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# New Aspects of the Renin Angiotensin System in Cardiovascular and Renal Diseases

Editors:
Dulce Elena Casarini, Danielle Yuri Arita
Juliana Almada Colucci
Tatiana Sousa Cunha

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# FOREWORD

Research on the pleiotropic actions and consequences of the renin angiotensin system (RAS) and its many effector components remains fascinating and interesting because new surprises continue to emerge and provide fodder for further analysis and new experimental directions. The older paradigm was so straightforward and simple focusing primarily on the circulating RAS and a single effector peptide, angiotensin II [Ang II]. However, we now know that the system is much more complex exhibiting multiple actions of various active components *via* different receptors and utilizing various important enzymatic pathways. In this exciting new e-book by Dr. Casarini and her colleagues, a number of important new concepts are presented which further demonstrate the complexity of the RAS system and the many effector components. As pointed out in the first few chapters, evidence obtained during the previous one or two decades has led to an expanded view of the RAS serving paracrine, intracrine, and endocrine functions. Our attention is also directed to the findings that many other angiotensin peptides also exert bioactive roles.

Several of the subsequent chapters then delve into the intricacies of the local tissue RAS activities focusing on the paracrine and intracrine functions of Ang II in the heart and kidneys as well as other angiotensin peptides including Ang 1-7. The more recently discovered components of the tissue RAS, including ACE2 and the prorenin receptor, are also integrated into the overall interactions that occur at the tissue and cellular level and also within the intratubular network in the kidneys. Our attention is then directed to a consideration of the complex enzymatic pathways that are responsible for the generation of the various angiotensin peptides. The roles of tonin, ACE and ACE2 and ACE polymorphisms are examined.

Additional chapters discuss the novel developments related to the ACE2/Mas axis and how it influences formation and action of Ang 1-7, and our evolving concepts regarding the renin-prorenin receptor and their possible roles in regulating the local formation and activities of Ang 1-7 and the prorenin receptor. Completing the book, the remaining chapters deal with RAS related pathophysiology and the roles of immunity and inflammation activated by Ang II, the interactions with the nitric oxide system and catecholamines, and how emotional stress and exercise may alter the activity of the RAS. Finally, a consideration of how the activity of the RAS is modulated in animal models of hypertension and diabetes provides further insights on pathophysiological contributions of the RAS in disease states.

In this e-book, Dr. Casarini and her associates have provided us with an outstanding array of topics which are covered in-depth and will be of tremendous value not only to investigators directly involved in studies of the RAS but also to all colleagues who are interested in

obtaining a comprehensive appreciation of the current state of knowledge regarding the regulation and roles of the many components of the "modern" RAS.

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# PREFACE

The idea of publishing a book on the new aspects of the renin-angiotensin system (RAS) in cardiovascular and renal diseases has been developed during the past few years. The need for a book that describes the new approaches of the RAS in the development of cardiovascular and renal diseases and the trend of future research became apparent. The book describes the important role of systemic and local RAS in the development of heart and kidney diseases and its relationship with the immune system, stress and exercise; as well as provides data on the biochemical and pathological changes in these diseases.

Understanding the mechanisms of participation of this system in cardiovascular and intrarenal modulation can lead us to better understand the pathogenesis of diseases such as diabetes, hypertension and chronic renal failure. Even knowing the therapeutic interventions for these diseases, there are many challenges in integrating traditional medical literature and increased information of new technologies that facilitate the comprehension of the molecular and cellular mechanisms.

We have been very fortunate to enlist a group of renowned experts in RAS research for this book, and very grateful to the dedication and contribution of our authors. We hope that book will be useful for researchers working in the field of the RAS.

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CHAPTER 1

# Renin Angiotensin System: Old System with New Different Components

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**Abstract:** Recently, the continuing and increasing interest in the renin angiotensin system (RAS) after discovery of new peptides and enzymes, has been stimulating us to review the new concepts described in the literature. The classical RAS is a system in which renin acts on angiotensinogen (Agt) to form angiotensin I (Ang I) that is cleaved to the active peptide angiotensin II (Ang II) by angiotensin converting enzyme (ACE). The Ang II pharmacological actions occur after their interaction with Ang II type 1 and type 2 receptors (AT1R; AT2R). In the last decade, a new concept of local tissue RAS systems has been described in different organs, and also intracellular RAS has been stated, allowing expansion of the concept of functions of this system as endocrine, paracrine, autocrine and intracrine. This first chapter provides brief overview on the history of the RAS components, circulating and tissue RAS, and outlines the physiological functions of the RAS major active substance, Ang II. Here, we also describe other bioactive angiotensins, such as Ang III, Ang IV, Ang (1-7), Ang (1-12), angiotensin A and alamandine. Moreover, we report the studies on ACE2 and chymase, enzymes identified during the last years. The recent advances in the understanding of the RAS will provide new opportunities to treat and prevent hypertension and cardiovascular diseases.

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**Keywords:** ACE, ACE2, Alamandine, Angiotensin A, Angiotensin converting enzyme 2, Angiotensin I-converting enzyme, Angiotensin II, Angiotensin III, Angiotensin IV, Angiotensin (1-7), Angiotensin (1-12), Chymases, Enzymes, RAS, Receptors, Renin, Renin angiotensin system.

## INTRODUCTION

Recently, the continuing and increasing interest in the renin angiotensin system (RAS) has been stimulating us to review and present the discoveries concerning the system. To briefly introduce this book, we will describe the classic and new components of the RAS. More than one hundred years ago, Tirgersted and Bergman (1898) discovered the RAS by detecting a pressor effect under intravenous injection when using an alcoholic extracts of renal cortex of rabbits. They called this substance renin [1]. The characterization of renin as an enzyme was proposed by Kohlstaedt *et al.*, in 1940 [2]. At the same period, Braun-Menendez and Irvine (1940) discovered the active substance angiotensin [3] and Skeggs *et al.* (1954) described two forms of this peptide, termed as angiotensin I (Ang I) and angiotensin II (Ang II) [4]. The RAS is a complex hormonal system; it is known that there are two RAS: plasma-localized, which acts *via* blood circulation regulating cardiovascular system function, and tissue-localized, regulating specific long-term changes. Both systems are crucial for hydro-electrolyte balance and blood pressure regulation. In the literature, it has been described that RAS is presently described as an endocrine, paracrine, autocrine and intracrine, system [5]. While, the intracellular system has important role in cellular homeostasis reflecting in renal function.

The RAS modulation is focused on the activity of Ang II, as well as other products of Ang I metabolism, as Ang (1-7), which has contrary actions of Ang II. The cascade is initiated with the formation of the decapeptide Ang I by renin, an aspartyl protease secreted and released by the juxtaglomerular apparatus of the kidney, after cleavage of Leu-Val peptide bond of angiotensinogen (Agt), which is produced by organs as liver, kidney, brain, heart. Prieto *et al.* (2009) described the renin production in the collecting duct cells that are able to liberate in this portion Ang I [6]. Renin produced by kidney is a rate-limiting regulator enzyme of the circulating and tissue RAS activity.

In the circulation renin is released as prorenin that can be converted to active renin by proteolytic and non-proteolytic cleavage. Intracellular renin was described to be derived from local synthesis, but also as a result of uptake from the circulation being active in cellular compartment [7, 8].

Ang I is hydrolyzed to Ang II by angiotensin converting enzyme (ACE), a kininase classified as metallo-endopeptidase [9]. Then, Ang II binds to two specific receptors, Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R), to follow up biological functions [5, 10]. ACE is expressed on the surfaces of the endothelial cells, epithelial and mesangial cells [8, 11, 12]. Besides of the pulmonary epithelial cells, ACE is also expressed in many other tissues in the organism. In the literature, two ACE forms are described in human as a product of alternative splicing, the germinal and somatic ones. Somatic ACE is expressed in the lung, kidney, intestine, placenta, pancreas, adrenal and choroid plexus. Whilst, the germinal ACE is present in testes during the spermatogenesis. Both forms of ACE are anchored to plasma membrane [13].

ACE have been described as soluble forms in circulation and in many fluids as intestinal and urine [14 - 18]. Further, ACE is able to inactivate bradykinin a vasodilator peptide from Kallikrein-Kinin System. ACE can interact with Ang II and evoke calcium and other signaling molecules in cells expressing only ACE, promoting an increase of reactive oxygen species [19, 20].

On the basis of classical RAS, the pharmacological actions of Ang II occur *via* AT1Rs resulting in cellular proliferation; vasoconstriction, cardiac contractility, increased renal tubule $Na^+$ reabsorption; aldosterone, vasopressin, and endothelin secretion; activation of the sympathetic nervous system (Fig. **1**).

Ang II also acts *via* AT2Rs but have opposite actions to the AT1Rs under most circumstances [21, 22].

## RAAS at Present

RAS profile in the last ten-fifteen years was extended, when new different components appeared, as well as independent actions were described for tissue and circulating systems demonstrating the independence of these two

CHAPTER 2

# The Important Role of Systemic and Local Renin-Angiotensin System

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**Abstract:** Knowledge of renin-angiotensin system (RAS) has evolved through the years from the classical endocrine system view, which explains the homeostasis and arterial blood pressure control, to a more complex system, including new components and independent local RAS acting intracellularly and within different organs. It is well-known that the circulating RAS plays a physiological and important role in blood pressure regulation through direct effects on vascular smooth muscle, aldosterone secretion and sodium, potassium and water equilibrium. The potent vasopressor peptide Angiotensin (Ang) II is the key regulator of the system and the Ang 1-7 counter-regulates Ang II actions. Components are generated in liver (angiotensinogen), kidneys [renin] and vascular endothelial cell membranes (angiotensin I -converting enzyme) and secreted to the circulation to generate systemic Ang II. Recently, the focus of interest in the RAS changed the role of tissue/local system in specific tissue. Ang II synthesis within tissues from angiotensinogen and enzymes is defined as local RAS. The activation of the circulating and/or local RAS plays a fundamental role in the pathogenesis of hypertension and chronic kidney disease. RAS blockade with angiotensin I-converting enzyme (ACE) inhibitors or Ang II receptor blockers is a major approach to treat cardiovascular and renal diseases. However, it is still unclear if a dual blockade exerts a better protection than single blockade or shows a higher risk for renal complications and hyperkalemia.

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**Keywords:** ACE inhibitors, Angiotensinogen, Angiotensins, Angiotensin converting enzyme 2, Angiotensin I-converting enzyme, Angiotensin II-receptor blockers, Cardiac diseases, Cells, Chronic kidney diseases, Heart, Kidney, Local RAS, RAS blockade, Renin, Renin-angiotensin system, Systemic RAS.

## CLASSICAL AND NEW VISION OF THE RAS

The renin-angiotensin system (RAS) has been studied for more than a century and the deep investigative research over the last years have well established the role of the RAS as a main component in the control of arterial blood pressure, cardiovascular, renal and adrenal function, and fluid and electrolyte homeostasis. RAS is identified as a dual vasoactive system, acting as both a circulating endocrine and a local tissue paracrine system [1 - 5].

In the classical view, the RAS is a hormone coordinated cascade of sequential enzymatic steps, which the first is the release of angiotensinogen (AGT) produced in the liver, cleaved by renin, which is released from the juxtaglomerular cells in the kidney generating angiotensin I (Ang I). This peptide is further processed by angiotensin-I converting enzyme (ACE) into angiotensin II (Ang II), the key regulator of the system. In addition, alternative enzymes generate Ang II, such as cathepsins and chymase, however it has been established that ACE is the key enzyme in the regulation of Ang II production in the cascate [1, 3, 6].

Ang II is a potent vasoconstrictor acting on different target organs, including blood vessels, kidney and heart. This peptide influences many physiological processes such as fluid and electrolyte balance, homeostasis and blood pressure. The effects of this potent peptide are mediated by two different receptors: AT1 and AT2. AT1 receptor (AT1R) is responsible for the majority action of Ang II in the heart, kidney and adrenal glands, including vasoconstriction, aldosterone and vasopressin release, sodium and water retention, cell proliferation and sympathetic facilitation. In most cases, the actions of the AT2 receptor (AT2R) counterbalance to those of the AT1R and are partly mediated by activation of kallikrein-kinin system [7].

Due to the great importance of the RAS, many studies have been conducted and important discoveries have been occurred and contributed to a new vision of this

system, such as the identification of new enzymes, new peptides, novel receptors and new functions. In 2000, two distinct groups described a new ACE homologue enzyme, called ACE2, which catalyzes Ang II into the biologically active peptide, angiotensin 1-7 (Ang 1-7) and also cleaves Ang I into angiotensin 1-9 (Ang 1-9), an inactive peptide. Importantly, the metabolization of Ang II into Ang 1-7 by ACE2 is much more efficient than the conversion of Ang I into Ang 1-9 [8, 9]. Ang 1-7 generation was also demonstrated by the cleavage of Ang I by the neutral endopeptidase also called neprilysin, a cell surface zinc metalloendopeptidase involved in peptide hydrolysis at the extracellular surface of the plasma membrane [10]. In 2007, Santos *et al.* [11] described the new receptor for Ang 1-7, the Mas proto-oncogene, which is mainly expressed in many organs, such as brain, testis, kidney, heart and vessels [12]. Studies demonstrate that Ang 1-7 contributes to the control of blood pressure in the cardiovascular and renal systems *via* actions that, within the heart, kidney, and the blood vessels, opposing the actions of Ang II [11, 13].

There are other active peptides generated through the cleavage of Ang II that may have important actions in the local RAS. Among them, the angiotensin III (Ang III) is generated by the cleavage of Ang II by aminopeptidase A, which is itself converted to angiotensin IV (Ang IV) by aminopeptidase N. These peptides have affinity to AT1R and AT2R, and AT2R and IRAP (insulin-regulated aminopeptidase), respectively. The interaction of Ang IV with its receptor, AT4R, has been comprehensively reviewed and research findings about Ang III and Ang IV lead to the conclusion that theses peptides are biologically active in the brain, supporting the possibility that Ang I and Ang II are precursor molecules for these active forms [5, 14]. Despite the existence of other components of the system, Ang II is still considered as the primary effector hormone of the RAS.

## Tissue Sites of Local RAS

Nowadays, the existence of local or tissue-based RAS and angiotensin production at tissue sites is firmly established, being described as a key player in the pathophysiology of cardiovascular and renal diseases [5, 15, 16]. In the tissue RAS, Ang II is generated from components that are mainly of local origin. In some tissues, the enzymes responsible for producing Ang II from AGT may be

CHAPTER 3

# Renin-Angiotensin System and Cardiovascular Physiology

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**Abstract:** Since the discovery of renin at the end of 19th century and the identification of angiotensin I (Ang I) and Angiotensin II (Ang II) seven decades ago, renin-angiotensin system (RAS) has been widely studied. The RAS is an important endocrine system that regulates blood pressure and fluid homeostasis. This system is composed of peptides, enzymes and receptors. The RAS plays a key role in the development and progression of cardiovascular diseases. In this sense, Ang II is an important peptide of this system, presenting vasoconstrictor and salt-retaining properties and, at cellular level, promoting proliferation, fibrosis and hypertrophy. This chapter focuses on RAS and cardiovascular physiology addressing to an overview about RAS and cardiovascular disease, RAS and its relation with elevated blood pressure, sympathetic overactivity, cardiac hypertrophy, endothelial and vascular dysfunctions, and immune response activation, as well as the physiological basis of RAS inhibitors and cardiovascular disease treatment.

**Keywords:** Angiotensin-converting enzyme inhibitors, Angiotensin receptor antagonists, Angiotensin II receptor blockers, Autonomic nervous system,

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Baroreflex, Blood pressure, Cardiac hypertrophy, Cardiovascular diseases, Endothelial dysfunction, Hypertension, Inflammation, Sympathetic nervous system, Vascular resistance.

## RAS & CARDIOVASCULAR SYSTEM: AN OVERVIEW

The renin–angiotensin system (RAS) plays a key role in regulating the systemic volume and vascular resistance; thus, influencing both the cardiac output and the blood pressure (BP). In this sense, it is well known that renin secretion by the juxtaglomerular cells depends on the increase of sodium concentration in the macula densa, the decrease in the perfusion pressure and the enhancement of the sympathetic activity. In addition, the production of renin by different tissues has been studied [1 - 4]. The cleavage of the angiotensinogen by renin action produces the inactive decapeptide, *i.e.,* Angiotensin I (Ang I). On the other hand, the *angiotensin converting enzyme (ACE)* cleaves Ang I, forming Angiotensin II (Ang II) and can promote the bradykinin degradation (Fig. **1**). Ang II can act directly on the AT1 receptors on the smooth muscle cells of the vessel [5], inducing vasoconstrictor effect as well as increasing the vascular tone by sympathetic nervous system interaction [6, 7]. Moreover, Ang II may induce volume expansion [8] and cellular proliferation and hypertrophy [9, 10].

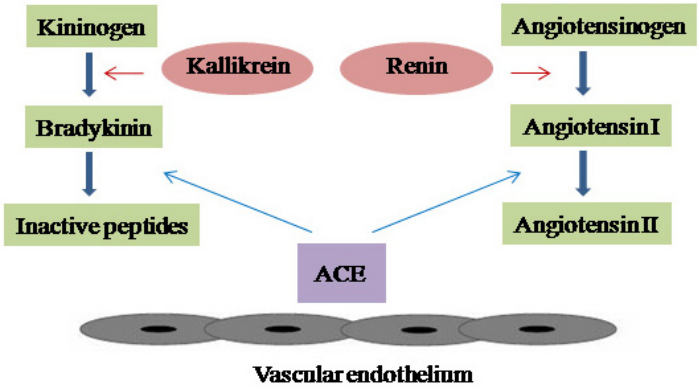


**Fig. (1). Schematic of the classical RAS and the kallikrein-kinin system.** ACE regulates the balance between Ang II and bradykinin. (*Modified of Brown and Vaughan* [11]).

In brief, the balance between natriuretic and vasodilatory characteristics of bradykinin and the vasoconstrictor and salt-retaining properties of Ang II are

regulated by ACE. ACE inhibitors decrease bradykinin degradation and Ang II formation (Fig. **1**).

It is important to emphasize that Ang II is the most studied molecule of the RAS and can act as a systemic hormone (endocrine) or as a locally generated factor (paracrine and autocrine). Ang II is also an omnipresent factor in several cardiovascular disorders, having an absolutely important influence on the pathophysiology and progression of cardiovascular diseases and on several cardiovascular risk factors (Fig. **2**).

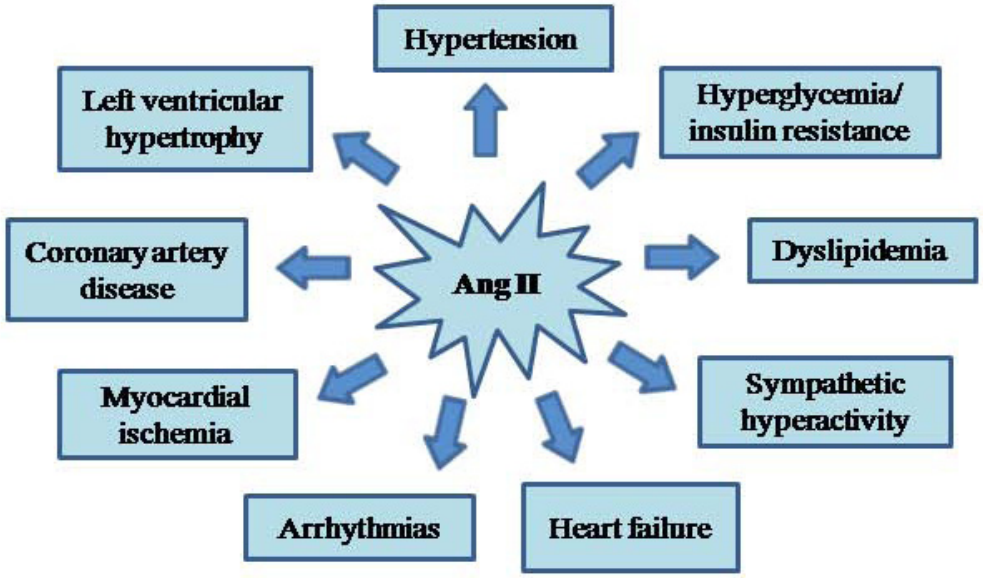


**Fig. (2).** Angiotensin II and its influences on several cardiometabolic disturbances.

Over the last decades, the view of the RAS has expanded to a more complex concept. The Ang II arm, classically known for its "harmful" responses through the interaction with AT1 receptor, can be counterbalanced by the Ang-(1- 7)-ACE2-Mas receptors axis, which can exert beneficial effects in the cardiovascular homeostasis in physiological and pathological conditions. Furthermore, recently was discovered a new element of RAS, the alamandine, which is derivative of Ang-(1-7), and its receptor, the Mas-related G-coupled receptor type D (MrgD) [12]. Alamandine is a vasoactive peptide that produces similar physiological

CHAPTER 4

# Cardiac Intracellular Renin-Angiotensin System

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**Abstract:** As a hormonal system, the renin-angiotensin system (RAS) is known for endocrine and autocrine/paracrine physiological functions. An intracrine role of the RAS has been proposed long ago; however, evidence for this function has started accumulating only recently. Angiotensin (Ang) II is the major hormone of the RAS and is the focus of research for the intracrine nature of this system. The intracrine functionality is characterized by intracellular actions of the hormone within the cell of synthesis or following internalization. Intracellular synthesis and actions of Ang II have been demonstrated in several cell types and tissues, with a significant amount of work in the heart. This review focuses on the cardiac intracellular RAS, delineating differences from the extracellular RAS that further consists of the circulatory and local RASs. The pathophysiological significance of the intracellular cardiac RAS has been discussed.

**Keywords:** Angiotensin converting enzyme, Angiotensin II, Angiotensinogen, Autocrine, Chymase, Diabetes, Diabetic cardiomyopathy, Heart, Hormone, Intracrine, Nucleus, Paracrine, Renin, Renin-angiotensin system.

## INTRODUCTION

### Hormones

Hormones, by definition, are factors released by cells in one part of the body that

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modulate the function of cells in other parts of the organism. Thus, hormones represent long distance control mechanisms to maintain proper coordination between different organs of an organism. Generally, hormones are carried from one part of the body to the other through the bloodstream. Sometimes, hormones affect the function of cells in close proximity to their cell of synthesis immediately after release. Depending on whether the hormone travels through the bloodstream to target distant organs or acts on neighboring cells through simple diffusion, their functions are categorized as endocrine and autocrine/paracrine functions, respectively (Fig. **1**). A single hormone may have one or both types of functionality. In either case, hormones act by interaction with specific receptors located generally on the plasma membrane of the target cells, for peptide hormones, or inside the cell, for steroid hormones.

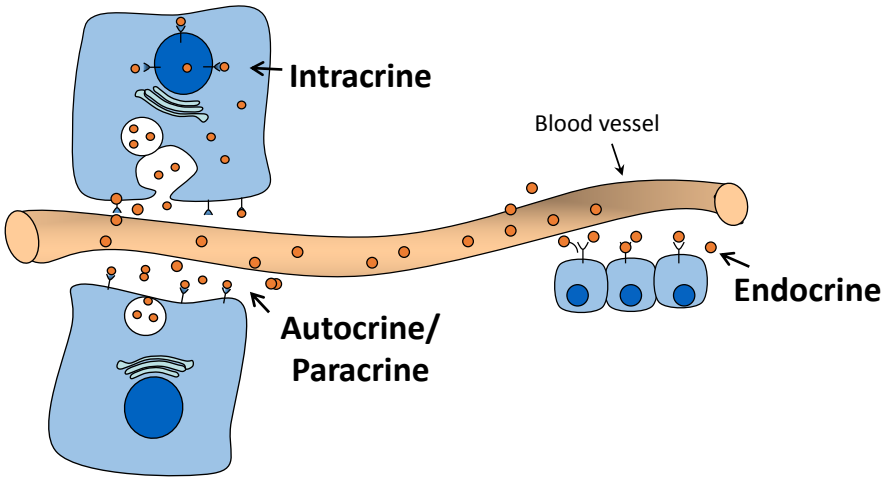


**Fig. (1). Modes of peptide hormone action:** Peptide hormones mainly act in two modes. One is endocrine mode, where the hormone released into circulation by endocrine glands targets distant organs. Another is autocrine/paracrine mode where the target site is on the same or adjacent cells. In both modes, the hormone binds to specific receptors on the cell surface, triggering intracellular signaling events. The third, mainly known for steroid hormones, is intracrine mode where the hormone acts intracellularly by binding to same receptors as on plasma membrane or other binding sites.

Another term, intracrine, has been used to describe certain hormone functions. According to a recent proposal, intracrine refers to intracellular actions of peptide hormones after internalization or within cells of their synthesis [1]. The latter part of the definition fits better with the definition of endocrine and auto-

crine/paracrine functionality that is based on the relationship between the site of synthesis and that of action of the hormone. Thus, a peptide hormone that also acts from an intracellular location is called an intracrine hormone; however, this term is used more commonly in relation to steroid hormones, as these generally act through intracellular receptors.

The significance of categorizing hormone functions, as described above, is not limited to the convenience of organization or the variety of physiological effects due to differences in the type of target cells at different locations. Recent evidence has shown that intracrine effects of peptide hormones can be similar or different than endocrine or autocrine/paracrine effects, in the same tissue or type of cells, due to differences in the intracellular and cell membrane receptors or in the signaling pathways activated by these receptors. Therefore, it is important to study all aspects of the hormone action to fully understand their physiological functions.

## The Renin-Angiotensin System

The renin-angiotensin system (RAS) comprises of several peptide hormones generated as a result of cleavage of angiotensinogen (AGT) by renin, followed by actions of various other enzymes. AGT and renin are secretory proteins produced in the liver and kidneys, respectively. Therefore, the peptide hormones of the RAS are believed not to be synthesized in a particular cell type of the body, rather in the bloodstream or interstitial space within tissues where AGT and renin encounter each other. Angiotensin (Ang) peptide hormones include Ang II, Ang III, Ang IV, and Ang (1-7). Although structurally similar, differing from Ang II by lack of only one or two N- or C- terminal amino acids, these peptides have distinct receptor affinities and biological functions. Given the multicomponent nature and different tissue origin of the circulating components, resulting in Ang peptide production in the blood, the RAS is primarily known as an endocrine system. Ang peptides are also generated at tissue sites, thereby affecting neighboring cells and acting as autocrine/paracrine hormones. Based on the above described site of synthesis of Ang II, the major peptide of the system, the RAS has been characterized as the circulating or the tissue/local RAS.

**CHAPTER 5**

# Angiotensin-(1-7) in the Heart

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**Abstract:** The renin-angiotensin system (RAS) is recognized as one of the most important modulators of the heart function, working intensely on maintaining cardiac homeostasis. Recent advances pointed out that this system is divided into two distinct counter-regulatory axes. The classical axis is well characterized and involves the formation and actions of the octapeptide Angiotensin (Ang) II, while the second axis has emerged in the last decades and has the heptapeptide Ang-(1-7) as the main effector. Ang-(1-7) modulates several aspects of the heart hemostasis, such as cardiac rhythm, contractility, hypertrophy, fibrosis and coronary flow. In this chapter, we will summarize the current literature addressing the role of Ang-(1-7) and its receptor Mas in the heart function and structure, highlighting its beneficial activities under pathological conditions.

**Keywords:** Angiotensin-converting enzyme, Angiotensin-converting enzyme 2, Angiotensin-(1-7), Angiotensin II, Cardiac homeostasis, Cardiac remodeling, Cardiac rhythm, Heart, Receptor Mas, Renin-angiotensin system.

## INTRODUCTION

For many years the renin-angiotensin system (RAS) has been recognized as one of the most important modulator of the cardiovascular system [1, 2]. In fact, great

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clinical achievements reached by angiotensin converting enzyme (ACE) inhibitors and AT1 blockers underscore the relevance of this system [3, 4]. Moreover, recent advances expanded the understanding of the pathophysiological role of RAS. The discovery of novel components of this system and their function are associated with new pathways which opposes angiotensin (Ang) II effects, thereby opening new avenues to target the RAS in the treatment of cardiovascular diseases [5 - 8].

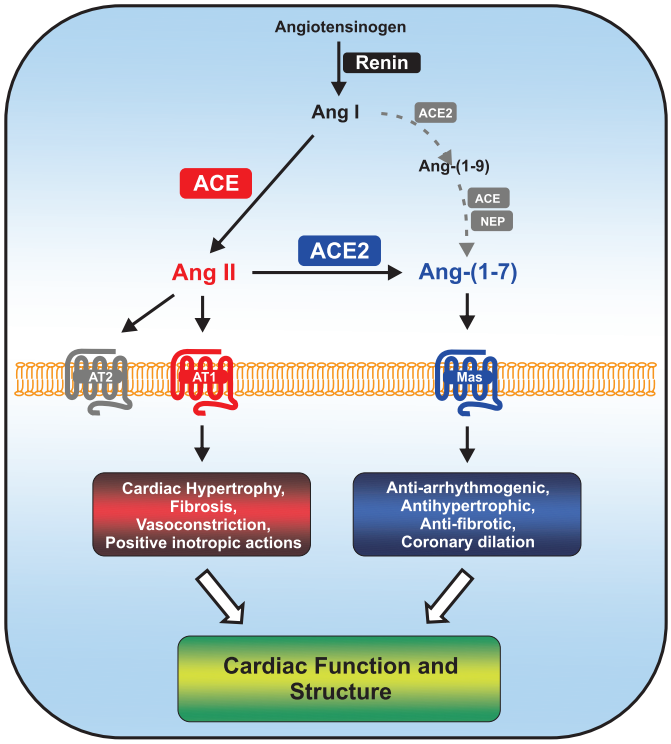


**Fig. (1). Schematic representation of the simplified view of the renin-angiotensin system (RAS) cascade**. The counterregulatory axes of the RAS are composed by ACE/Ang II/ AT1 and ACE2/Ang-(1–7)/Mas. ACE: angiotensin-converting enzyme; Ang: angiotensin; AT1: Ang II type 1 receptor; AT2: Ang II type 2 receptor; Mas: Ang- (1-7) receptor; NEP: neutral-endopeptidase 24.1.

One of the most important targets of RAS is the heart. This system modulates many features of the cardiac function and structure, such as cardiac rhythm, contractility, coronary perfusion, hypertrophy and fibrosis [9, 10]. Nowadays, the RAS concept consists of two opposite axes (Fig. **1**). One axis composed by ACE

forming Ang II, which activates AT1 receptor, and another composed by ACE2 forming Ang-(1-7), which activates Mas receptor [5, 8, 9]. In general terms, ACE/Ang II/AT1 axis evokes deleterious effects under pathological conditions. For instance, Ang II stimulation induces positive inotropic actions, cardiac hypertrophy and fibrosis [8, 11]. Contrarily, ACE2/Ang-(1-7)/Mas axis induces beneficial actions [5, 7]. In the heart, Ang-(1-7) produces antiarrhythmogenic, antihypertrophic and antifibrotic actions [10, 12 - 17].

In this chapter, we summarize the recent literature about the role of Ang-(1-7) and Mas in heart function, highlighting their activities under pathophysiological conditions.

## Renin Angiotensin-System Cascade

The RAS is a hormonal system which acts not only in an endocrine manner, but also as a paracrine and autocrine system [8]. Thus, in many organs, this system can be locally modulated and/or disturbed, influencing specific organ functions independent of the circulating system [8, 18]. The system cascade starts with the conversion of the precursor angiotensinogen to Ang I by the enzyme renin [8] (Fig. **1**) Sequentially, ACE cleaves Ang I and forms Ang II [1, 8, 19]. Ang II produces its effects *via* two distinct G-protein coupled receptors, named AT1 and AT2 [1, 20]. AT1 receptor is involved in most of the Ang II-mediated deleterious effects under pathological conditions, such as blood pressure increase, cardiac hypertrophy, fibrosis and arrhythmia [3, 7]. On its turn, AT2 appears to antagonize AT1 actions [4, 21]. Consistent evidences suggest that an augmented AT2 activation is one of the indirect mechanisms implied in the AT1 blockers actions [22, 23]. Moreover, it has been suggested that selective AT2 agonists may represent a potential therapeutic tool against cardiovascular diseases [23, 24]. However, the relevance of AT2 receptor in human pathophysiology still needs to be demonstrated [24].

In the last decades, the classical RAS, described as a linear cascade forming Ang II and promoting deleterious effects, has been expanded and became more complex [5, 7, 8]. A counter-regulatory arm of the RAS mediated by Ang-(1-7) has been established [5, 10]. Ang-(1-7) is a heptapeptide that acts mainly

# CHAPTER 6

# Essential Roles of Renin-Angiotensin System in the Kidney

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**Abstract:** The renin angiotensin system (RAS) has crucial action in the kidney; it is able to modulate intrarenal hemodynamics, glomerular filtration, and fluid and electrolytes homeostasis. Currently, six components of this system mediate their action through receptor(s). Four peptides, Ang II, Ang-(1-7), Ang III and Ang IV; and two enzymes, renin and prorenin through the renin and prorenin receptor, respectively. Angiotensin II (Ang II), the main peptide of RAS, through its type 1 receptor (AT1R) alters intrarenal hemodynamics, glomerular filtration, and fluid and electrolytes homeostasis readjusting blood pressure and body fluid balance. In the later functions, direct action of Ang II on the sodium and water transport was observed and related to diuretic/anti-diuretic and natriuretic/anti-natriuretic action depending on Ang II concentration. Angiotensin-(1-7) also influences the glomerular filtration rate but without changing the blood pressure. This heptapeptide showed biphasic direct action on tubular transport of sodium and water, but there is no consensus which receptor translates its tubular effect. Reports showed that Angiotensin III and Angiotensin IV could present natriuretic action; the pressor effect of both peptides is unclear. Direct action on tubular transport via renin and prorenin receptor has not yet been reported.

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**Keywords:** Angiotensin II (Ang II), Angiotensin III (Ang III), Angiotensin IV (Ang IV), Angiotensin-(1-7) (Ang-(1-7)), AT1R, AT2R, Blood pressure, Distal tubule, Diuresis, Loop of Henle, Kidney, MAS receptor, Natriuresis, Pro(renin) receptor, Proximal tubule, Renin-angiotensin system.

## INTRODUCTION

The kidney is a pivotal organ for regulation of blood pressure and water and electrolytes balance. These functions, regardless of exposure to environment fluctuation, must be maintained in restricted range of variation. Thus, the body orchestrates production and release of humoral factors that interact with the uniquely engineered cells within the kidney adjusting the process to maintain water and electrolyte balance. One of these factors is the renin-angiotensin system (RAS).

The fascinating history of RAS began in 1898 with the discovery of a substance isolated from the kidney, later named renin, capable of increasing blood pressure [1]. Since then, the system has been constantly unraveled by the discovery of other members and the existence of a local RAS in different organs.

Classically, under physiological conditions, angiotensin II (Ang II) formation initiates with the secretion of renin by the juxtaglomerular apparatus stimulated by decrease of blood pressure detected by the baroreceptors. Decrease of sodium levels is sensed by the macula densa, and responds with overactivity of sympathetic nervous system. Since its discovery, the RAS has been characterized in many organs. Despite of that, the action of Ang II is fascinating in the kidney, where the peptide is able to alter intrarenal hemodynamics, glomerular filtration, and fluid and electrolytes homeostasis readjusting blood pressure and body fluid balance. The critical role of RAS in renal physiology is supported by the observation that intrarenal Ang II blockade augmented glomerular filtration rate, sodium and water excretion, and renal plasma flow without any alteration of systemic pressor responses [2]. During many years, Ang II was used as synonym for RAS because it was believed that only Ang II possessed biological action. However, in the present scenario, there are strong physiological evidence supporting that smaller peptides of this system, such as angiotensin III (Ang III),

angiotensin IV (Ang IV), and angiotensin-(1-7) (Ang-(1-7)), also modulate their activities through receptor(s). It was observed that in the nephron these peptides participated in the fluid and electrolytes homeostasis by their action on sodium and water transporters.

## RAS – Bioactive Angiotensins and Receptors

The RAS has been constantly expanded by the discovery of other member including new enzymes, bioactive peptides and receptors, thus increasing its importance. The current versatile and complex system has six biological active components that translate its activity through receptor(s): four peptides (Ang II, Ang III, Ang IV, and Ang-(1-7)); and enzymes (renin and prorenin).

The mentioned peptides are formed by the action of an array of enzymes. It starts with renin hydrolyzing angiotensinogen (AGT) to form angiotensin I (Ang I) followed by the conversion of Ang I to Ang II by angiotensin-converting enzyme (ACE) [3]. Besides this classical pathway formed by two enzymatic steps, AGT can also be hydrolyzed by cathepsin D to form Ang I and in turn Ang I can be cleaved by chymase to form Ang II [4]. Additionally, Ang II can be produced directly from AGT in one enzymatic step by the following enzymes: either tonin, cathepsin G or chymotrypsin [5 - 7].

Furthermore, Ang I and Ang II can also be substrates to produce Ang-(1-7). Direct and indirect pathways are described to form Ang-(1-7) from Ang I. Direct formation of this heptapeptide occurs by the activity of thimet oligopeptidase (TOP), neprilysin (NEP) or prolyl endopeptidase (POP) on Ang I [8]. Indirect production of this heptapeptide occurs *via* two enzymatic steps: angiotensin-converting enzyme 2 (ACE2) cleaves Ang I to Angiotensin-1-9, and in turn angiotensin-1-9 is hydrolyzed by ACE to produce Ang-(1-7). Ang II can also be hydrolyzed to Ang-(1-7) by ACE2 [9, 10].

Likewise, Ang II can generate Ang III by the action of aminopeptidase A (APA), and Ang III in turn can be hydrolyzed into Ang IV by aminopeptidase N (APN) [11]. Ang II, Ang-(1-7), and Ang IV are further cleaved into smaller inactive fragments.

CHAPTER 7

# Renin-Angiotensin System Along the Nephron and its Role in Hypertension

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**Abstract:** There is increasing evidence regarding the pivotal role of the intrarenal renin angiotensin system (RAS) in the pathogenesis of hypertension and its independent role in the regulation of interstitial and intratubular fluids. The presence of high intrarenal angiotensin II (Ang II) levels and the localization of Ang II type 1 receptor (AT1R) in proximal and distal nephron segments suggested a physiological role for this receptor. In the collecting duct, luminal AT1R activation directly stimulates the activity of epithelial sodium channel (ENaC), contributing to enhance sodium reabsorption. Several reports have demonstrated the expression of angiotensinogen (AGT) and prorenin/renin in proximal tubules and collecting ducts, respectively. Both, tubular AGT and prorenin/renin are upregulated during Ang II-dependent hypertension despite the suppression of renin in juxtaglomerular cells. The (pro)renin receptor (PRR) is a new member of the RAS. PRR by binding renin or prorenin, enhances renin activity and activates the non active enzyme prorenin. The PRR and its soluble form are increased in the renal tissue and urine of Ang II hypertensive rats; however, the contribution of PRR to enhance renin activity in the distal nephron remains unclear. The presence of angiotensin converting enzyme (ACE), the enzyme that converts Ang I into Ang II in the nephron, along with the augmentation of intratubular AGT, renin and PRR may contribute to additional production of Ang II in the tubular lumen, which enhances sodium reabsorption and high blood pressure.

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**Keywords:** Angiotensin, Angiotensinogen, Angiotensin converting enzyme, Blood pressure, Collecting duct, Cyclic AMP, Cyclooxygenase-2, Hypertension, Intracellular pathways, Kidney, Prorenin, Protein kinase C, Renal medulla, Renin, Sodium, Transport, Urine.

## INTRODUCTION

The renin-angiotensin system (RAS) plays crucial role in controlling blood pressure and body volume. Beside the systemic RAS, which is mainly controlled by the production and release of renin from the kidneys into the circulation, locally acting RAS in abnormal or sustained activated conditions has been related to the pathogenesis and development of hypertension. A change in the paradigm occurred when it was discovered that proximal intratubular concentrations of Ang II were greater than plasma [1 - 3]. The presence of angiotensin (Ang) type 1 receptors (AT1R) along the luminal membrane of the proximal and distal nephron [4] also suggested a physiological role for the AT1R, despite the abundant expression of degrading enzymes in the proximal tubules. Evidence demonstrating the increased expression and secretion of AGT by the proximal tubule cells [5, 6] and prorenin and renin in the collecting duct in Ang II-dependent hypertension [7 - 10], further support the increased generation of local intratubular Ang II during intrarenal RAS activation [11, 12]. ACE is another key enzyme of the RAS that is also present in collecting duct cells [13]. This enzyme is augmented in Ang II-dependent hypertension [14, 15] and other models of kidney injury [16]. These findings, along with the demonstration of the PRR expression in the collecting ducts establish the basis for a physiologically important role of the tubular RAS.

### Intratubular Ang II and AT1R Along the Nephron

Ang I and Ang II concentrations in the proximal tubule are around 5-10 pmol/ml [17]. Which is similar to what have been found in interstitial fluid [18, 19]. these concentrations are elevated in Ang II-dependent hypertensive rats [20], 2K1C Goldblatt hypertensive rats [21] and TGR(mRen2) rats [22]. The AT1aR are essential for in the regulation of blood pressure and the increase in blood pressure in response to Ang II [23]. Furthermore, AT1aR knockout mice fail to develop hypertension in response to unilateral renal arterial constriction [24]. There is

evidence that Ang II concentrations are about 0.5 pmol/ml; this value increases during chronic Ang II infusions in mice [25, 26]. These responses can be blocked by AT1R antagonists in rats [27]. Ang II concentrations are sufficiently high to influence distal transport [28, 29]. AT1R are responsible for internalizing Ang II in proximal tubules, since AT1R blockade prevents this effect [30]. Internalized Ang II can migrate to the nucleus and exert transcriptional effects [31], including the activation of several profibrotic and proliferative genes [32]. Thus, intrarenal and intratubular Ang II may contribute to sustained de novo Ang II formation and profibrotic responses in the kidney.

## Increased Expression of AGT in Proximal Tubules in Hypertension

Angiotensinogen (AGT) mRNA has been found in proximal tubules [5, 33, 34]. This observation generated interest about its function. The augmentation of AGT mRNA and AGT protein in the urine during chronic Ang II infusion (Fig. **1**) [5, 6, 35, 36] are mediated by AT1R, since AT1R blockers prevented AGT upregulation [18]. The same phenomenon occurs *in vitro* using proximal tubule cell [36]. Also, augmentation of AGT in proximal tubules requires the presence of inflammatory factors like IL-6, and oxidative stress [37 - 39].

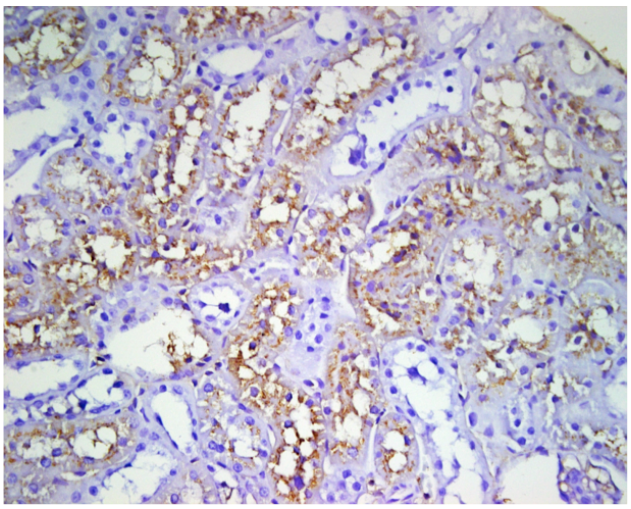

**Fig. (1).** Angiotensinogen (AGT) specific immunostaining in the proximal tubules of an Angiotensin II-infused rat kidney section (5 μm). Sheep anti-AGT antibody was used at a dilution of 1:60,000.

**CHAPTER 8**

# Tonin: An Overview and Functional Analysis

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**Abstract:** Tonin, a serinoproteinase, is capable of releasing angiotensin II by the hydrolysis of Phe[8]-His[9] bond in the angiotensinogen, angiotensin I and synthetic peptides corresponding to the N-terminal portion of angiotensinogen. Tonin is able to hydrolyze beta-lipotropic hormone releasing an opiate-like segment. It is capable of degrading adrenocorticotropic hormone, substance P but not bradykinin. Hydrolysis of Phe.His bond depends on a minimum sequence involving residues Ile.His.Pro.Phe.His.Leu. Tonin activity is present in human and rat tissues. In the rat, tonin is present in various tissues including kidney, brain and heart, and the activity levels vary according to age and sex being higher in males. Tonin is released into bloodstream and saliva after beta-adrenergic stimulation. There is evidence that tonin is involved in blood pressure control and participates in the hydromineral balance. Intracerebroventricular injection of tonin induces salt appetite and water intake and increases urinary volume and blood pressure. TGM(rTon), a transgenic mouse that expresses rat tonin, presents increased blood pressure. The levels of angiotensin II in the plasma are increased in TGM(rTon) and the AT1 receptors desensitized when compared to the wild type. A significant increase in the plasmatic and a decrease in urinary sodium were observed in TGM(rTon), suggesting alterations in the renal function. Induction of cardiac hypertrophy by isoproterenol injection in rats showed that tonin may be involved in this process. TGM(rTon) presents resistance to develop isoproterenol-induced hypertrophy. The molecular basis for the hypertrophy resistance

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is to be determined. These results lead us to conclude that tonin, an angiotensin II liberating enzyme may represent an alternative pathway with effects on the renal and cardiovascular systems.

**Keywords:** Angiotensin I, Angiotensin II, Angiotensinogen, Antinociceptive effect, Blood pressure, Converting enzyme, Heart, Hypertension, Hypertrophy resistance, Isoproterenol, Renin, Serine proteinase, Tonin, Transgenic mouse, Water intake.

## HISTORICAL REVIEW

The first citation of tonin was in the 70s, when a group of researchers, coordinated by Jacques Genest from the Clinical Research Institute of Montreal, Canada, described the presence of this enzyme in large amounts in the submandibular gland (SMG) of the rat. It was described as a new angiotensin-converting enzyme (ACE) and named as ß- converting enzyme [1]. Due to several differences in the properties of this enzyme, compared to renin and ACE, as specificity, catalytic activity, factors of activation and inhibition it was renamed as tonin.

While ACE is able to release angiotensin II (Ang II) by hydrolysis of the $Phe^{8}$-$His^{9}$ bond of angiotensin I (Ang I), tonin is also capable of releasing Ang II by hydrolysis of the $Phe^{8}$-$His^{9}$ bond from angiotensinogen (AG) [2] and from synthetic substrates homologues to the tetradecapeptide, a synthetic substrate corresponding to the 14 residues of N-terminal portion of angiotensinogen (AG(1-14)) [3, 4] as shown in the Fig. (**1**).

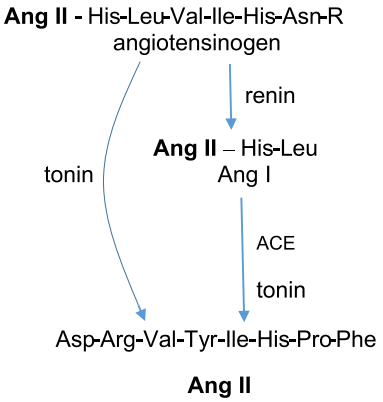


**Fig. (1).** Tonin-angiotensin II system.

Tonin activity was observed in various rat tissues such as the testes, liver, renal cortex and medulla, spleen, brain, lung, parotid, prostate, pancreas and submandibular gland where it is present at higher levels [3, 5 - 8]. Tonin, in the rat SMG, represents around 8% of the protein content. Woodley-Miller *et al.* (1987) [8] found that the levels of immunoreactive tonin in the SMG are approximately 10% of the protein content (105.27 ± 2.71 μg/mg protein) [8]. A similar result was found by Johansen *et al.* (1987) [7] who observed that the levels are of the order of 69 μg/mg of protein [7]. Tonin activity in this tissue varies according to age and sex being higher in males. The difference in sex becomes more evident after 60-80 days of age [9].

Orstavik and colleagues (1982) [10] investigated the cellular localization of tonin in the rat salivary glands by indirect immunofluorescence and observed that the enzyme is abundantly present in the granular tubular cells. In the striated duct cells tonin-specific staining was detected only as a thin luminal rim and no signal in any other structure of the gland [10]. Ledoux and collaborators (1982) [11] determined, by the indirect immunofluorescence and by the unlabeled antibody technique, the localization of tonin in salivary glands and kidney. The results were identical with both techniques. Immunoreactive tonin was localized in the cytoplasm of granular convoluted tubular cells and on the apical surface of striated duct cells and collecting duct cells. In the parotid and sublingual glands tonin was only found on the apical surface of striated duct and collecting duct cells. In the kidney, immunoreactive tonin was found associated with cells of the distal convoluted tubules [11]. Zacharatos *et al.* (1983) [12] also determined the levels of tonin in the rat SMG and tonin release. Their results show that a tonin-like substance appears, in the ducts, late, during gestation, where it is present at high levels in the adult. They also found that tonin amount in females increases during lactation; the granules are discharged during stimulation and are rapidly replaced [12]. Lis and collaborators (1977) [9] verified that hypophysectomy led to a sharp drop of tonin activity, whereas adrenalectomy, thyroidectomy, and gonadectomy have little effect. Treatment of hypophysectomized male rats with growth hormone and testosterone restored tonin activity. Treatment with prolactin, alone or in combination with testosterone had no effect in hypophysectomized animals [9]. Shih and collaborators (1986) [13] found that

# CHAPTER 9

# ACE Gene "Dosage" and Cardiovascular and Renal Disease

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**Abstract:** Complex phenotypes are those depending on a complex interaction between the genotype and environmental factors. Genetic studies on different human populations have shown a great variability in the genetic background due to the presence of genetic polymorphisms. The renin-angiotensin system (RAS) is closely involved in regulation of several physiological processes, such as body fluids homeostasis and blood pressure. Angiotensin-converting enzyme (ACE) plays a central role in the RAS functioning because this enzyme activates angiotensin II (Ang II) generation (vasoconstrictor) and inactivates bradykinin (vasodilator) simultaneously. ACE activity in plasma is variable in different families, and this variability is related with the genomic structure of the ACE gene dependent of a 287 base-pairs Insertion (presence) or Deletion (absence) in the DNA sequence. Variation in the number of copies of a single gene has been interpreted as representing different "dosage" of a specific gene product. Presence of D allele in ACE gene, mainly when in the DD polymorphism, has been associated with a higher generation of Ang II either in the systemic and in the local RAS leading to higher blood pressure levels and incidence of hypertension. These associations, however, have not been found in Caucasians, but are stronger in populations with Asiatic genetic background (where presence of the D allele is less frequent), such as the Amerindian populations. Individuals with DD polymorphism also have higher predisposition to development of accelerated atheroscl-

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erosis as compared with those presenting the DI and II ACE genes. However, these findings are not found in all studies because associations are generally weak and complex phenotypes, such as blood pressure levels and atherosclerosis development, depend on many genetic and environmental factors. Therefore, genetic polymorphisms may contribute to different phenotypes. Since the relationships between genes and the gene-environmental interactions are nonlinear, their practical use in medicine should be done with caution. However, they can give important insights in relation to different prevalence of diseases in populations with different genetic background.

**Keywords:** ACE polymorphism, Aldosterone, Angiotensinogen, Angiotensin converting enzyme, Angiotensin gene polymorphism, Angiotensin II, Atherosclerosis, Blood pressure, Bradykinin, Gene polymorphism, Hypertension, Potassium, Renin, Renin-angiotensin system, Sodium.

## INTRODUCTION

The renin-angiotensin system (RAS) exerts a key role for the homeostatic regulation of the circulation since the main effector peptide of this system, angiotensin II (Ang II), are closely involved in the homeostasis of the body fluids and blood pressure. Classical components of RAS are the renin, angiotensinogen, angiotensin I (Ang I) and Ang II peptides. All effects of Ang II are mediated by its interaction with two membrane-bound receptors, named AT1 and AT2 receptors [1]. Renin is an intracellular protein produced in cells of the juxtaglomerular apparatus as a proenzyme (pro-renin). Once secreted into the plasma, the active enzyme (renin) cleaves the decapeptide Ang I from its precursor peptide angiotensinogen. Angiotensinogen is produced in many tissues and cells, but the liver accounts for the most circulating Ang I level [2]. Beyond the systemic or endocrine RAS, experimental research led to the description of several local or tissues RAS in different organs, including the brain, heart, kidneys and blood vessels [3]. Since these local systems are complete, that is, they have all the components of the systemic RAS, the entire downstream cascade of the RAS peptides can also be generated as paracrine or local secretions. Therefore, Ang I produced in plasma as well as locally in organs and tissues can be transformed locally in the highly active peptide Ang II by the angiotensin-converting enzyme (ACE). Ang II in turn shows ubiquitous actions in almost all tissues and organs of the body, particularly in the heart, blood vessels and kidneys.

The systemic RAS was originally described as a key component related to the control of body volume because Ang II shows an important participation on $Na_+$ and $K_+$ metabolism thus affecting the concentration of these minerals in the body fluids. The volume of the extracellular space and the $Na_+$ concentration outside and inside cells affect the systemic vascular resistance and the cardiac output, thus interfering in the long-term blood pressure regulation [1, 3 - 5]. Moreover, the local production of the biologically active peptides of the RAS has shown that, beyond their systemic effects, they exert also local regulatory actions related to the structure and function of several organs, such as the heart and blood vessels. These findings were relevant to understand the participation of some components of the RAS, mainly Ang II, in several physiological processes, such as cardiac and vascular remodeling after myocardial infarction [6, 7], the long term glomerular damages determined by diabetes and hypertension [8] and other tissue injuries occurring in several other systemic diseases, such as in sarcoidosis [9]. Ang II is the most powerful endogenous vasoconstrictor. It also retains $Na_+$ in the kidneys, stimulates twist and salt appetite and induces endothelial dysfunction by reducing nitric oxide availability in resistance arterioles. Furthermore, Ang II, mainly when produced locally in the heart and in the kidneys also stimulates cell proliferation and growth and the deposition of new molecules in the extracellular matrix, contributing to alter tissue structure and function. The development of orally active ACE inhibitors and lately the AT1 receptor blockers opened new perspectives for the treatment of several highly prevalent diseases, such as hypertension, heart failure, diabetic nephropathy, and others. More recently, another pathophysiologic and therapeutic perspectives are being opened with the description of physiological actions of new components of the RAS, such as angiotensin-1-7 (Ang 1-7), angiotensin converting enzyme 2 (ACE 2), angiotensin 2-5 (Ang 2-5) and MAS-receptors [10, 11]. Since all these substances are produced in many different sites of the organism, these new players may represent other important therapeutic targets with potential importance for the treatment of several diseases. However, even considering these new components of the classical RAS, ACE still represents the key step to our actual understanding of the multiple physiological and pathological processes influenced by a deregulation of the systemic RAS. Therefore, our aim in this chapter is to give an overview of the ACE gene expression and how the different forms of organization of this enzyme

**CHAPTER 10**

# The ACE2 – Angiotensin (1-7)-Mas Receptor Axis in the Kidney

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**Abstract:** The renin-angiotensin-system (RAS) constitutes a key hormonal system in both the acute and long-term maintenance of blood pressure. Indeed, inappropriate regulation of this system is a major contributor to various pathologies that impact kidney function and blockade of the RAS either through attenuation of angiotensin converting enzyme (ACE) activity or angiotensin type 1 receptor ($AT_1R$)-dependent signaling has important therapeutic benefit. The RAS is no longer considered a monolithic peptidergic system whereby Ang II is the sole effector acting through the $AT_1R$, but a diverse system that reflects multiple peptides with distinct actions that are mediated by multiple receptors. The ACE-Ang II-$AT_1R$ axis is considered the classic pathway of the RAS that upon activation contributes to a number of peripheral and central mechanisms to effectively regulate blood pressure. However, the dysregulation of the $AT_1R$ axis may lead to sustained hypertension, inflammation, and an imbalance in redox mechanisms, cellular fibrosis, and other pathological responses. The ACE2-Ang-(1-7)-$AT_7R$ axis is now defined as the non-classical pathway of the RAS that in many situations exhibits actions that are opposite those of the Ang II-$AT_1R$ axis. The cellular actions of the Ang-(1-7)-$AT_7R$ axis primarily reflect the stimulation of both nitric oxide and prostaglandin pathways that would contribute to lower blood pressure and attenuation of inflammation, fibrosis and cellular injury. Moreover, the progression of various pathologies attributed to a stimulated Ang II-$AT_1R$ axis may, in part, reflect

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a reduced Ang-(1-7) tone. The current review assesses the non-classical axis of the RAS regarding the cellular and intracellular pathways for the expression and metabolism of Ang-(1-7), as well as the influence of the peptide in fetal-programmed hypertension.

**Keywords:** Angiotensin converting enzyme 2, Epithelial to mesenchymal transition, Fetal programming, Glucocorticoids, Natriuresis, Neprilysin, Peptide metabolism, Renin-angiotensin system, Thimet oligopeptidase.

## INTRODUCTION

The concept of peptide pathways that are distinct from the Ang II-$AT_1$ receptor axis has emerged over the last two decades that ostensibly originated from our demonstration of the endogenous expression [1] and biological actions of Ang-(1-7) (Fig. **1**) [2 - 4]. Over the course of this period, key evidence has established a novel Ang-(1-7) receptor (Mas protein) that is widely distributed in cardiovascular tissues, identification of $AT_7$/Mas receptor antagonists and agonists, a selective peptidase (ACE2) that directly hydrolyzes Ang II to Ang-(-7), the ACE-dependent metabolism of Ang-(1-7), and the elucidation of various signaling pathways that are activated by Ang-(1-7) [5, 6]. Apart from the identification of the biochemical components of the Ang-(1-7)-$AT_7$ receptor ($AT_7R$) axis expressed throughout the body, accumulating data on the novel functions of the peptide add to the physiological significance of this system [7, 8]. Ang-(1-7) was originally investigated on the peptide's ability to elicit vasorelaxation and to reduce blood pressure, particularly in lieu of the higher circulating Ang-(1-7) levels following treatment with ACE inhibitors; however, the functional effects of the Ang-(1-7) axis that are not directly related to blood pressure regulation should be considered as well [2, 9, 10]. The functional arc of Ang-(1-7) appears to beneficially influence numerous pathological conditions including diabetes, cancer, vascular and skeletal muscle dysfunction, wound healing and cognitive decline [3, 6, 11]. The span of Ang-(1-7)'s actions is not altogether unexpected given that the RAS, and in particular, the Ang-(1-7) axis is widely expressed in the body and this system may be important in the buffering of the Ang II-$AT_1R$ pathway [12]. In the current review, we critically assess recent studies on the ACE2-Ang-(1-7)-MasR axis as they pertain to the expression and metabolism of the peptide, the renal actions of the peptide and the emerging role

of Ang-(1-7) in fetal-programmed hypertension.

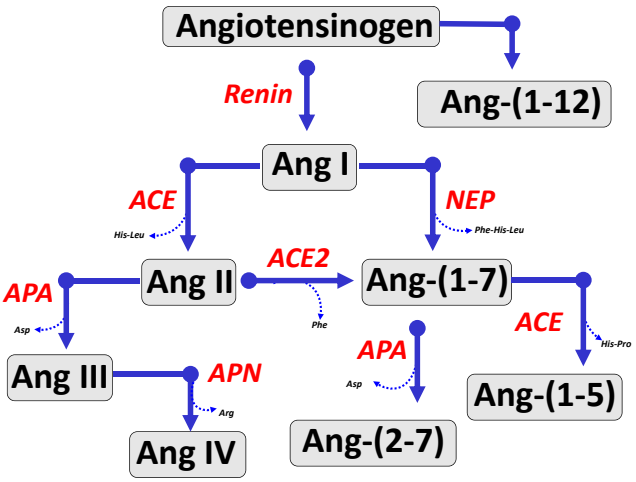


**Fig. (1). Cascade of the renin-angiotensin system elucidating the peptide pathways.** Angiotensinogen is initially hydrolyzed to angiotensin I (Ang I) by the aspartyl protease Renin. Angiotensin converting enzyme (ACE) hydrolyzes Ang I to Ang II. The carboxypeptidase ACE2 cleaves the $Pro^{7}$-$Phe^{8}$ bond of Ang II to Ang-(1-7). Alternatively, Ang I is hydrolyzed by endopeptidases (NEP - neprilysin, thimet oligopeptidases, prolyl oligopeptidase) to Ang-(1-7). Aminopeptidases including aminopeptidase A (APA) and aminopeptidase N (APN) process Ang II to Ang-(2-8) (Ang III) and Ang-(3-8) (Ang IV), respectively. ACE degrades Ang-(1-7) to the pentapeptide Ang-(1-5) and APA may contribute to the generation of Ang-(2-7). Angiotensinogen may undergo alternative processing by a non-renin dependent pathway to form Ang-(1-12) in rat.

## Renal Angiotensin-(1-7)

Angiotensinogen, a 453 amino acid protein, is the sole precursor for all angiotensins [13]. Angiotensinogen is the only known substrate for the aspartyl protease renin that forms Ang I and des-[Ang I]-angiotensinogen; circulating Ang I is rapidly hydrolyzed by the metallopeptidase ACE to the active peptide Ang II (Fig. **1**). Following ACE inhibitor treatment, circulating Ang I and Ang-(1-7) are markedly higher [14]. The higher the expression of Ang-(1-7) in the circulation provided the first initial the evidence that the peptide may impart a therapeutic benefit to ACE inhibitors and that the peptide's actions may be distinct from that of the Ang II-$AT_1$R axis. Importantly, these data suggested that Ang-(1-7) formation in the circulation is likely independent from the generation of Ang II by ACE [4]. Subsequent studies revealed that the endopeptidase neprilysin, originally

CHAPTER 11

# Renin Receptors in Cardiovascular and Renal Diseases

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**Abstract:** Local angiotensin generation depends on the uptake of circulating renin and/or its precursor prorenin. Such uptake may involve binding to a receptor. In the past 3 decades, three potential receptor candidates have been evaluated: a renin-binding protein, the mannose 6-phosphate/insulin-like growth factor II receptor, and the (pro)renin receptor. The most promising candidate seemed to be the (pro)renin receptor; however its affinity for renin and prorenin is several orders of magnitude above their actual levels in blood, raising doubt on whether this interaction truly occurs *in vivo*. In addition, conflicting *in-vivo* data have been reported regarding the putative (pro)renin receptor blocker, handle region peptide, while (pro)renin receptor knockout studies revealed lethal consequences that are (pro)renin-independent. The latter is most likely due to the fact that the (pro)renin receptor colocalizes with vacuolar $H^+$-ATPase, and possibly determines the stability of this vital enzyme. This chapter briefly discusses the various receptors, and ends with the conclusion that (pro)renin-(pro)renin receptor interaction, if it occurs *in vivo*, is limited to (pro)renin-synthesizing organs like the kidney.

**Keywords:** Angiotensin, Cyclo-oxygenase-2, ATP6AP2, Extracellular signal-regulated kinase, Glomerulosclerosis, Handle region peptide, Kidney, Mannose 6-phosphate/insulin-like growth factor II, Plasminogen-activator inhibitor-1, Prorenin, (Pro)renin receptor, Renin, Renin-binding protein, Transforming growth factor ß1, Vacuolar $H^+$-ATPase, Wnt signaling.

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## INTRODUCTION

Renin is formed from its precursor, prorenin, by cleavage of a 43-amino acid aminoterminal prosegment in the juxtaglomerular cells of the kidney. It is then either secreted into the circulation or stored in granules. Prorenin is also secreted from the kidney, without storage, and, unlike renin, originates simultaneously from several other organs, like the adrenal, eye and ovaries [1]. In general, plasma prorenin concentrations are much higher than plasma renin concentrations [2].

It is widely accepted that angiotensin (Ang) II, the end-product of the renin-angiotensin system (RAS), is generated not only in the circulation, but also locally. Given the exclusive synthesis of renin in the kidney, 2 scenarios have emerged to explain such tissue angiotensin generation: either renal renin accumulates at tissue sites (*e.g.*, through binding to a receptor), or prorenin (of renal or extrarenal origin) is activated at tissue sites. In support of renin uptake, tissue renin levels (expressed per g wet weight), *e.g.* in the heart, are too high to be explained based upon the amount of renin- and prorenin-containing extracellular fluid in tissue [3, 4]. In addition, studies in rat and pigs have shown that part of tissue renin is membrane-associated [3, 5]. Moreover, isolated perfused hearts of rats transgenic for human angiotensinogen release Ang I during renin infusion and this release continues after stopping the renin infusion [6].

These data strongly support the idea that circulating renin binds to a renin-binding protein/receptor, and that bound renin is catalytically active. It would be of even greater interest if such receptors would also bind prorenin, particularly if this would result in prorenin activation. Three potential renin/prorenin (here together denoted as (pro)renin) receptors have been described in the past 3 decades, a renin-binding protein, the mannose 6-phosphate/insulin-like growth factor II receptor, and the (pro)renin receptor ((P)RR). This chapter, based on a recent review [7], describes each of these receptors.

### Renin-Binding Protein

An intracellular renin-binding protein (RnBP) was discovered almost thirty years ago in humans, rats and pigs [8 - 10]. Binding of renin to this RnBP strongly inhibited its capacity to convert angiotensinogen to Ang I, suggesting a role as an

*in-vivo* renin inhibitor. Later it was discovered that RnBP is the enzyme N-acy--D-glucosamine 2-epimerase [11, 12]. *In-vitro* studies subsequently showed that RnBP not only inhibited renin, but that the reverse is also true [13]. Yet, mice lacking RnBP were normotensive and did not display any major alteration in their circulating or renal RAS [14]. Moreover, RnBP was located in the collecting duct and tubules, as opposed to the juxtaglomerular location of renin, and this situation did not change following renin induction in two kidney, one clip (2K1C) rats [15]. Taken together therefore, a role for the RnBP in the regulation of renin/prorenin and/or RAS activity in plasma or kidney seems unlikely.

## Mannose 6-Phosphate/Insulin-Like Growth Factor II Receptor

Lysosomal enzymes, following their synthesis in the endoplasmic reticulum, are targeted to their destination *via* a mechanism involving phosphomannosyl residues. These residues are recognized by so-called mannose 6-phosphate (M6P) receptors. The secretory granules of the juxtaglomerular cells resemble lysosomes, and renin transport in these cells involves the M6P receptor [16]. Extracellular lysosomal enzymes bind to cell surface M6P receptors and are subsequently internalized *via* clathrin-coated pits. They dissociate from the receptor in acidified endosomal compartments and are delivered to lysosomes.

M6P receptors are divided in two groups: the large M6P receptors are cation-independent and bind ligands independently of divalent cations; the small M6P receptors are cation-dependent and require divalent cations for optimal binding [17]. The large M6P receptor is identical to the insulin-like growth factor II (IGFII) receptor [18], and hence this receptor is now known as the M6P/IGFII receptor [19, 20].

The M6P/IGFII receptor binds phosphomannosylated (*i.e.*, M6P-containing) renin and prorenin. Such binding resulted in their internalization in a variety of cells, including cardiomyocytes, fibroblasts, vascular smooth muscle cells and endothelial cells [21 - 26]. Moreover, after internalization, prorenin was converted to renin by an as yet unidentified enzyme [23]. Thus, at least theoretically, M6P/IGFII receptors might contribute to the uptake of M6P-containing (pro)renin from the circulation at tissue sites, as well as the local activation of this prorenin.

**CHAPTER 12**

# Renal Physiology and Immune System: The Role of Renin-Angiotensin System, Nitric Oxide and Catecholamines

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**Abstract:** The renin-angiotensin system (RAS) affects both the innate and adaptive immune responses. Since hyperactive RAS has been associated with several diseases, the contribution of tissue RAS to the progression of immune and non-immune conditions has been considered in the recent years. It has a well-established role in fibrinogenesis, leukocyte infiltration, activity of T cells and has been shown to be chemotactic to macrophages, T cells, and natural killer cells. Nitric oxide (NO) is synthesized by many cell types involved in immunity and inflammation and plays an important role in hypotension and regulates the functional activity, growth and death of many immune and inflammatory cell types. Current evidences suggest that catecholamines (CAs) play a key role in activating and limiting inflammatory and immune reactions. In this chapter, we will discuss some aspects related to the role of these molecules in inflammatory process and immunologically mediated conditions.

**Keywords:** Catecholamines, HPA axis, Immune system, Nitric oxide, Renal physiology, Renin-angiotensin system, Sympathetic nervous system.

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## RENIN-ANGIOTENSIN SYSTEM

Since its discovery, the renin-angiotensin system (RAS) is recognized as a hormone system by which the kidney influences systemic cardiovascular regulation. Several studies on into molecular biology of RAS revealed that angiotensin II (Ang II), a potent vasoconstrictor, is synthesized not only in the circulation but also locally in tissues [1]. Accumulating data indicates that RAS participates in the induction and progression of several diseases, including immune and inflammatory conditions. Several clinical and experimental observations suggest that activated local RAS is involved in the pathogenesis of renal damage.

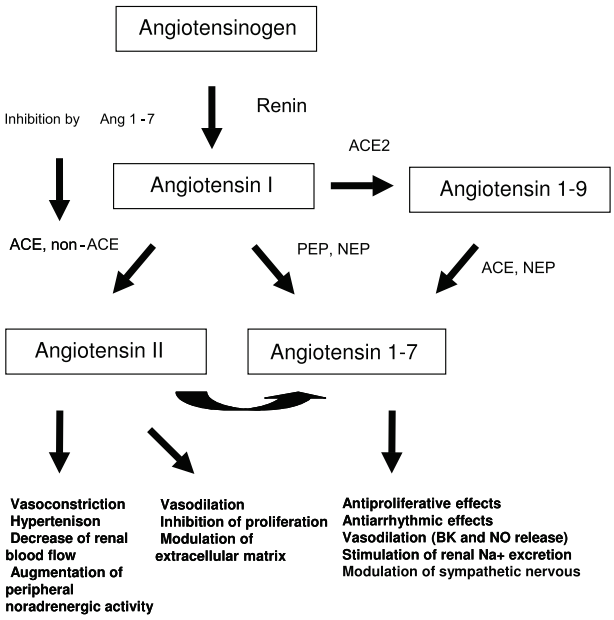


**Fig. (1). Pathways for the formation of biologically active angiotensin peptides.** Abbreviations: ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral endopeptidase; PEP, prolylendopetidase. (Adapted from Schindler *et al.* 2007 [62]).

The classical RAS has important molecules that participate in several aspects of physiology and pathophysiology including renin, angiotensin converting enzyme (ACE), angiotensins I (Ang I) and II and Ang II type 1 and 2 ($AT_1$ and $AT_2$) receptors. The identification of new angiotensins, such as angiotensin-(1-7) (Ang

1-7) and angiotensin IV (Ang IV), renin/prorenin receptors, Mas receptor and angiotensin-converting enzyme 2 (ACE2) increased the complexity of the system, suggesting that further investigations are necessary to better understand the role of RAS (Fig. **1**) [2, 3].

Circulating angiotensinogen (AGT) concentration is an important factor for the RAS tone being the precursor of the angiotensin peptides [4]. Apart from important role of renin in the generation of circulating angiotensin peptides, several other enzymes convert AGT to either Ang I or Ang II directly, and non-ACE enzymes may convert Ang I to Ang II, which is able to increase AGT expression *via* nuclear factor-kappa B (NF- κB) activation in hepatocytes and other tissues [5] particularly at inflammation sites.

Both local and circulating Ang II exert their activities through $AT_1$ receptor, a protein with a typical structure of 7-transmembrane G protein-coupled receptors [6], or $AT_2$ receptor, which is highly expressed in fetal stage, and is up-regulated in response to injury in the adult [7]. $AT_2$ receptor is known to stimulate the production of bradykinin, nitric oxide (NO) and cGMP, thus, mediating vasodilatation, cell differentiation, and apoptosis [8]. Stimulation of $AT_1$ receptors activates downstream effectors including phospholipase $A_2$, C and D. Activation of phospholipase C produces inositol-1,4,5- triphosphate ($IP_3$) and diacylglycerol inducing calcium efflux into the cytoplasm and a cascade of events causing contraction of vascular smooth muscle cell (VSMC). Diacylglycerol is an effector of the RAS, mitogen activated protein kinase (MAPK) and MEK pathways. $AT_1$ receptor also activates kinases such as PKC and MAPK including ERK1/2, p38MAPK and c-Jun NH2-terminal kinase that are implicated in inflammation and hypertrophy [9].

Recently, new angiotensin receptors were described including Mas and angiotensin $AT_4$ receptors, which are able to bind Ang 1-7 and Ang IV peptides, respectively [10, 11]. The $AT_4$ receptor has been implicated in sodium transport in proximal tubules [12] and promotes thrombosis and fibrosis *via* induction of plasminogen activator inhibitor-I shown in both endothelial and vascular smooth muscle cells [13]. Mas receptor induces natriuresis and diuresis, [14] and mediates the effect of Ang 1–7 on prostaglandins and NO release [10].

**CHAPTER 13**

# Cardiovascular and Behavioral Effects of Emotional Stress: The Participation of Renin-Angiotensin System

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**Abstract:** Stress reaction aims to preserve body homeostasis. During stress, the renin-angiotensin system (RAS) increases the effects of the sympathetic nervous system and the hypothalamic-pituitary- adrenal (HPA) axis so that the organism will adapt to stressors. However, during chronic stress, these responses are sustained, adaptation does not occur and pathologies might be developed. In this chapter, we address evidences towards the correlation between RAS and the negative effects that stress has on behavior and cardiovascular system. Both circulating and local RAS are involved in stress – related hypertension. Among the mechanisms involved, it has been demonstrated that RAS activates HPA axis, catecholamines upregulate the renin synthesis and RAS activity in the peripheral organs, and vascular effects of angiotensin II (Ang II) impair the balance between vascular relaxing and contracting agents. Considering cardiac hypertrophy, hyperactivity of RAS and SNS promotes growth factors increase, fibroblast proliferation and collagen synthesis in the myocardium. Ang

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II triggers the production of superoxide and acts as a pro-inflammatory molecule in the blood vessels, and, consequently, might initiate and aggravate the process of atherosclerosis. Considering emotional and cognitive effects of stress, there is a link between depression and dysfunction of stress response systems involving the activation of RAS. In treating hypertension, patients having depression might also benefit from RAS blockers as these agents are known to have antidepressant effects. Inhibitors of ACE have been reported to improve cognition and memory, *via* Ang II conversion to Ang IV in the central nervous system. Stress-induced brain RAS has also been suggested as having a role in Alzheimer's disease.

**Keywords:** Anxiety, Atherosclerosis, Cognition, Depression, Emotion, Stress, Hypertension, Hypothalamic-pituitary-adrenal axis, Memory, Renin-angiotensin system, Sympathoadrenal axis.

## INTRODUCTION

According to the modern concept of stress, our regulatory physiological systems fluctuate within operating ranges to match environmental challenges. Such condition is known as allostasis. The stress is initiated by sensory pathways, which trigger adaptive responses in the central nervous system and, subsequently, hormonal secretion by the hypothalamic-pituitary-adrenal and sympathoadrenal axes [1].

During stress, the circulating and local renin-angiotensin systems (RAS) are also stimulated, increasing plasma renin activity [2], concentration of angiotensin II (Ang II) in blood [3], and expression of Ang II type 1 receptors (AT1R) [3]. Most organs are capable of producing Ang II, and many tissues express Ang II receptors. Therefore, Ang II can be considered a multitask peptide that regulates many systemic functions, including those involved in stress reactions [1].

Activates the peripheral and central responses to preserve body homeostasis [4]. These responses depend on genetic factors, underlying diseases, and stressors' intensity and frequency. Such conditions might cause hyperactivity in the hypothalamic-pituitary-adrenal and sympathoadrenal axes and RAS, resulting in a variety of stress-related diseases, such as hypertension, coronary heart disease, pathological cardiac hypertrophy, atherosclerosis, depression, anxiety [3, 5, 6].

Clinical and experimental data show that RAS is involved in stress-related

diseases [2]. In animals and humans, the inhibition or blockade of the $AT_1R$ has been reported to have positive effects on the treatment of atherosclerosis and hypertension-induced organ injury [7, 8], improves hypertensive patients quality of life [9], and decreases anxiety and depression in diabetic patients [10]. In addition, $AT_1R$ receptors antagonists and angiotensin-converting enzyme (ACE) inhibitors improve mood and well-being [11, 12], increase the efficacy of antidepressants [13] and decrease anxiety and depression in normotensive subjects [14].

Moreover, the actions of aldosterone in the brain and other organs *via* mineralocorticoid receptors seem to induce deleterious effects like myocardial necrosis and fibrosis, endothelial dysfunction, catecholamine release and cardiac arrhythmias on depressive patients at risk of cardiovascular dysfunction [15 - 17]. Besides increasing the renin-angiotensin activity, chronic mild and unpredictable stress can also induce anhedonia, hypothalamic-pituitary-adrenal (HPA) axis dysfunction and activation of proinflammatory cytokines in laboratory animals [18].

## Circulating and Local Renin-Angiotensin System

Circulating and local RAS are stimulated during acute and chronic stress. During stress, the renin-angiotensin system (RAS) increases the effects of the sympathetic nervous system (SNS) and the hypothalamic-pituitary- adrenal to maintain the homeostasis. In this way, the brain RAS stimulates the SNS, and consequently there is an increase in the secretion of renin and epinephrine in the kidney and blood [19], peripheral and central Ang II concentrations, AT1R expression in the HPA axis [2]. The renin released from kidney circulates in plasma and cleaves angiotensinogen to form angiotensin I. In the pulmonary vasculature, angiotensin I is converted to angiotensin II, which is transported by blood and activates specific angiotensin II receptors in the peripheral tissues. Also, the function of the circulating RAS is carry renin and angiotensinogen to the tissues, which produces locally the most angiotensin II by action of ACE. In the brain, stressful events can upregulates the $AT_1R$ expression in the hypothalamic paraventricular nucleus (PVN) and anterior pituitary [20], and these effects induces synthesis and releasing of corticotrophin hormone (CRH), which stimulates releasing of

# CHAPTER 14

# Exercise and Renin Angiotensin System

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**Abstract:** The number of people affected by cardiovascular disease is increasing in the Western world, and it is partially explained by urbanization, industrialization, work condition and inadequate diet. Cardiovascular disease killed nearly 17 million people in 2011, and of these, 7 million died of ischaemic heart disease and 6.2 million from stroke [1]. This chapter will focus on the contribution of regular physical exercise for prevention and reversal of cardiovascular disease, and on the role of renin angiotensin system (RAS) in these processes. Experimental and clinical studies show that exercise training is efficient to block RAS overactivity, thus preventing and/or reversing cardiac dysfunction and deleterious remodeling of the heart in pathological conditions such as hypertension, myocardial infarction, heart failure and obesity. Indeed, studies show that the association between RAS inhibition and exercise training can bring major benefits to individuals with heart disease (especially those with mild, moderate or severe heart failure) and also cardiometabolic alterations, acting synergically as a successful combination to the recovery and maintenance of health of these patients.

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**Keywords:** ACE polymorphism, Angiotensin receptors, Barorreflex, Cardiovascular disease, Central nervous system, Endothelium, Exercise, Hypertension, Hypothalamus, Nephropathy, Obesity, Oxidative stress, Renin angiotensin system, $VO_2$.

## INTRODUCTION

The number of people affected with cardiovascular and renal disease is increasing in the Western world, and it is partially explained by urbanization, industrialization, work condition and inadequate diet. Cardiovascular disease killed nearly 17 million people in 2011, and of these, 7 million died of ischaemic heart disease and 6.2 million from stroke [1]. This chapter will focus on the contribution of regular physical exercise to prevention and reversal of cardiovascular disease, and on the role of renin angiotensin system (RAS) on these processes.

### Role of RAS on Central and Peripheral Responses to Exercise

#### *Brain*

After the original demonstration that angiotensin II (Ang II)increased blood pressure by a direct action on the central nervous system [2] it became clear that this peptide had several central effects [3]. Subsequent studies demonstrated that angiotensin receptors were located in circumventricular organs as well in areas inside the blood-brain barrier [3, 4]. Since Ang II does not cross blood-brain barrier, it seemed unlikely that effects, other than those mediated by the circumventricular organs, were due to the circulating peptide. These observations raised the possibility that Ang II could be synthesized within the central nervous system. Indeed in the seventies, Ganten *et al.* [5] using refined biochemical techniques, highly sensitive radioimmunoassay and immunohistochemical methods, showed that the components required for the generation of Ang II are present within the brain [5, 6]. The brain was the first extrarenal tissue in which all components of the RAS were systematically demonstrated and confirmed by the local expression of its mRNA message [7]. These findings led to new hypothesis and functional concept of a ‘local’ RAS actions based on brain

synthesis of Ang II and other biologically active angiotensins.

Renin activity in the brain was first reported by Ganten *et al.* [5] and confirmed by subsequent molecular biology techniques and transgenic and knock-out animal models [7]. Levels of renin expression in the brain are low, but renin activity is high and present in many brain areas as the hypothalamus, pituitary, pineal gland of different species, although decreasing with ageing [7]. It has been suggested that renin might be taken up from circulation [8]. Indeed a high prorenin receptor (it binds the inactive precursor prorenin and renders it active) expression was detected in the brain [9]. mRNA and protein for the angiotensin converting enzyme isoforms (ACE and ACE2) are largely expressed in several brain areas, with high levels found in the choroid plexus, circumventricular organs, caudate putamen, brain stem, cerebellum, hippocampus and intermediate levels in the hypothalamus, thalamus, pituitary and basal ganglia [7, 10]. Other angiotensin-forming enzymes as tonin, cathepsin, chymase, aminopeptidadese A and N were also present in the brain [11].

Besides the enzymatic machinery, the precursor - angiotensinogen (Aogen) - is highly expressed in the brain. Aogen mRNA is mainly localized in cells stained for glial fibrillary acid protein, suggesting the glia as the main source for Aogen synthesis in the brain, although it was also found in pure neuronal cultures, indicating the possibility of intraneuronal synthesis as well. Following synthesis, Aogen is secreted into the extracellular since high concentration of the precursor was detected in the cerebrospinal fluid in dogs and rats [7]. It is also possible that part of the intraneuronal Aogen content could be taken from the cerebrospinal fluid pool. Aogen mRNA and Aogen immunoreactivity were found in the basal ganglia, preoptic region, hypothalamus, midbrain, pons, medulla oblongata and cerebellum, the highest levels being observed in the hypothalamus and brain stem [12].

In the presence of Aogen and biosynthetic enzymes all components of angiotensin family (Ang I, Ang II, Ang (1-7), Ang III, Ang IV) are synthesized in the brain. Highest levels of Ang II (the active peptide of the vasoconstrictor, trophic, pro-fibrotic and pro-inflammatory ACE-Ang II-$AT_1R$ axis of RAS) were found in the paraventricular and supraoptic nuclei of the hypothalamus, pituitary and cortex,

**CHAPTER 15**

# Renin-Angiotensin System in Animal Models of Diabetes and Hypertension

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**Abstract:** Translational research has currently become the focus of many ongoing studies. The use of inbred animals represents an advantage to human studies to a certain point because of the elimination of several uncontrollable variables. However, we need to consider the limitation of such approach in the translational potential to humans. Within the field of hypertension and diabetes research, animal models are irreplaceable research tools providing insight into human diseases. These two diseases independently predispose to renal and cardiovascular complications but, more importantly, can aggravate each other. Although some of the best models for diabetes and hypertension are spontaneous, the use of transgenic models provides a better control of the pathological mechanisms to be studied and the combination of the available tools will most likely make a difference in understanding how the RAS is modulated in diabetes and hypertension. Although these animals add a few layers of complexity and are sometimes closer to the human pathological mechanism, there are still many challenges to overcome.

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**Keywords:** Animal models, Basic research, Diabetes, Hypertension, *In vivo* studies, Knock out, Mice, Rats, RAS, Renin-angiotensin system, SHR, Spontaneously hypertensive rats, Transgenic, Translational medicine, Type 1 diabetes, Type 2 diabetes.

## INTRODUCTION

Diabetes and hypertension are comorbid diseases that independently predispose to renal and cardiovascular complications. Both diseases aggravate each other in terms of subsequent renal and cardiovascular complications [1, 2]. Achieving proper blood pressure control is key in hypertension, although even with a vast choice of antihypertensive drugs it remains a challenge, especially with concomitant diabetes. The metabolic side effects of the most used treatments is an important issue and whether blood pressure reduction *per se* is the most important target of antihypertensive treatment or if there are specific benefits associated with certain antihypertensive regimens, in particular inhibitors of the renin-angiotensin system (RAS) is still controversial. In general, agents that at the same time suspend the RAS and enhance the aldosterone pathway appear to be notably useful in diabetic renal and cardiovascular disease [1, 3, 4]. Most patients with diabetes and hypertension will require more than 2 or 3 antihypertensive drugs as recommended in the latest US, European, and World Health Organization (WHO) guidelines, but there is still no consensus on the most beneficial combination [1].

This chapter describes the use of animal models of diabetes and hypertension in the search for better diagnostic and therapeutical tools.

## ANIMAL MODELS IN RAS RESEARCH

Animal models have provided massive data in the growing complexity of the RAS. Ever since Tigerstedt and Bergmann discovered renin in 1898, RAS has been extremely important in the regulation of blood pressure (BP) and volume homeostasis. Subsequently, an aberrant activity of the RAS has been suggested as a pathogenic factor and/or a complicating factor in the context of hypertension and diabetes [5]. Through the course of research history, the use of relevant models to mimic human diseases is certain to have contributed with vast information by allowing us to better understand the cause and progression of the disease and also by giving insights of potential therapeutic interventions [6]. On

the other hand their value in predicting the efficiency of treatment strategies in clinical trials is still controversial [7, 8]. Animal models have been used for several reasons. Firstly, animal studies provide unique insights into the pathophysiology and causes of disease and usually unveil unknown targets for directed treatments. Secondly, animal studies provide a genetic manipulation rarely achievable in humans and finally, if preliminary testing on animals show that they do not have a clinical importance, the human testing phase may not be necessary [9].

## Animal Models of Hypertension and RAS

The use of relevant models to mimic human cardiovascular disease provides useful information by giving us an overview of the cause and progression of the disease status and also by revealing potential therapeutic interventions, which would not be possible in the context of clinical research [6]. Many animal models have been extensively used in the study of hypertension but it is important to point out that each one has a particular role in the development of the disease [6].

### *Genetic Models*

#### ***Spontaneously Hypertensive Rat***

Spontaneously hypertensive rats (SHRs) originates from Wistar and non-hypertensive Wistar–Kyoto (WKY) rats inbreeding [10]. These rats spontaneously become hypertense at about 4 to 6 weeks of age but environmental factors can affect the development of hypertension [11]. In 1999, Zicha *et al.* stated that >90% of the papers available in this field of research used this model [11]. In the early stages of hypertension, SHRs have normal total peripheral resistance and increased cardiac output. By the time the cardiac output returns to normal hypertrophied blood vessels promote an increase in peripheral resistance, establishing hypertension [12]. Between 6 and 24 months of age, as the hypertension advances, the SHR develops structural alterations in the heart, which are associated with progressive cardiac hypertrophy [6, 13]. Later, using the SHR background, Okamoto *et al.* developed a stroke-prone spontaneously hypertensive rat (SHRSP) [14].

CHAPTER 16

# Quantification of Angiotensins: Is there a Consensus in the Literature?

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**Abstract:** The renin angiotensin system (RAS) is an essential regulator of renal and cardiovascular function. The components of this system are present in the circulation, organs, tissues and various cell types; and their syntheses in the organs are independent of the circulation. Understanding how the circulatory and organ RAS interact in the maintenance of the body function is knowledge that is not only key, but absolutely essential in helping us to unravel the implications of the RAS in a disease process. In this regard, this chapter aims to verify whether or not there is a consensus in the literature for the reported quantities and concentrations of Angiotensin I (Ang I), Angiotensin II (Ang II) and Angiotensin 1-7 (Ang1-7) in normal physiological states in mice, rats and humans. Because of the various methods for quantification of angiotensins, there is not presently a clear consensus for the absolute quantities of these peptides in plasma and the different tissues.

**Keywords:** Angiotensins, Angiotensin converting enzyme (ACE), Angiotensin converting enzyme 2 (ACE2), Angiotensin I, Angiotensin II, Angiotensin 1-7,

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Consensus, Enzyme-linked immunosorbent assay (ELISA), Extraction of angiotensins, High performance liquid chromatography (HPLC), Liquid chromatography (LC) mass spectrometry (MS), Plasma angiotensins, Protease inhibitors, Radioimmunoassay (RIA), Tissue angiotensins.

## INTRODUCTION

The renin angiotensin system (RAS) is an essential regulator of arterial blood pressure, and cardiovascular and renal functions. The first observation as a blood pressure regulator was in 1898 when Tigerstedt and Bergman discovered the rate-limiting enzyme renin [1]. The initial description of this system was related to the discovery of the circulating RAS. The circulating RAS starts with renin released from the kidney, which cleaves angiotensinogen (Agt) to form angiotensin I (Ang I). The decapeptide Ang I ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-$His^{9}$-$Leu^{10}$-COOH is converted by angiotensin converting enzyme (ACE) to form angiotensin II (Ang II: $Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-COOH) that is the first and major active angiotensin peptide (Ang). Since then, many studies have described other angiotensin peptides (Angs) and enzymes in tissues and fluids. The Angs can be biologically active or inactive, within the active peptides are: Ang III (Ang 2-8: $Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-COOH), Ang IV (Ang 3-8: $Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-COOH), Ang 1-9 ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-$His^{9}$-COOH), Ang 1-7 ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-COOH), Ang 3-4, Ang A and Alamandine ($Ala^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-COOH). The inactive angiotensins are Ang 1-12 ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-$His^{9}$-$Leu^{10}$-$Leu^{11}$-$Val^{12}$-COOH), Ang 1-5 ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-COOH), Ang 1-4 ($Asp^{1}$-$Arg^{2}$-$Val^{3}$-$Tyr^{4}$-COOH), Ang 2-7 ($Arg^{2}$-$Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-COOH), Ang 3-7 ($Val^{3}$-$Tyr^{4}$-$Ile^{5}$-$His^{6}$-$Pro^{7}$-COOH), Ang 5-8 ($Ile^{5}$-$His^{6}$-$Pro^{7}$-$Phe^{8}$-COOH), and Ang 5-7 ($Ile^{5}$-$His^{6}$-$Pro^{7}$-COOH). All the Angs are formed by the action of a diverse range of enzymes such as aspartyl, serine, thiol, and metallo-proteases among other enzymes (Fig. **1**) [2 - 6].

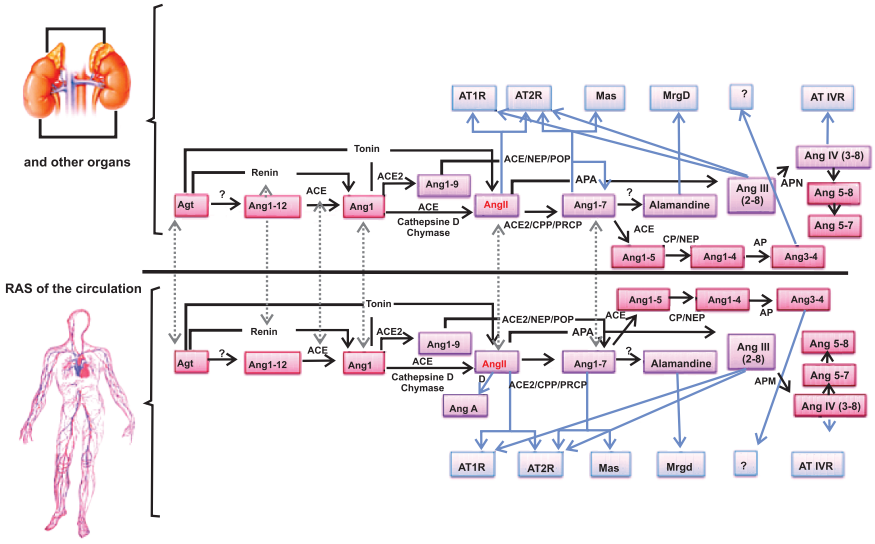


**Fig. (1). Renin-angiotensin system cascade.** Active peptides: Ang1-9, Ang II, Ang A, Ang1-7; Alamandine; and inactive peptides: Ang1-12, Ang I, Ang 1-5, Ang1-4, Ang 3-4, Ang5-8, Ang5-7. Protein: Agt: angiotensinogen Enzymes: renin, angiotensin I-converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2) -, AP – aminopeptidase (AP), aminopeptidase A (APA), aminopeptidase N (APN), –carboxypeptidase (CP), - endopeptidase (EP), –carboxypeptidase P (CPP), prolyl carboxypeptidase (PRCP), neprilysin (NEP), prolyl oligopeptidase (PO). Receptors: AT1, AT2, Mas, MrgD, ATIVR.

Two Angs have been extensively studied, Ang II and Ang 1-7, that are present in the circulation, organs, tissues and cell types, and have endocrine, paracrine, autocrine and intracrine functions. Pathophysiologically, Ang II has been implicated in hypertension, inflammation, fibrosis, and proteinuria. On the other hand, Ang 1-7 has been shown to act as an anti-hypertensive, anti-inflammatory and anti-fibrosis peptide [7 - 12]. However, these phenotypes for Ang II and Ang 1-7 are highly generalized concepts. Further dissection of the function of Angs is necessary, so that we can better understand their pathophysiology. Indeed, Ang II acts on vascular smooth muscle cells (VSMC) causing vasoconstriction while acting on the endothelial cells to elicit vasodilation by the same angiotensin type 1 receptor (AT1R) [13]. This specific study demonstrated that what is true for one tissue may not be for others. Can Ang 1-7 behave in the same pattern as Ang II,

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