23 Hormones of the Kidney

Masashi Mukoyama, MD, PhD
and Kazuwa Nakao, MD, PhD

CONTENTS

INTRODUCTION
COMPONENTS OF RAS
PATHOPHYSIOLOGY OF RAS
COMPONENTS OF NATRIURETIC PEPTIDE SYSTEM
PATHOPHYSIOLOGY OF NATRIURETIC PEPTIDE SYSTEM
KALLIKREIN-KININ SYSTEM
ADRENOMEDULLIN AND ENDOTHELINS
ERYTHROPOIETIN

1. INTRODUCTION

The kidney plays an essential role in the maintenance of life in higher organisms, not only through regulating the blood pressure and body fluid homeostasis and clearing the wastes, but also by acting as a major endocrine organ. The kidney secretes (1) renin, a key enzyme of the renin-angiotensin system (RAS) that leads to the production of a potent pressor hormone angiotensin, and produces the following hormones and humoral factors: (2) kallikreins, a group of serine proteases that act on blood proteins to produce a vasorelaxing peptide bradykinin; (3) erythropoietin (EPO), a peptide hormone essential for red blood cell (RBC) formation by the bone marrow; and (4) 1,25-$(OH)_2$ vitamin D_3, the active form of vitamin D essential for calcium homeostasis, which is produced by the proximal tubule cells via the enzyme 1α-hydroxylase.

In addition, the kidney serves as an important endocrine target organ for a number of hormones, thereby controlling the extracellular fluid volume, electrolyte balance, acid-base balance, and blood pressure. Among these hormones, angiotensin and aldosterone, both key products in the axis of the RAS, and the natriuretic peptide family, comprising potent diuretic and vasorelaxing hormones secreted from the heart, are regarded as the most important players. Furthermore, the kidney is a major organ for the production and action of various "local hormones," or autocrine/paracrine regulators, such as prostaglandins (PGs), adrenomedullin (AM), and endothelins (ETs). These factors are thought to provide an integrated mechanism for the fine-tuning of microcirculation, solute transport, and various cellular functions in the kidney.

This chapter discusses the roles of the hormones that are produced or have major actions in the kidney, focusing on their functional relationships and implications in physiologic and pathophysiologic conditions. The roles of vitamin D and the kidney in calcium homeostasis as well as the prostanoid system are detailed in other chapters.

2. COMPONENTS OF RAS

The RAS is a proteolytic cascade, composed of a group of proteins and peptides that ultimately produce

From: *Endocrinology: Basic and Clinical Principles, Second Edition*
(S. Melmed and P. M. Conn, eds.)

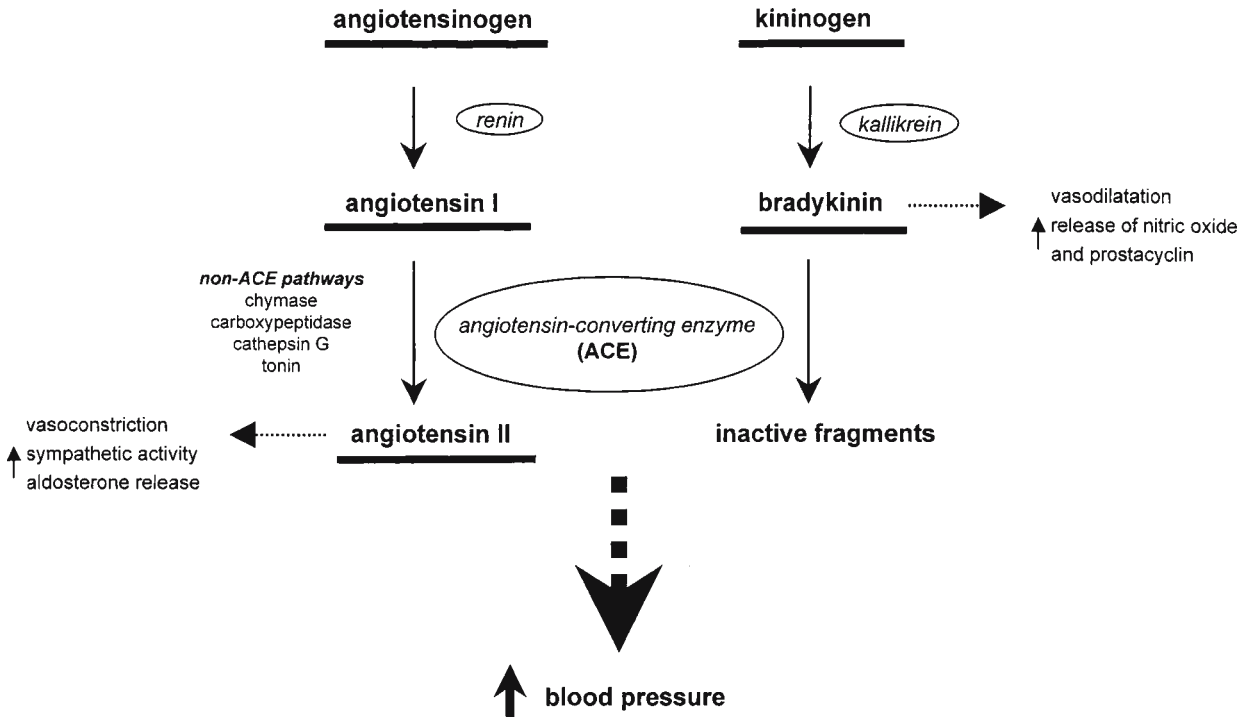

Fig. 1. Biosynthetic cascade of the RAS.

a potent octapeptide, angiotensin II (Ang II) (Fig. 1). Classically, the cascade starts with the proteolytic enzyme renin, released from the juxtaglomerular cells of the kidney (Fig. 2). Renin acts on a liver-derived plasma α_2-globulin, angiotensinogen, to cleave the N-terminal decapeptide sequence and produce Ang I. Subsequently, the C-terminal dipeptide His^9-Leu^{10} is cleaved from Ang I to form Ang II, by angiotensin-converting enzyme (ACE), primarily within the pulmonary circulation. Ang II then acts on various target tissues, resulting in vasoconstriction in the resistance vessels, increased intraglomerular pressure and sodium reabsorption in the kidney, and stimulated biosynthesis and secretion of the mineralocorticoid aldosterone in the adrenal cortex. In addition to such a well-described circulating hormonal RAS, it is now recognized that there are components of the RAS that allow local synthesis of Ang II. Such a system is referred to as the tissue RAS and may serve local actions of Ang II in an autocrine/paracrine manner.

The biologic actions of the RAS are mediated by Ang II via at least two types of the specific membrane receptors: angiotensin type 1 (AT_1) and type 2 (AT_2) receptors. With the availability of pharmacologic and genetic tools that inhibit ACE and block Ang II receptors, as well as data from a number of clinical studies, it is now revealed that the RAS plays a critical role in

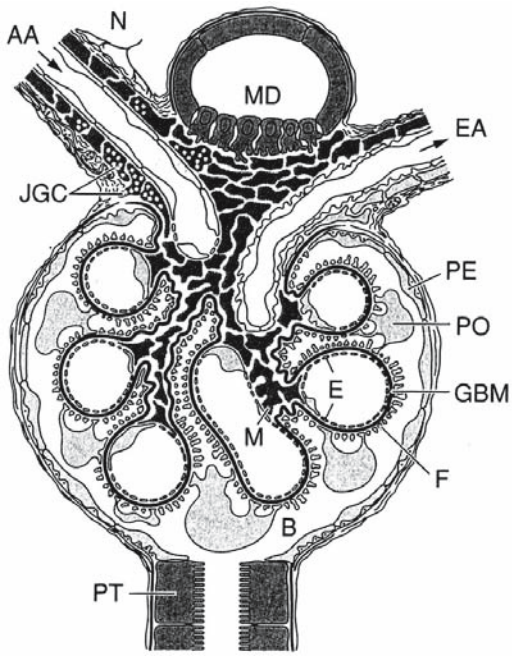

Fig. 2. Juxtaglomerular apparatus. MD = macula densa; JGC = juxtaglomerular cells; AA = afferent arteriole; EA = efferent arteriole; N = sympathetic nerve terminal; M = mesangium; GBM = glomerular basement membrane; E = endothelium; PO = podocyte; F = foot process; PE = parietal epithelium; B = Bowman's space; PT = proximal tubule.

maintaining cardiovascular and renal homeostasis physiologically, and in developing disease states pathologically. Accordingly, interruption of the RAS has become an increasingly important therapeutic strategy for various cardiovascular disorders such as hypertension, heart failure, and renal disease.

2.1. Renin

2.1.1. Synthesis and Biochemistry of Renin

More than a century ago, Tigerstedt and Bergman found a potent pressor activity in rabbit kidney extract. They named a putative substance secreted from the kidney *renin*, after the Latin word *ren* (kidney). Forty years later, Braun-Menéndez et al. and Page et al. showed that this material was of a protease nature, acting on a plasma protein to release another pressor substance, which was later named angiotensin.

Renin (EC 3.4.25.15) is classified as an aspartyl protease and synthesized as a preproprotein. Renin is stored and secreted from the renal juxtaglomerular cells located in the wall of the afferent arteriole, which is contiguous with the macula densa portion of the same nephron (Fig. 2). The human renin gene, spanning 12 kb, is located on chromosome 1 (1q32-1q42) and consists of 10 exons and 9 introns. Hormonal-responsive elements in the 5´-flanking region of the renin gene include consensus elements for cyclic adenosine monophosphate (cAMP) and steroids (glucocorticoid, estrogen, and progesterone). In certain strains of the mouse, there are two renin genes (*Ren-1* and *Ren-2*), both located on chromosome 1, and in the rat, the renin gene is located on chromosome 13. In most mammals, the kidney is the primary source of circulating renin, although renin gene expression is found in a number of extrarenal tissues, including the brain, adrenal, pituitary, submandibular glands, gonads, and heart.

The initial translation product preprorenin, consisting of 406 amino acids, is processed in the endoplasmic reticulum to a 47-kDa prorenin by removal of a 23-amino-acid presegment. Prorenin then enters either a regulated or a constitutive secretory pathway. A substantial portion of prorenin is further processed, when a 43-amino-acid prosegment is removed, to the active 41-kDa mature renin, which is a glycosylated single-chain polypeptide that circulates in human plasma. Prorenin also circulates in the blood at a concentration several times higher than active renin. Active renin can be generated from prorenin by cold storage (cryoactivation); acidification; or a variety of proteolytic enzymes including trypsin, pepsin, and kallikrein. The N- and C-terminal halves of active renin are similar, and each domain contains a single aspartic residue in the active center, which is essential for its catalytic activity. Angiotensinogen (renin substrate) is the only known substrate for renin. This reaction appears to be highly species specific. Human renin does not cleave mouse or rat angiotensinogen, and human angiotensinogen, in turn, is a poor substrate for rodent renin.

2.1.2. Regulation of Renin Release

Because renin is the rate-limiting enzyme in circulating Ang II production, control of renin release serves as a major regulator of the systemic RAS activity. Restriction of salt intake, acute hemorrhage, administration of diuretics, or acute renal artery clamping results in a marked increase in renin release. The regulation of renin release is controlled by four independent factors: renal baroreceptor, macula densa, renal sympathetic nerves, and various humoral factors:

1. Mechanical signals, via the baroreceptor or vascular stretch receptor, of the juxtaglomerular cells sensing the renal perfusion pressure in the afferent arteriole (Fig. 2): The renal baroreceptor is perhaps the most powerful regulator of renin release, and reduced renal perfusion pressure strongly stimulates renin release.
2. Tubular signals from the macula densa cells in the distal convoluted tubule: The cells function as the chemoreceptor, monitoring the delivery of sodium chloride to the distal nephron by sensing the sodium and/or chloride load through the macula densa cells, and decreased concentrations within the cells stimulate renin release.
3. The sympathetic nervous system in the afferent arteriole: Juxtaglomerular cells are directly innervated by sympathetic nerves (Fig. 2), and β-adrenergic activation stimulates renin release. Renal nerve–mediated renin secretion constitutes an acute pathway by which rapid activation of the RAS is provoked by such stimuli as stress and posture.
4. Circulating humoral factors: Ang II suppresses renin release (as a negative feedback) independent of alteration of renal perfusion pressure or aldosterone secretion. Atrial natriuretic peptide (ANP) and vasopressin inhibit renin release, whereas PGE_2 and prostacyclin (PGI_2) stimulate renin release.

In addition to the major regulators just described, a series of other humoral factors is implicated, considering the finding that the primary stimulatory second messenger for renin release is intracellular cAMP whereas the inhibitory signal is increased intracellular calcium and increased cyclic guanosine monophasphate (cGMP). For example, local paracrine regulators, such as adenosine and nitric oxide (NO), may have significant influences on renin release, perhaps more importantly in certain pathologic conditions.

2.2. Angiotensinogen

Angiotensinogen is the only known substrate for renin capable of producing the family of angiotensin

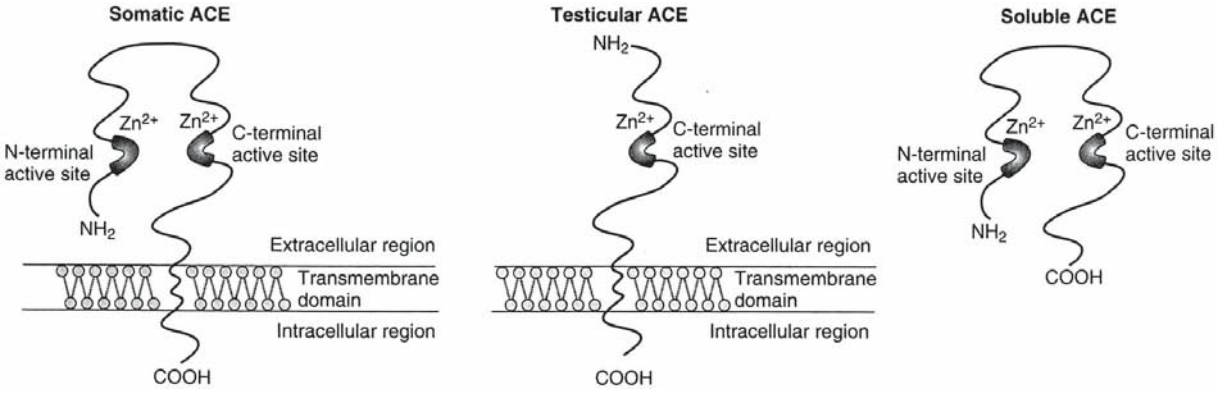

Fig. 3. Schematic representation of three isoforms of ACE.

peptides. In most species, angiotensinogen circulates at a concentration close to the K_m for its cleavage by renin, and, therefore, varying the concentration of plasma angiotensinogen can affect the rate of Ang I production. Because angiotensinogen levels in plasma are relatively constant, plasma concentrations of active renin, not angiotensinogen, would be the limiting factor for the rate of plasma Ang I formation in normal conditions, as determined by the plasma renin activity. However, in certain conditions such as pregnancy and administration of steroids, when angiotensinogen production is enhanced, circulating angiotensinogen would have a major effect on the activity of the systemic RAS. Furthermore, recent studies on the linkage analysis between angiotensinogen gene and human essential hypertension suggest that the alterations in plasma angiotensinogen levels may have a significant impact on the total RAS activity, affecting blood pressure.

Angiotensinogen shares sequence homology with α_1-antitrypsin and belongs to the serpin (for serine protease inhibitor) superfamily of proteins. The human angiotensinogen gene (~12 kb long) is located on chromosome 1 (1q42.3) close to the renin gene locus. The angiotensinogen gene consists of five exons and four introns, and cDNA codes for 485 amino acids, of which 33 appear to be a presegment. The first 10 amino acids of the mature protein correspond to Ang I. The 5´-flanking region of the human angiotensinogen gene contains several consensus sequences for glucocorticoid, estrogen, thyroid hormone, cAMP, and an acute phase–responsive element.

The liver is the primary site of angiotensinogen synthesis and secretion. However, angiotensinogen mRNA is expressed in a variety of other tissues, including brain, large arteries, kidney, adipose tissues, reproductive tissues, and heart, which constitutes an important part of the tissue RAS.

2.3. Angiotensin-Converting Enzyme

ACE, or kininase II (EC 3.4.15.1), is a dipeptidyl carboxypeptidase, which is a membrane-bound ectoenzyme with its catalytic sites exposed to the extracellular surface. It is a zinc metallopeptidase that is required for the final enzymatic step of Ang II production from Ang I (Fig. 1). ACE also plays an important role in the kallikrein-kinin system, by inactivating the vasodilator hormone bradykinin. In vascular beds, ACE is present on the plasma membrane of endothelial cells, where it cleaves circulating peptides; vessels in the lung, as well as in the brain and retina, are especially rich in ACE. ACE is also abundantly present in the proximal tubule brush border of the kidney.

There are primarily two molecular forms of ACE (somatic and testicular) that are derived from a single gene by different utilization of two different promoters. Although the majority of ACE is membrane bound, somatic ACE can be cleaved near the C-terminus, leading to the release of ACE into the circulation. This results in three main isoforms of ACE: somatic ACE, testicular (or germinal) ACE, and soluble (or plasma) ACE (Fig. 3). The human ACE gene consisting of 26 exons and 25 introns, is located on chromosome 17q23. The somatic promoter is located in the 5´-flanking region of the gene upstream of exon 1, whereas the testicular promoter is present within intron 12. Somatic ACE is a 170-kDa protein consisting of 1306 amino acids encoded by a 4.3-kb mRNA, which is transcribed from exons 1 to 26 except exon 13. It is an extensively glycosylated protein, containing two highly homologous domains with an active site in each domain. Testicular ACE is an approx 90-kDa protein consisting of 732 amino acids, harboring only one C-terminal active site. This isoform is found only in the testes. Testicular ACE is encoded by a 3-kb mRNA, transcribed from

exons 13 to 26, with exon 13 encoding the unique N-terminus of the testicular isoform.

Somatic ACE is distributed in a wide variety of tissues, including blood vessels, kidney, heart, brain, adrenal, small intestine, and uterus, where it is expressed in the epithelial, neuroepithelial, and nonepithelial cells as well as in endothelial cells. Somatic ACE in these tissues (tissue ACE) is postulated to play a crucial role in the rate-limiting step of the tissue RAS activity. In addition, studies on the human ACE gene revealed the presence of a 287-bp insertion (*I*)/deletion (*D*) polymorphism within intron 16, which may account for the high degree of individual variability of ACE levels. The *D* allele is associated with high plasma and tissue ACE activity and has been linked to cardiovascular diseases such as acute myocardial infarction.

In addition to ACE, it is now known that there are other ACE-independent pathways of Ang II generation from Ang I (Fig. 1). Among them, chymase, which is present abundantly in the human heart, is thought to be most important. The relative importance of such alternative pathways in physiologic and pathophysiologic states, however, is the subject of continuing debate and awaits further clarification.

2.4. Angiotensin Receptors

For many years, it was thought that Ang II exerts its effects via only one receptor subtype that mediates vasoconstriction, aldosterone release, salt-water retention, and tissue remodeling effects such as cell proliferation and hypertrophy. This receptor subtype is now termed the AT_1 receptor. In the late 1980s, it became clear that there was another Ang II–binding site that was not blocked by the AT_1 receptor antagonists. This receptor subtype is now known as the AT_2 receptor. Pharmacologic examinations may suggest the presence of other receptor subtypes, but to date, no other receptors have been isolated or cloned.

Most known biologic effects of Ang II are mediated by the AT_1 receptor. The AT_1 receptor consists of 359 amino acids, with a relative molecular mass of 41 kDa, and belongs to the G protein–coupled, seven-transmembrane receptor superfamily. The principal signaling mechanism of the AT_1 receptor is through a G_q-mediated activation of phospholipase C (PLC) with a release of inositol 1,4,5-trisphosphate and calcium mobilization. Activation of the protein tyrosine kinase pathway may also be involved. In humans, there is a single gene for this receptor, located on chromosome 3. The human AT_1 receptor gene consists of five exons and four introns, with the coding region contained within exon 5. The promoter region contains putative elements for cAMP, glucocorticoid, and activating protein-1 sites for immediate early gene products. In rodents, there are two isoforms of this receptor, named AT_{1A} and AT_{1B}, encoded by different genes. These isoforms show a very high sequence homology (94%) and AT_{1A} is considered to be a major subtype, although the functional significance of each isoform is not fully clarified. AT_1 receptor mRNA is expressed primarily in the adrenals, vascular smooth muscle, kidney, heart, and specific areas of the brain implicated in dipsogenic and pressor actions of Ang II, and it is also abundantly present in the liver, uterus, ovary, lung, and spleen.

The AT_2 receptor consists of 363 amino acids, with a relative molecular mass of 41 kDa. This receptor also exhibits a seven-transmembrane domain topology but shares only 32% overall sequence identity with the AT_1 receptor. It is likely coupled to a G protein, although it may also be coupled to a phosphotyrosine phosphatase. The AT_2 receptor gene, located on chromosome X, is composed of three exons and two introns, with the entire coding region contained within exon 3. Expression of the AT_2 receptor is developmentally regulated. It is abundantly expressed in various fetal tissues, especially in mesenchyme and connective tissues; it gets downregulated on birth and is not expressed at significant levels in adult tissues including the cardiovascular system at normal conditions, being limited to adrenal medulla, brain, and reproductive tissues. Interestingly, however, the AT_2 receptor is reexpressed under certain pathologic conditions, such as on tissue injury and remodeling, especially in the cardiovascular system. The signaling mechanism and functional role of the AT_2 receptor have not been fully elucidated, but recent studies have shown that stimulation of the AT_2 receptor induces apoptosis and exerts cardioprotective actions by mediating vasodilatation, probably via activation of NO and cGMP production. Furthermore, the AT_2 receptor exerts an antiproliferative action on vascular smooth muscle cells, fibroblasts, and mesangial cells. Thus, it is now recognized that the AT_2 receptor should act to counterbalance the effects of the AT_1 receptor.

2.5. Angiotensins

A family of angiotensin peptides is derived from Ang I through the action of ACE, chymase, aminopeptidases, and tissue endopeptidases. There are at least four biologically active angiotensin peptides (Table 1). Ang I, decapeptide cleaved from angiotensinogen, is biologically inactive. Ang II acts on AT_1 and AT_2 receptors, with equally high affinities. Ang II can be processed by aminopeptidase A or angiotensinase, to form Ang III. Like Ang II, Ang III circulates in the blood and shows somewhat less vasoconstrictor activity but exerts an almost equipotent activity on aldosterone secretion.

Table 1
Angiotensin Peptides

Peptide	*Sequence*
Ang I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
Ang II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Ang III	Arg-Val-Tyr-Ile-His-Pro-Phe
Ang IV	Val-Tyr-Ile-His-Pro-Phe
Ang 1-7	Asp-Arg-Val-Tyr-Ile-His-Pro

Ang III can be further converted by aminopeptidase B into Ang 3–8, or Ang IV. In addition, Ang 1–7 can be produced from Ang I or Ang II by endopeptidases. It is reported that the fragments Ang IV and Ang 1–7 have pharmacologic and biochemical properties different from those mediated by the AT_1 or AT_2 receptors, perhaps exerting an opposite effect of Ang II such as vasodilatation. The functional significance and receptors of these peptides, however, still remain elusive.

3. PATHOPHYSIOLOGY OF RAS

3.1. Biological Actions of Ang II

Ang II has short-term actions related to maintaining normal extracellular fluid volume and blood pressure homeostasis as well as long-term actions related to cardiovascular remodeling, most of which are mediated via the AT_1 receptor. Six primary short-term actions are as follows:

1. Increasing aldosterone secretion.
2. Constricting vascular smooth muscle, thereby increasing blood pressure and reducing renal blood flow.
3. Increasing the intraglomerular pressure by constriction of the efferent arteriole, contracting the mesangium, and enhancing sodium reabsorption from the proximal tubule.
4. Increasing cardiac contractility.
5. Enhancing the sympathetic nervous activity by increasing central sympathetic outflow, and releasing norepinephrine and epinephrine from the adrenal medulla.
6. Promoting the release of vasopressin.

Long-term actions of Ang II include the following:

1. Increasing vascular smooth muscle hypertrophy and hyperplasia.
2. Promoting cardiac hypertrophy.
3. Enhancing extracellular matrix synthesis, thereby causing tissue fibrosis.
4. Promoting inflammatory reactions by stimulating the migration and adhesion of monocytes to the vessel wall.

These actions are closely associated with the cardiovascular structural manifestations, or cardiovascular remodeling, in both human and experimental hypertension. Ang II also acts on the central nervous system, increasing thirst and sodium craving. In addition, Ang II may have potential actions in regulating ovarian and placental function.

3.2. Tissue RAS

Many tissues and organs can synthesize Ang II independent of the classic circulating RAS, and locally formed Ang II can exert multiple effects acting as an autocrine and paracrine regulator. Ang II levels may be much higher in tissues than in plasma. A variety of tissues express angiotensinogen, renin, ACE, and other Ang II–generating enzymes, as well as angiotensin receptors. These additional enzyme systems are referred to as the tissue RAS.

The effects of locally generated Ang II are long term, i.e., not just vasoconstriction or salt-water retention, but the induction of tissue remodeling, modulation of cell growth, and inflammation. These effects could be mediated by alternative pathways; thus, these multiple pathways in tissues allow more ways to synthesize Ang II, particularly in the areas of inflammation where mast cells release chymase, monocytes release ACE, and neutrophils secrete cathepsin G. With the presence of such non-ACE pathways of Ang II generation, the inhibition of ACE alone is not theoretically sufficient to completely inhibit Ang II production. Although the importance of the tissue RAS has been suggested and tissue Ang II should be a target for antihypertensive, antihypertrophic, and antiinflammatory effects, it is recognized that many of the data available so far are experimental and there is no definitive proof in humans. The availability of and analysis with several AT_1 receptor blockers in clinical settings should provide an answer to this issue.

3.3. Transgenic and Knockout Approaches

Several types of transgenic and knockout animals have been established to study the functional significance of the RAS in vivo. Transgenic lines of mice and rats harboring both the human renin and angiotensinogen genes develop severe hypertension. Hypertension in the mice likely represents pathologic conditions brought about by the inappropriate secretion of renin from outside the kidneys, including pregnancy-associated hypertension (preeclampsia). Transgenic rats harboring the mouse *Ren-2* gene exhibited fulminant hypertension, which overexpressed the transgene in the adrenal gland. Cardiac-specific overexpression of the AT_1 receptor resulted in hypertrophy and arrhythmia, whereas overexpression of the AT_2 receptor in the heart and vessels showed reduction in hypertrophy and tissue damage. These models may indicate the functional significance of the tissue RAS in cardiovascular control.

Knockout studies of the components of the RAS reveal that each component of the cascade (angiotensinogen, renin, ACE, and AT_{1A} receptor) is indispensible to the maintenance of normal blood pressure. These knockout animal models invariably show low blood pressure by ~30 mmHg. Moreover, mice deficient in any component exhibit severe abnormality in kidney development, characterized by cortical atrophy and hypoplasia. ACE-null male mice show greatly reduced fertility. The AT_2 receptor–knockout mice reveal enhanced pressor response to Ang II and exaggerated cardiovascular remodeling in response to noxious stimuli, again suggesting a potential cardioprotective role of this receptor.

3.4. Genetic Studies and Clinical Implication

Linkage and association studies have been performed using polymorphic markers of ACE, angiotensinogen, renin, and Ang II receptors. In rats, significant linkage has been demonstrated between the ACE locus and blood pressure. In humans, on the other hand, no relation was found between the ACE gene and hypertension. However, affected sib-pair analysis has found a strong linkage between the human angiotensinogen gene and hypertension. Among the polymorphic markers of the angiotensinogen gene, amino acid conversion at codon 235 from methionine to threonine (M235T) was significantly associated with hypertension. 235T subjects also have higher angiotensinogen levels in plasma. In addition, M235T polymorphism was found to be linked with several polymorphisms in the 5´-promoter region of the human angiotensinogen gene, such as A(-20)C, C(-18)T, and A(-6)G.

The human ACE gene contains an *I*/*D* polymorphism (ACE *I*/*D*), characterized by the presence/absence of a 287-bp fragment in intron 16. A significant linkage has been shown between a deletion polymorphism of the human ACE gene (ACE *DD*) and myocardial infarction. The deletion allele is associated with significantly increased ACE levels in the tissue and circulation. In addition, several reports have shown an association between the ACE *DD* polymorphism and an increased risk of cardiovascular events such as restenosis after coronary intervention, and progression of renal disease such as IgA nephropathy and diabetic nephropathy. Multiple lines of evidence have shown that ACE inhibitors and AT_1 receptor blockers are particularly effective in reducing morbidity and mortality in heart failure, and in retarding the progression of diabetic and nondiabetic nephropathies. Therefore, the presence of the ACE *DD* polymorphism should provide more compelling indications of these antihypertensive agents.

4. COMPONENTS OF NATRIURETIC PEPTIDE SYSTEM

Following the discovery of atrial natriuretic peptide (ANP) from human and rat atrial tissues, two endogenous congeners, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), were isolated from the porcine brain. These natriuretic peptides share a common ring structure of 17 amino acids formed by a disulfide linkage (Fig. 4), which is the essential part of their biologic actions. The natriuretic peptide system is a potent natriuretic, diuretic, and vasorelaxing hormone system, comprising at least three endogenous ligands and three receptors (natriuretic peptide receptor A [NPR-A], NPR-B, and the clearance receptor) (Fig. 4). The accumulated evidence indicates that this system plays an essential role in the control of blood pressure and body fluid homeostasis by acting on the kidney and vasculature as cardiac hormones, as well as by regulating cardiovascular and renal remodeling, neural control, and bone metabolism as local regulators. Furthermore, the importance of this system in the clinical setting has now been established not only as an excellent diagnostic marker but also as a useful therapeutic agent for cardiovascular diseases.

4.1. Natriuretic Peptide Family

4.1.1. ANP and BNP as Cardiac Hormones

ANP (28-amino-acid peptide) and BNP (32-amino-acid peptide in humans) act as cardiac hormones. ANP is predominantly synthesized in the cardiac atrium as pro-ANP (also called γ-ANP, with 126 amino acids) in healthy subjects, whereas BNP (from pro-BNP, with 108 amino acids) is mainly produced in the ventricle. Active peptides reside at the C-terminus of the prohormones and are cleaved during storage or in a process of secretion. Plasma ANP levels are well correlated with atrial pressure, thereby providing a good marker of blood volume status. Although BNP was first isolated from the brain, only small amounts of BNP are detected in the brain in humans and rodents.

Synthesis and secretion of ANP and BNP are markedly augmented in animal models of ventricular hypertrophy and in patients with congestive heart failure (CHF) in accordance with the severity, in which ventricular production of ANP as well as BNP is significantly enhanced. In humans, elevation of BNP becomes more prominent than ANP in relation to the severity of heart failure. Therefore, the plasma BNP level is now the most reliable biochemical marker for left ventricular dysfunction. In addition, plasma BNP levels are markedly increased in the early phase of acute myocardial infarction, when plasma ANP is increased only slightly.

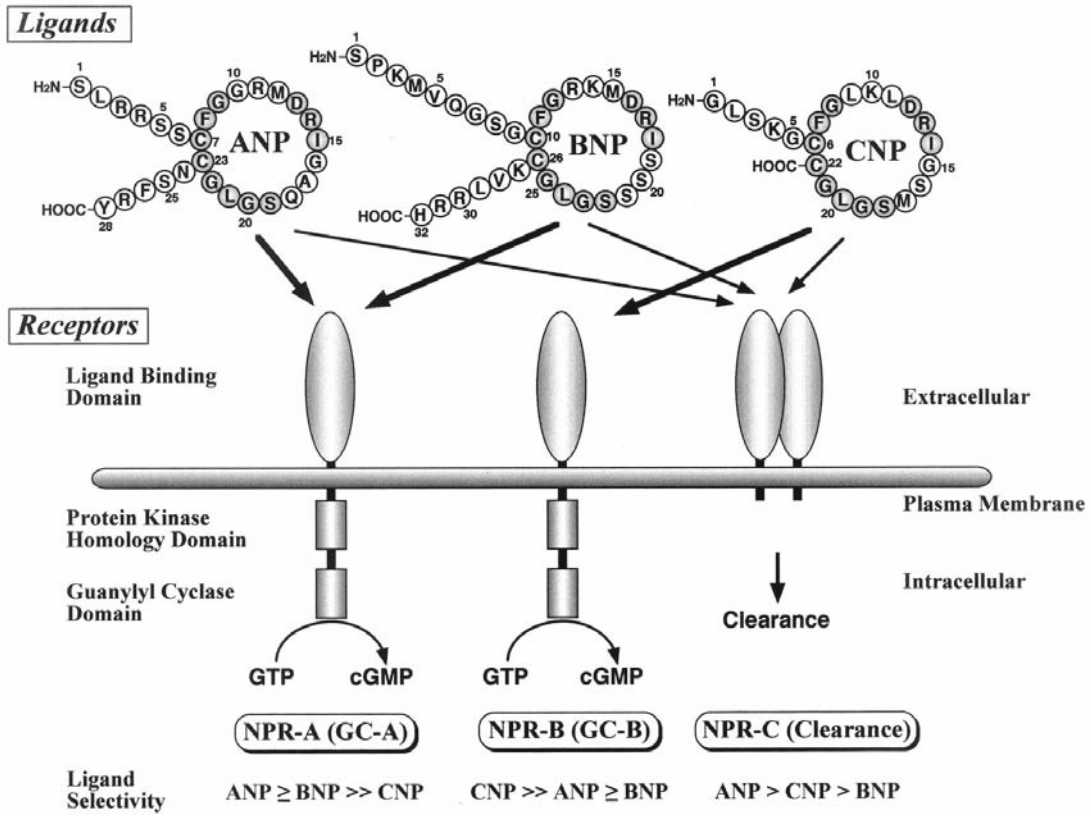

Fig. 4. Natriuretic peptide system.

It is also shown that a sustained increase in plasma BNP is associated with decreased ventricular contractility, increased stiffness, and poor prognosis. These observations suggest that BNP plays an important role in ventricular remodeling.

ANP and BNP activate a common guanylyl cyclase (GC)–coupled receptor subtype, NPR-A or GC-A, that is expressed in a wide variety of tissues. The main distribution of GC-A includes the kidney, blood vessels, heart, lung, adrenal, and brain. Human urine contains another peptide called urodilatin, an N-terminally extended form of ANP by four amino acids, which is synthesized in the kidney and secreted into the tubular lumen. A functional significance of urodilatin is still unclear, but it may act as a local regulator of tubular reabsorption in the distal nephron.

4.1.2. CNP as a Local Hormone

CNP, a 22-amino-acid peptide, is the third member of the natriuretic peptide family with a highly conserved ring structure, but uniquely it lacks the C-terminal extension. The precursor structure of CNP is well preserved among species, and the concentrations of CNP are much higher than those of ANP and BNP in the brain, indicating the significance of CNP as a neuropeptide. CNP is found in the cerebral cortex, brain stem, cerebellum, basal ganglia, and hypothalamus. Furthermore, CNP is expressed in a variety of peripheral tissues, including vascular endothelium, kidney tubules and glomeruli, adrenal gland, thymus, uterus, and macrophages. Endothelial production of CNP represents a potent peptide-type endothelium-derived relaxing factor. Vascular CNP expression may be induced in pathologic states such as septic shock and in injured tissues during vascular remodeling. Notably, CNP and its receptor, NPR-B or GC-B, are abundantly expressed in the chondrocytes in the growth plate of the bone. Transgenic and knockout approaches now reveal that the CNP/GC-B system is an essential regulator of endochondral bone growth.

4.2. Natriuretic Peptide Receptors

The natriuretic peptide family elicits most of its biologic actions by the activation of particulate GC. Three classes of NPRs have been identified (Fig. 4), two of which are the monomeric 130-kDa protein initially designated as the biologically active receptor, containing GC-A and GC-B. The other type of receptor not coupled

to GC, the clearance receptor (C receptor), forms a homodimer of a 70-kDa protein and is thought to be involved in the clearance of natriuretic peptides from the circulation. The rank order of ligand selectivity of GC-A is ANP ≥ BNP >> CNP, whereas that of GC-B is CNP >> ANP ≥ BNP. Thus, GC-A is a receptor for ANP and BNP, whereas GC-B is selective to CNP. The rank order of affinity for the clearance receptor is ANP > CNP > BNP, which is consistent with the lower clearance of BNP than ANP from circulation.

The cDNA sequences of GC-A and GC-B predict the presence of a single transmembrane domain. The extracellular putative ligand-binding domains of these two receptors are 43% identical at the amino acid level and ~30% identical to that of the clearance receptor. Just within the plasma membrane lies a protein kinase–like domain, which may function as a negative regulatory element of GC. A cyclase catalytic domain is present at the C-terminus. The gene for the rat GC-A spans approx 17.5 kb and is organized into 22 exons and 21 introns. Exon 7 encodes the transmembrane domain, and the protein kinase–like and cyclase catalytic domains are encoded by exons 8–15 and 16–22, respectively. The clearance receptor sequence consists of 496 amino acids, with a large extracellular domain and a 37-amino-acid cytoplasmic domain. The bovine gene for the clearance receptor spans more than 85 kb and comprises eight exons and seven introns. Exon 1 contains a coding sequence for the large portion of the extracellular domain, and exons 7 and 8 encode the transmembrane and cytoplasmic domains, respectively.

Genes of three subtypes of NPRs are widely expressed with different tissue and cell specificity. GC-A is expressed in the renal glomeruli, lung, adrenal zona glomerulosa, heart, and adipose tissue. GC-B exists in the brain, lung, kidney (mainly in the tubule), placenta, heart, and bone. The clearance receptor is abundantly present in the renal glomeruli, lung, placenta, and heart.

5. PATHOPHYSIOLOGY OF NATRIURETIC PEPTIDE SYSTEM

5.1. Biologic Actions of Natriuretic Peptides

Natriuretic peptides exert their actions by activating GC-A or GC-B, thereby leading to an increase in intracellular cGMP concentrations. The sites of actions of ANP and BNP are paralleled with the distribution of GC-A, whereas CNP actions are dependent on the expression of GC-B. The effects of natriuretic peptides can be viewed as a "mirror image" of the RAS, by generally antagonizing the actions of the RAS both systemically and locally.

Table 2
Biological Actions of Natriuretic Peptides

Peripheral actions
• Diuresis, natriuresis
• Vasodilatation, reduction in blood pressure
• Inhibition of hormone release: renin, aldosterone
• Inhibition of cell proliferation and hypertrophy: vascular smooth muscle, mesangium, cardiomyocytes
• Antifibrosis
• Angiogenesis, endothelial regeneration
• Stimulation of endochondral ossification
Central actions
• Inhibition of drinking
• Inhibition of salt appetite
• Reduction in blood pressure
• Inhibition of hormone release: vasopressin, ACTH

Actions of natriuretic peptides include peripheral and central effects (Table 2). Renal effects of natriuretic peptides involve (1) increased glomerular filtration rate, by potent afferent arteriolar dilation with the modest efferent arteriolar constriction plus mesangial relaxation; (2) increased renal perfusion and medullary blood flow; and (3) inhibited reabsorption of water and sodium in the collecting duct and proximal tubule. Together with the inhibited secretion of renin and aldosterone and potent vasodilatation, these effects participate in their diuretic and antihypertensive effects. The potent antiproliferative effects on vascular and mesangial cells may also play important roles in various pathologic conditions.

5.2. Transgenic and Knockout Approaches

Transgenic and knockout animal models have been established to study the functional roles of the natriuretic peptide system in vivo. Transgenic mice of ANP or BNP with high circulating levels of these peptides showed significantly low blood pressure. Moreover, BNP-transgenic mice appeared to be quite resistant against various nephropathies and cardiovascular disease states, suggesting the potential renal and cardiovascular protective effects brought about by chronic excess of circulating natriuretic peptides. Activation of the CNP/GC-B system in transgenic mice resulted in skeletal overgrowth.

Knockout studies of the components of the natriuretic peptide system have elucidated their distinct roles. ANP-null mice showed salt-sensitive hypertension. BNP-null mice, by contrast, were normotensive but revealed enhanced cardiac fibrosis in response to pressure overload. Mice lacking GC-A resulted in severe salt-resistant hypertension, cardiac hypertrophy and

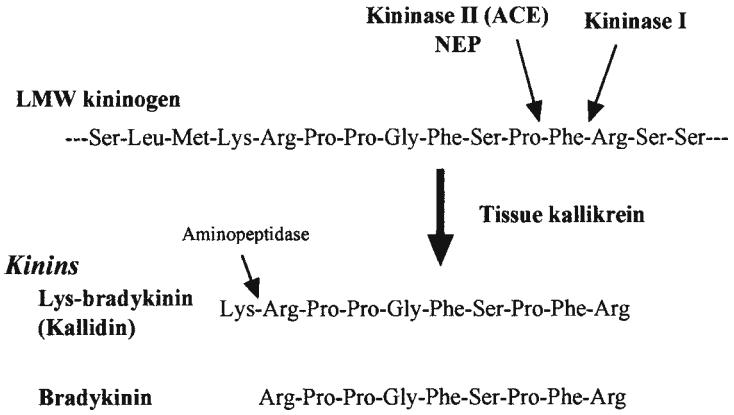

Fig. 5. Biosynthetic pathway of kallikrein-kinin system.

fibrosis, and increased sudden death. Therefore, the ANP/GC-A system is important in regulating blood pressure and sodium handling, whereas the BNP/GC-A system plays a role in antifibrosis as a local regulator of ventricular remodeling. CNP-null mice exhibited dwarfism owing to impaired endochondral ossification, indicating that the CNP/GC-B system is essential during skeletal development. These studies will provide plausible evidence for applications of natriuretic peptides to various disease states in clinical settings.

5.3. Clinical Implications

ANP and BNP are elevated in CHF, renal failure, and hypertension, but their levels appear inappropriately low for cardiomyocyte stretch caused by chronic volume and pressure overload. Thus, these disease states may represent relative natriuretic peptide deficiency. Therefore, therapeutic strategies are emerging that amplify the actions of ANP and BNP. One strategy is to administer these peptides directly, and another is to retard their metabolic clearance. The latter includes blockade of the clearance receptor, and inhibition of their degradation by neutral endopeptidase 24.11 (NEP). Recently, NEP inhibition has been combined with ACE inhibition in a series of new antihypertensive agents called vasopeptidase inhibitors.

Administration of ANP and BNP has been demonstrated to exert fairly beneficial effects in patients with CHF, and this is now considered to be one of the standard therapeutic strategies in heart failure. Clinical trials with vasopeptidase inhibitors in hypertension are now ongoing. ANP has also been shown to exert potential beneficial effects in experimental and clinical acute renal failure. Clinical efficacy of ANP and vasopeptidase inhibitors in chronic renal dysfunction should await further clarification.

6. KALLIKREIN-KININ SYSTEM

The kallikrein-kinin system consists of four major components: kininogen, kallikreins, kinins, and kininases (Fig. 5). The kallikrein gene family is a subset of closely related serine proteases with a narrow range of substrate specificity. The main function of kallikrein is the cleavage of a plasma α_2-globulin known as kininogen to generate kinins, of which bradykinin and Lys-bradykinin (kallidin) are the main peptides. Kinins are potent vasodilators with natriuretic, diuretic, and proinflammatory properties, stimulating the release of NO, PGs and other mediators. Kinins are short-lived in vivo because of the presence of kininases (I and II), which degrade kinins into inactive fragments. Kininase II is identical to ACE. The multiple roles of the kallikrein-kinin system still remain elusive, but recent pharmacologic and genetic studies suggest the potential significance of this system in regulating renal salt and water handling as well as in mediating part of the cardiovascular and renal protective effects of ACE inhibitors.

6.1. Synthesis of Kinins

There are two main forms of kininogen: high molecular weight and low molecular weight. They are encoded by a single gene and generated by alternative mRNA splicing. Kininogens are synthesized primarily in the liver and are present at high concentrations in plasma.

Kininogen is cleaved to release kinins by kallikreins. Two classes of kallikreins have been identified: plasma and tissue (glandular). These are separate enzymes that are derived from different genes and differ in function. Plasma kallikrein (100 kDa) releases bradykinin only from high molecular weight kininogen and does not cleave low molecular weight kininogen. Plasma kallikrein is involved in coagulation, fibrinolysis, and possibly activation of the complement system. By contrast,

tissue kallikrein (24–44 kDa), found principally in the kidney and in the exocrine and endocrine glands such as salivary gland and pancreas, cleaves both low molecular weight and high molecular weight kininogens to release Lys-bradykinin (Fig. 5). The tissue kallikrein gene family comprises a large number of closely related genes. The sizes of this gene family vary among species, up to 20 genes in the rat, 24 in the mouse, and 3 in the human. These members exhibit high sequence homology, suggesting that they share a common ancestral gene.

6.2. Kinin Receptors and Their Function

Kinins act on two receptors, B_1 and B_2 receptors, which differ in tissue distribution, regulation, pharmacologic properties, and biologic activities. The B_2 receptor has a high affinity to bradykinin and Lys-bradykinin, whereas the B_1 receptor is selectively activated by des-Arg^9-bradykinin or des-Arg^{10}-kallidin. These receptors belong to a seven-transmembrane-domain, G protein–coupled receptor superfamily. On stimulation, both B_1 and B_2 receptors lead to activation of PLC with inositol phosphate generation and calcium mobilization. The B_2 receptor gene contains three exons and two introns; the third exon encodes a whole receptor protein of 364 amino acids, which shows 36% amino acid identity with the B_1 receptor. The promoter region of the B_2 receptor gene contains consensus interleukin-6 (IL-6) and cAMP-responsive elements. The B_1 receptor is generally not expressed in normal conditions but appears in pathologic states such as administration of lipopolysaccharide, inflammation, and injury. The B_2 receptor, on the other hand, is widely distributed in many tissues including the kidney, heart, lung, brain, and testis. Therefore, in normal conditions, most of the physiologic effects of kinins are mediated by the B_2 receptor.

Kinins have prominent effects in the cardiovascular, pulmonary, gastrointestinal (GI), and reproductive systems. Kinins, via the B_2 receptor, appear to play an important role in the regulation of local blood flow. In the vasculature, kinins induce vasodilatation with release of various mediators, such as NO, PGs, platelet-activating factor, leukotrienes, and cytokines, and may be involved in vasodilatation and edema formation observed during inflammation. Kinins induce smooth muscle contraction in the GI tract, uterus and bronchioles. The B_2 receptor is also likely to be involved in renal salt handling and in blood pressure regulation in individuals consuming a high-sodium diet. The B_1 receptor may be implicated in the chronic inflammatory and pain-producing responses to kinins, but studies are still needed to clarify their functional significance.

6.3. Renal Kallikrein-Kinin System

Tissue kallikrein is synthesized in the kidney and excreted in urine. Filtered kinins, which are active on the glomerular vasculature, would not be found downstream in the nephron because of the high activity of kininases in the proximal tubule. Renal kallikrein has been localized by immunohistochemical techniques to the distal nephron segments, mostly in the connecting tubule. Kinin receptors are present in the collecting duct. Therefore, a paracrine role for the renal kallikrein-kinin system near the site of action has been proposed to explain the importance of this system. In addition, kinins generated in the cortical distal nephron segments may act on the glomerular vasculature, because the sites are in close association with the glomerular tuft.

Pharmacologic evidence shows that kinins play an important role in the regulation of renal microcirculation and water and sodium excretion. Renal actions of kinins involve glomerular and tubular actions. Bradykinin dilates both afferent and efferent arterioles and can increase renal blood flow without significant changes in glomerular filtration rate, but with a marked increase in fluid delivery to the distal nephron. It appears that natriuresis and diuresis are the result of an effect of kinins on renal papillary blood flow, which inhibits sodium reabsorption. Kinins also inhibit vasopressin-stimulated water permeability and sodium transport in the cortical collecting duct. Because the effect of bradykinin is greatly attenuated by cyclooxygenase inhibition, the natriuretic and diuretic actions of kinins may be mediated mostly, or at least partly, by PGs.

6.4. Pathophysiology of the Kallikrein-Kinin System

Decreased activity of the kallikrein-kinin system may play a role in hypertension. The urinary excretion of kallikrein is significantly reduced in patients with hypertension or in children with a family history of essential hypertension, and the urinary kallikrein levels are inversely correlated with blood pressure. Reduced urinary kallikrein excretion has also been described in various models of genetic hypertension. A restriction fragment length polymorphism for the kallikrein gene family in spontaneously hypertensive rats has been linked to high blood pressure. Collectively, these findings suggest that genetic factors causing a decrease in renal kallikrein activity might contribute to the pathogenesis of hypertension.

Endogenous kinins clearly affect renal hemodynamics and excretory function. This notion is supported by studies using kininogen-deficient Brown Norway rats, which show a brisk hypertensive response to a high-

sodium diet. Furthermore, B_2 receptor knockout mice have provided more definitive data supporting the conclusion that kinins can play an important role in preventing salt-sensitive hypertension.

Increased tissue concentrations of kinins and potentiation of their effect may be involved in the therapeutic effects of ACE inhibitors. This hypothesis is supported by the finding that a kinin antagonist partially blocks the acute hypotensive effects of ACE inhibitors. Moreover, beneficial effects on the heart and kidney by ACE inhibition are significantly attenuated or reversed by treating with the kinin antagonist, or in mice lacking the B_2 receptor and kininogen-deficient rats. These data strongly suggest a potential role of kinins in mediating part of the cardioprotective and renoprotective effects exerted by treatment with ACE inhibitors.

7. ADRENOMEDULLIN AND ENDOTHELINS

7.1. Adrenomedullin

AM is a potent vasorelaxing peptide with 52 amino acids that is isolated from the adrenal medulla and shares structural homology with calcitonin gene–related peptide. The preproadrenomedullin gene encodes two active peptides, AM and proadrenomedullin N-terminal 20 peptide (PAMP), which are generated by post-translational processing of the same gene. AM is produced primarily in the vasculature; is released as an endothelium-derived relaxing factor; and is also expressed in the adrenal medulla, brain, heart, and kidney. AM exerts its effects via activation of cAMP production and nitric oxide synthesis. PAMP, on the other hand, does not activate cAMP or NO synthesis and exerts its vasodilatory effects via presynaptic inhibition of sympathetic nerves innervating blood vessels. AM receptors are composed of two components, a seven-transmembrane calcitonin receptor-like receptor and a single-transmembrane receptor–activity-modifying protein, whereas PAMP receptors remain elusive and are yet to be cloned. AM has potent diuretic and natriuretic actions, and AM and PAMP also inhibit aldosterone secretion. Thus, the AM gene encodes two distinct peptides with shared biologic activity, but unique mechanisms of action.

AM increases renal blood flow and has tubular effects to stimulate sodium and water excretion. AM also has a potent inhibitory effect on proliferation of fibroblasts, mesangial cells, and vascular smooth muscle cells. In addition, experiments of AM infusion and AM gene delivery have shown that it has a potent vasodilatory and antifibrotic property, resulting in cardiovascular and renal protective effects. Furthermore, AM exerts a potent angiogenic activity, as demonstrated by AM-deficient mice that exhibit a profound defect in fetal and placental vascular development, leading to embryonic death. In humans, plasma concentrations of AM are elevated in various cardiovascular disorders including CHF, hypertension, and renal failure, which may represent a compensatory role of AM in these disorders. Furthermore, preliminary clinical studies have revealed that the administration of AM causes beneficial effects on CHF and pulmonary hypertension, suggesting the possibility of potential clinical usefulness of AM in such diseases.

7.2. ET Family

The vascular endothelium is able to modulate the vascular tone in response to various mechanical and chemical stimuli, and such modulation is achieved, at least partly, by endothelium-derived humoral factors, relaxing factors and constricting factors. ET was isolated as an endothelium-derived constricting peptide with 21 amino acids that is the most potent endogenous vasoconstrictor yet identified. The first peptide identified is called ET-1, and the ET family now consists of three isoforms, ET-1, ET-2, and ET-3, acting on two receptors, ET_A and ET_B. ET-1 is the primary peptide secreted from the endothelium and detected in plasma, and its mRNA is also expressed in the brain, kidney, lung, uterus, and placenta. Endothelial ET-1 production is stimulated by shear stress, hypoxia, Ang II, vasopressin, thrombin, catecholamines, and growth factors and inhibited by CNP and AM. ET-2 is produced in the kidney and jejunum, and ET-3 is identified in the intestine, adrenal, brain, and kidney. The ET_A receptor is relatively specific to ET-1, whereas the ET_B receptor has an equal affinity to three isoforms. Both receptors are coupled to G proteins, leading to activation of PLC with inositol phosphate generation and calcium mobilization.

Plasma ET-1 concentrations are elevated in renal failure, acute myocardial infarction, atypical angina, essential hypertension, and subarachnoid hemorrhage. ET-1 exerts a positive inotropic action and potent vasoconstriction (coronary, pulmonary, renal, and systemic vasculature) as well as vascular and cardiac hypertrophy. An important synergism exists between ET-1 and Ang II, especially in the heart during cardiac hypertrophy, which is counteracted by ANP and BNP. Pharmacologic blockade of ET receptors has been effective in some forms of experimental hypertension and heart failure, and the nonselective antagonist bosentan has been approved for treatment of primary pulmonary hypertension. In the kidney, the receptors are mainly present in the blood vessels and mesangial cells.

Although these are predominantly the ET_A subtype, the ET_B receptor may have pathophysiologic significance, particularly in the distal tubules, where ET_B receptor activation causes sodium excretion. Involvement of renal ET_B receptor in sodium-sensitive hypertension remains to be clarified. Furthermore, gene knockout approaches have revealed that the ET system plays an essential role during development; the ET-1/ET_A system is crucial in branchial arch development and cardiac septum formation, whose mutation causes mandibulofacial and cardiac abnormalities. By contrast, the ET-3/ET_B system is essential for migration of neural crest cells (melancytes and neurons of the myenteric plexus), whose mutation results in aganglionic megacolon (Hirschsprung disease) and vitiligo.

8. ERYTHROPOIETIN

The kidney is the primary organ responsible for regulating the production of the protein hormone EPO, in response to perceived changes in oxygen pressure. A number of experimental and clinical studies have demonstrated an essential role of the kidney in erythropoiesis, including the development of severe anemia by renal ablation, and in renal failure patients. EPO is a glycosylated protein composed of 165 amino acids with a relative molecular mass of 34 kDa. Plasma concentrations of EPO normally range from 8 to 18 mU/mL and may increase 100- to 1000-fold in anemia. EPO mRNA levels are highly sensitive to changes in tissue oxygenation, and, therefore, its synthesis is regulated primarily at the level of gene transcription.

The site of EPO production in the kidney is now shown to be the interstitial cells of the renal cortex, around the base of the proximal tubule. Oxygen deficiency is sensed effectively by the "oxygen sensor" in these cells. Reduced capillary blood flow may also induce the increased production of EPO. Studies on the EPO gene have shown that its production in response to hypoxia is induced by a transcription factor, hypoxia-inducible factor-1.

Erythropoiesis begins when the pluripotent stem cells in the bone marrow are stimulated by nonspecific cytokines, such as IL-3 and granulocyte-macrophage colony-stimulating factor, to proliferate and transform into the erythroid-committed progenitor cells. EPO then acts on these early progenitor cells bearing its receptor to expand and differentiate into colony-forming unit-erythroid (CFU-E). EPO further continues to stimulate CFU-E to erythroid precursors, which eventually reach the stage of mature RBCs. CFU-E is the key target cell for EPO, which indeed regulates RBC production.

Anemia can develop relatively early in the course of chronic renal failure, which is referred to as renal anemia. The impairment of EPO production appears to parallel the progressive reduction of functional nephron mass, and plasma EPO levels are relatively very low for the degree of severity of anemia in these patients. Recombinant human EPO can potently reverse anemia in such states, and its administration has now widely been performed routinely for correcting anemia in hemodialysis and peritoneal dialysis patients, as well as in patients with moderate renal impairment.

SELECTED READINGS

Cambien F, Poirier O, Lacerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641–644.

Candido R, Burrell LM, Jandeleit-Dahm KA, et al.. Vasoactive peptides and the kidney. In: Brenner BM, ed. *The Kidney*, 7th Ed., vol. 1. Philadelphia, PA: W. B. Saunders 2004:663–726.

Chao J, Chao L. New experimental evidence for a role of tissue kallikrein in hypertension. *Nephrol Dial Transplant* 1997;12: 1569– 1574.

Chusho H, Tamura N, Ogawa Y, et al. Dwarfism and early death in mice lacking C-type natriuretic peptide. *Proc Natl Acad Sci USA* 2001;98:4016–4021.

Drewett JG, Garbers DL. The family of guanylyl cyclase receptors and their ligands. *Endocr Rev* 1994;15:135–162.

Dzau VJ. Circulating versus local renin-angiotensin system in cardiovascular homeostasis. *Circulation* 1988;77:I-4–I-13.

Horiuchi M, Akishita M, Dzau VJ. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* 1999;33:613–621.

Inagami T. Molecular biology and signaling of angiotensin receptors: an overview. *J Am Soc Nephrol* 1999;10(Suppl 11):S2–S7.

John SW, Krege JH, Oliver PM, et al. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science* 1995; 267:679–681.

Kitamura K, Kangawa K, Kawamoto M, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993;192:553–560.

Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell* 2001;104:545–556.

Lopez MJ, Wong SKF, Kishimoto I, et al. Salt-resistant hypertension in mice lacking the guanylyl cyclase-A receptor for atrial natriuretic peptide. *Nature* 1995;378:65–68.

Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans: evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. *J Clin Invest* 1991;87:1402–1412.

Nakao K, Ogawa Y, Suga S, Imura H. Molecular biology and biochemistry of the natriuretic peptide system. I: Natriuretic peptides. *J Hypertens* 1992;10:907–912.

Suganami T, Mukoyama M, Sugawara A, et al. Overexpression of brain natriuretic peptide in mice ameliorates immune-mediated renal injury. *J Am Soc Nephrol* 2001;12:2652–2663.

Takahashi N, Smithies O. Gene targeting approaches to analyzing hypertension. *J Am Soc Nephrol* 1999;10:1598–1605.

Tamura N, Ogawa Y, Chusho H, et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci USA* 2000; 97:4239–4244.

Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411–415.