the role of angiotensin II (Ang II) in hypertension, vascular disease, and lipid and carbohydrate metabolism does not necessarily match with the outcomes of clinical trials testing the system's contribution by way of using angiotensin converting enzyme (ACE) inhibitors[1,2], Ang II receptor blockers (ARBs)[3], and now the new class of direct renin inhibitors[4–8]. It will be inappropriate to assume that this relative gap between the lessons that are learnt from testing the effects of these agents in the clinical setting and the information gained from meticulous studies of the renin-angiotensin system in animal models and cell systems suggests that one or the other has gone astray. What we need to remember is that homeostasis, in both health and disease processes, depends on the interplay of multiple regulatory mechanisms, which in the normal state act in coordination while they may become discordant in disease states.

In this chapter, we will address these issues from a viewpoint that for one of us (CMF) originates from perspectives gained from his association with Dr. Irvine H Page and from the research we conducted since the first demonstration of the biological actions of angiotensin-(1-7). Throughout this time, the slow process of unraveling pieces of this puzzle provides a more cogent understanding of the harmonious and dis-harmonious ways by which the renin-angiotensin system works to regulate normal blood pressure and its contribution to the expression of the disease, we call, essential hypertension.

A Revolving Story

Although a discussion of the biochemical pathways accounting for the formation of angiotensin peptides should begin with a description of the role of renin in the formation of angiotensin I (Ang I), for our objective we will begin with the discovery and analysis of the functions of the heptapeptide angiotensin-(1-7) [Ang-(1-7)], since its characterization became the stepping stone for a new understanding of the renin-angiotensin system. At the time of the first report of a biological effect of Ang-(1-7)[9], investigators were adamantly focused on finding a receptor for the actions of Ang II. Work on Ang II analogs and Ang II peptide antagonists suggested that the Pro^7–Phe^8 bond of the Ang II molecule was an essential requisite for binding to the as yet to be identified receptor[10–12]. Therefore, our first report that Ang-(1-7), having a truncated C-terminus, showed biological activity did not meet with any enthusiasm. Over the ensuing years, and as reviewed elsewhere, our laboratory continued to unravel the participation of Ang-(1-7) in the regulation of

blood pressure[13–33], characterize the effects of Ang-(1-7) in blocking the proliferative actions of Ang II[34–36], and decipher the biochemical pathways of Ang-(1-7) processing and metabolism[18,37–40]. Our studies and those of others further demonstrated a role for Ang-(1-7) in mediating a part of the antihypertensive actions of ACE inhibitors and ARBs[21–23,31,41,42]. As work progressed, the concept advanced that Ang-(1-7) may act in tissues as a paracrine hormone opposing the actions of Ang II[43,44]. A paracrine role for this component of the renin-angiotensin system explains the relative higher concentrations required for Ang-(1-7) effects, as it is known that at the vicinity of a tissue receptor, the concentration of the ligand may be in the nanomolar range. The characterization of the *mas*-receptor as the binding site expressing the cellular actions of Ang-(1-7) completed a critical step in defining the role of Ang-(1-7) in cardiovascular homeostasis and opened new avenues for exploring alternate approaches in competing against the pathological actions of Ang II[45,46].

A second critical step in gaining acceptance for the functional activity of Ang-(1-7) came about from the identification of angiotensin converting enzyme 2

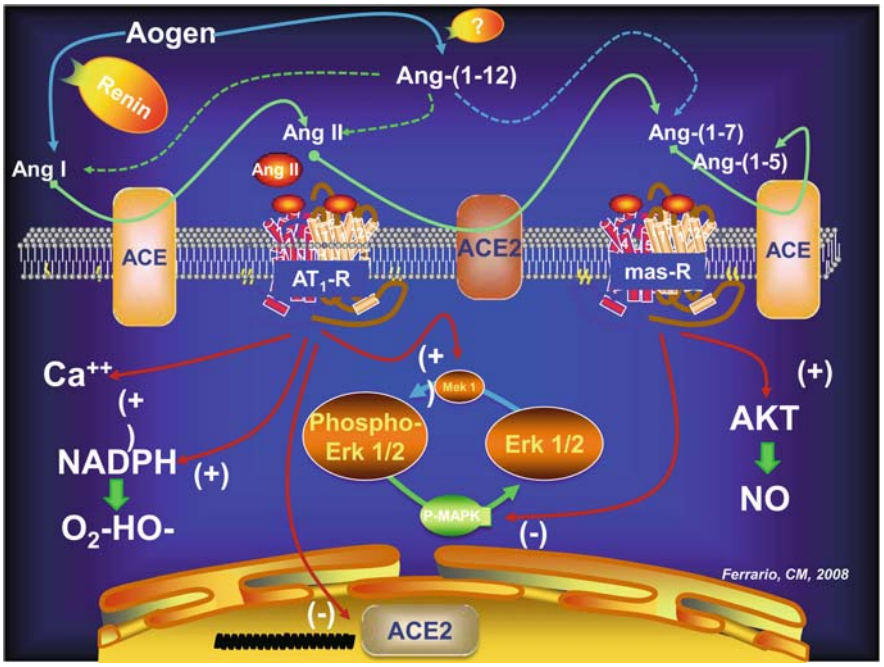

Fig. 2.1 Schematic diagram illustrating the pathways for the formation of angiotensin peptides and their actions on cellular signaling mechanisms. Abbreviations other than those described in the text are NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; O_2—HO—, a form of active oxygen species or free radical; AKT, protein kinase B; MEK 1, dual threonine and tyrosine recognition kinase that phosphorylates and activates mitogen-activated protein kinase (MAPK); ERK 1 and ERK 2, mitogen-activated protein or extracellular signal-regulated kinase p^{44ERK1} and p^{42ERK2}

(ACE2) by two separate laboratories[47,48]. These studies led to the demonstration of a high efficiency of ACE2 in converting Ang II into Ang-(1-7)[49–51], the demonstration that Ang II negatively regulated ACE2 gene expression[52–55] and that genetic or molecular approaches to suppress ACE2 function were associated with cardiac abnormalities, vascular proliferative responses, and worsening of type 2 diabetes[56–62]. Several review articles detail the knowledge that was gained from exploring the role of ACE2 in cardiovascular pathology, its interaction with Ang-(1-7), and its role as the cellular entry point for the severe acute respiratory syndrome (SARS) virus[63–65]. Figure 2.1 illustrates the biochemical pathways leading to the formation of the biologically active angiotensin peptides.

New Precursor of Angiotensin Peptides

Our original view of the RAS as a complex system entailing several levels of regulation and processing expanded with the identification of proangiotensin-12 [angiotensin-(1-12), Ang-(1-12)] as an upstream propeptide to Ang I. Nagata et al.[66] first isolated this novel angiotensinogen-derived peptide from the rat small intestine. Consisting of 12 amino acids, this peptide was termed proangiotensin-12 [Ang-(1-12)], based on its possible role as an Ang II precursor. Ang-(1-12) constricted aortic strips and, when infused intravenously, raised blood pressure in rats[66]. The vasoconstrictor response to Ang-(1-12) was abolished by either captopril or the Ang II type I receptor blocker, CV-11974. Over the years, questions arose regarding the capability of tissues other than the kidneys to synthesize Ang II, in part because gene expression for some of the RAS components occurs at low levels (i.e., renin and Aogen). The heart is a critical example. Although a large body of evidence suggests a participation of local tissue RAS in the regulation of cardiac function and remodeling[67–76], most studies showed low levels of gene expression for both cardiac renin and Aogen. Neither the identification of renin in cardiac mast cells[77] nor the finding of renin activation by prorenin binding to the prorenin/renin receptor[78–80] can be construed as evidence for local production of cellular renin, as an uptake mechanism from the blood compartment cannot be excluded.

Many cell types in myocardial tissue, including cardiomyocytes, contain receptors for Ang II, but as indicated above the activation of these receptors requires angiotensin concentrations in the micromolar range, which do not occur in plasma in vivo. However, angiotensins formed locally in the heart can activate these receptors in a paracrine and autocrine mode[81]. Indeed, recent studies have provided compelling evidence for the existence of an intracellular RAS that functions as an *autocrine* system[67,68,74–76,81]. It has been suggested that intracellular generation of Ang II may occur via a non-renin pathway with rerouting of Aogen to other subcellular structures[67,68,71,74–76,81–89]. With this in mind, we began to explore the potential role of Ang-(1-12) in the formation of angiotensin peptides as well as to inquire into the mechanisms that may regulate the processing of Aogen into

Ang-(1-12). Work in progress documents the expression of Ang-(1-12) in cardiac myocytes of Wistar Kyoto (WKY) rats, increased expression of Ang-(1-12) in the spontaneously hypertensive rat (SHR)[90], and evidence that Ang-(1-12) is a functional substrate for the formation of Ang I, Ang II, and even Ang-(1-7) in the isolated heart from several rat strains[91]. In rodents, the sequence of Ang-(1-12) is Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}-Leu^{11}-Tyr^{12}. Since renin specifically cleaves the Leu^{10}–Leu^{11} bond of rat Aogen to form Ang I, the cleavage between the two aromatic residues Tyr^{12}–Tyr^{13} for the liberation of Ang-(1-12) may not be accounted for by the action of renin. Assessment of the processing of Ang-(1-12) into angiotensin peptides in the isolated heart confirmed that renin is not involved in processing Ang-(1-12) into Ang I. In addition, we have recently extended these observations in rats in which bilateral nephrectomy was employed as a tool for elimination of renal renin[92]. The anephric state resulted in divergent effects on circulating and cardiac content of Ang-(1-12), Ang I, and Ang II since these peptides fell in the plasma, but increased markedly in the left ventricle of 48 h bilateral nephrectomized WKY rats compared to sham-operated controls. A 34% decrease in plasma Ang-(1-12) levels 48 h post-nephrectomy was associated with a 78 and 66% decrease in plasma Ang I and Ang II, respectively ($p < 0.05$ versus sham-animals). In contrast, cardiac content of Ang-(1-12) in anephric rats averaged 276 ± 24 fmol/mg compared to 144 ± 20 fmol/mg in sham-operated controls ($p < 0.005$). A representative example of Ang-(1-12) in cardiac myocytes is illustrated in Fig 2.2.

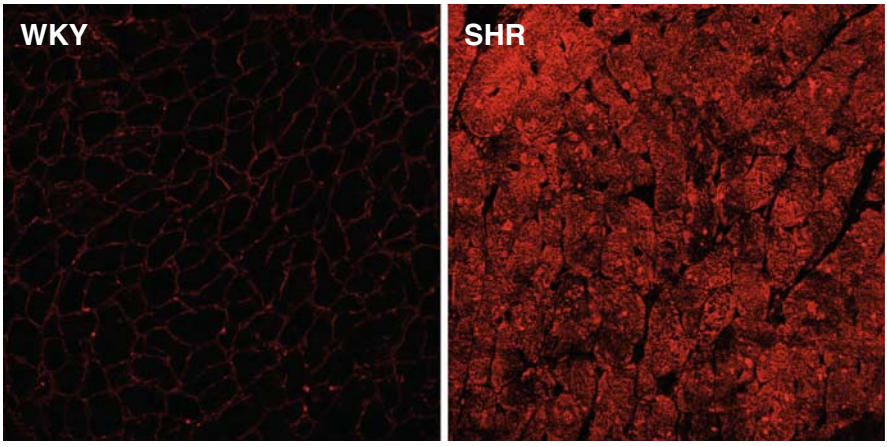

Fig. 2.2 Representative Ang-(1-12) immunofluorescence in the left ventricle of WKY and SHR rats. Data from our reference[90]

Further research on Ang-(1-12) may resolve the nature of the precursor protein accounting for the local synthesis of angiotensins (Ang II and Ang-(1-7)) in cardiovascular tissues, particularly the heart. The lower molecular weight Ang-(1-12) peptide (compared to Aogen) may represent a stored form of a precursor

to angiotensins formation since its amino acid sequence is composed of 12 rather than the 458 amino acids of the parent Aogen compound. These findings are also germane to the demonstration by others of the existence of an intrinsic mechanism for the intracellular generation of Ang II in cardiac myocytes that is independent from uptake from the circulation or the interstitial environment[68,82].

Conclusions

Our past work on Ang-(1-7) and ACE2 has been seminal in bringing about a better understanding of the biochemical and functional role of the renin-angiotensin system in cardiovascular regulation. Furthermore, the discovery of the biological actions of Ang-(1-7) provided the underpinning for the latter characterization of ACE2 and the establishment of the *mas*-receptor as a component of the system. Altogether, these studies contributed much to the affirmation of a role of this system in tissues and established a newer understanding of how the counter regulatory actions of Ang-(1-7) oppose the effects of Ang II on arterial pressure and cardiovascular remodeling. A new phase in unraveling the biochemical pathways that determine the generation of angiotensin peptides arises from studies now identifying Ang-(1-12) as an alternate Ang I forming substrate for the cellular processing of Ang II and even Ang-(1-7). The discovery of Ang-(1-12) as an endogenous substrate for the formation of angiotensin peptides may be critical to the understanding of intracellular mechanisms associated with the actions of the renin-angiotensin system in health and disease. It will be incorrect to argue that characterization of this potentially alternate substrate may have no major consequences in terms of the functions of the biologically active angiotensins. First, characterization of Ang-(1-12) as a cellular substrate may explain the observations (both clinical and experimental) of incomplete blockade of Ang II formation with the currently approved orally active renin inhibitor [aliskiren (Tekturna®)]. We have reviewed in detail the data[93] that demonstrates that the antihypertensive effects of aliskiren are not greater than those obtained with amlodipine[94] and that the addition of an angiotensin receptor blocker (ARB) markedly potentiates the response to renin inhibition[4,7]. These data suggest that aliskiren does not eliminate formation of Ang II. Further evidence for an incomplete blockade of Ang II generation was obtained in normal volunteers in whom the reduction of plasma Ang II following administration of Ang II was not complete as plasma Ang II levels returned toward control values within 12 h after oral administration[95]. To date no studies of the fate of Ang II following direct renin inhibition have been performed in essential hypertensive subjects. Thus, our recent studies on Ang-(1-12) have much significance in determining the efficacy of direct renin inhibition as the current data suggest that Ang-(1-12) generation is not dependent on renin.

If we are correct in assuming that the primary factor involved in the expression of hypertension and the remodeling of the heart and blood vessels originates from the rupture of the equilibrium between on one hand the ACE/Ang II/AT_1 receptor axis and on the other the state of the counterbalance actions of ACE2/Ang-(1-7)

/*mas*-receptor axis, a further inquiry into the mechanisms that result in reduced expression of the later regulatory arm of the renin-angiotensin system should lead to new therapies and a better understanding of the cellular processes at which these two systems act to maintain homeostasis.

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