Tissue Renin-Angiotensin-Aldosterone Systems: Targets for Pharmacological Therapy

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Key Words

reactive oxygen species, bradykinin, nitric oxide, hypertension, cardiac hypertrophy, signaling

Abstract

The renin-angiotensin-aldosterone system is one of the most important systems in cardiovascular control and in the pathogenesis of cardiovascular diseases. Therefore, it is already a very successful drug target for the therapy of these diseases. However, angiotensins are generated not only in the plasma but also locally in tissues from precursors and substrates either locally expressed or imported from the circulation. In most areas of the brain, only locally generated angiotensins can exert effects on their receptors owing to the blood-brain barrier. Other tissue renin-angiotensin-aldosterone systems are found in cardiovascular organs such as kidney, heart, and vessels and play important roles in the function of these organs and in the deleterious actions of hypertension and diabetes on these tissues. Novel components with mostly opposite actions to the classical renin-angiotensin-aldosterone systems have been described and need functional characterization to evaluate their suitability as novel drug targets.

INTRODUCTION

Classical Renin-Angiotensin-Aldosterone System

RAAS: renin-angiotensin-aldosterone system

Ang II: angiotensin II

ACE: angiotensin-converting enzyme

The renin-angiotensin-aldosterone system (RAAS) generates peptide hormones with great impact on cardiovascular regulation and the pathogenesis of cardiovascular diseases. Tigerstedt and Bergman discovered renin over 100 years ago (1). The enzyme is produced in the kidney and metabolizes its only substrate, angiotensinogen, in the plasma to liberate the decapeptide angiotensin (Ang I) (**Figure 1**). Ang I is then converted into the octapeptide Ang II by the angiotensin-converting enzyme (ACE), which is primarily present on endothelial cells. The actions of Ang II are transmitted by two main G-protein-coupled receptors with seven-transmembrane domains, AT1 and AT2. However, most cardiovascular effects of Ang II are conveyed by the AT1 receptor.

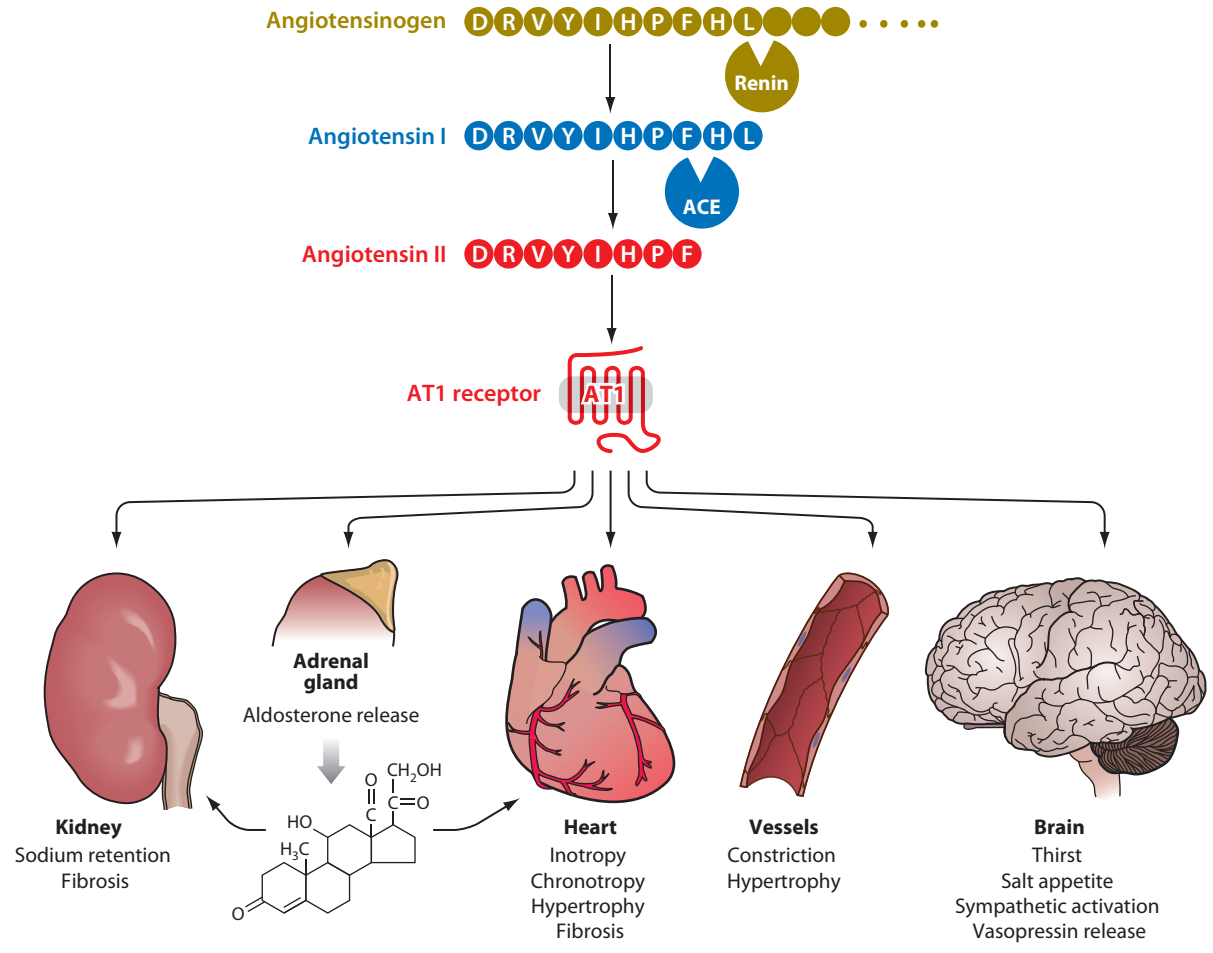

Figure 1

Classical RAAS. Angiotensinogen mainly expressed in the liver is cleaved by renin from the kidney to release Ang I in plasma. ACE on endothelial cells metabolizes Ang I further to Ang II. This peptide interacts mainly with the AT1 receptor in numerous cardiovascular organs to elicit the indicated effects, all leading to sodium retention, hypertension, or organ damage. In the adrenal cortex, Ang II induces aldosterone secretion, which aggravates the effects of Ang II.

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Ang II elicits an increase in blood volume and blood pressure by stimulating vasoconstriction, sodium retention, thirst, the sympathetic nervous system, and aldosterone synthesis and secretion from the adrenal cortex. In turn, the steroid hormone aldosterone interacts with the mineralocorticoid receptor (MR) in the renal collecting ducts and enhances the sodium retaining effect (2).

Renin-Angiotensin System and Kallikrein-Kinin System

The actions of the AT2 receptor are, in most cases, opposite to those of the AT1 receptor and are partly mediated by activation of the kallikrein-kinin system (KKS) (3). The links between the RAAS and the KKS are numerous (4). ACE is not only the activating enzyme of the RAAS but is also a major moderating component of the KKS by degrading active kinins. Furthermore, a direct physical and functional interaction between the AT1 receptor and the kinin B2 receptor has been described (5), but these data have been challenged recently (6).

AT1 Receptor Signaling

Effects of the AT1 receptor are mediated by multiple intracellular signaling pathways, starting with G-protein and phospholipase activation, followed by an increase in intracellular inositol 1,4,5-trisphosphate (IP3) and calcium (7–9). Downstream, protein kinase C, small GTP-binding proteins such as Ras and RhoA, and tyrosine kinase cascades are amplified, affecting several members of the mitogen-activated protein (MAP) kinase family such as extracellular regulated kinase 1/2 (ERK1/2) and p38 and the JAK-STAT (Janus kinase–signal transducers and activators of transcription) pathway. Finally, transcription factors, such as AP1, NF-κB, and the STATs, are activated and initiate the expression of growth-related genes. Moreover, the mTOR/S6 pathway, and thereby protein synthesis, is increased. Furthermore, Ang II elicits the production of reactive oxygen species (ROS) by nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] oxidase activation in several cell types (10), which in turn transactivate other receptors such as epidermal growth factor receptor (EGFR) and MR.

Local Actions of Angiotensin II

The concept of the RAAS as a purely endocrine system had to be revised in the past decades on the grounds of clinical and experimental observations. Drugs targeting the RAAS, such as ACE inhibitors and AT1 antagonists, independently from their antihypertensive action, efficiently protect cardiovascular organs from damage by hypertension and diabetes. Furthermore, the antihypertensive effect of ACE inhibitors did not correlate with their capacity to block the generation of Ang II in the circulation. After several weeks of treatment, plasma Ang II levels returned to normal, but blood pressure remained low (11). A possible explanation for this observation is the local generation of Ang II in tissues such as kidney, vessels, heart, and brain, where the peptide is synthesized from precursors and enzymes either locally generated or imported from the plasma. These tissue RAASs are autonomously regulated and have important functions inside the respective organs and a significant pathophysiological impact (12, 13). They are the subject of this review summarizing information about their characteristics and the therapeutic implications of their actions.

Extrarenal Actions of Aldosterone

Besides local Ang II synthesis, the generation of aldosterone in extraadrenal tissues has been postulated, and aldosterone synthase (CYP11B2) and MR have been discovered there (14–16). Such a local aldosterone system could explain the extraordinary efficiency of MR antagonists in heart

MR: mineralocorticoid receptor

Kallikrein-kinin system (KKS): hormone system with two receptors, B1 and B2, for the effector peptides, kinins, released from kininogen precursors by kallikrein enzymes

Inositol 1,4,5-trisphosphate: second messenger molecule released from the membrane phospholipid phosphatidylinositol 4,5-bisphosphate by phospholipase C

ERK1/2: extracellular regulated kinase 1/2

JAK-STAT pathway: Janus kinases (JAKs) are activated by extracellular cytokines and growth factors and phosphorylate signal transducers and activators of transcription (STATs)

NF-κB: nuclear factor κB is a homo- or heterodimeric transcription factor particularly involved in cellular responses to stress and infection

mTOR/S6: the mammalian target of rapamycin (mTOR) is a serine-threonine kinase phosphorylating p70-S6 kinase, which phosphorylates the ribosomal protein S6

ROS: reactive oxygen species

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failure patients with normal or even reduced plasma aldosterone levels [RALES and EPHESUS trials (17, 18)]. However, recent studies have shown that the local generation of aldosterone in tissues outside the adrenal gland is negligible, and adrenalectomy reduces its tissue concentrations to extremely low levels (19, 20). Thus, the situation is obviously more complex (21, 22). It is known that glucocorticoids bind to MR with equal affinity as aldosterone but are about 1000-fold more abundant in blood and tissues than the mineralocorticoid. In the aldosterone target cells in the kidney, unwanted activation of the MR by glucocorticoids is avoided by high expression of the enzyme 11ß-hydroxysteroid dehydrogenase type 2 (11ßHSD2), which converts glucocorticoids to metabolites that cannot bind to MR anymore. However, the levels of this enzyme are too low to block glucocorticoid actions on MR in other organs such as heart and vessels. Nevertheless, glucocorticoid-MR complexes are normally inactive at least in cells outside the renal tubules. Consequently, glucocorticoids exert essential protective actions by detaining aldosterone from binding to MR. Mice overexpressing 11ßHSD2, and thus decreased glucocorticoid binding to the MR, develop cardiac hypertrophy and die early (23). However, when the redox state of a cell changes, for example, by increased generation of ROS following Ang II stimulation, the MR becomes effective again (24). Either the glucocorticoid-MR complexes are activated under oxidizing conditions, or glucocorticoids are oxidized and lose their binding affinity to MR, granting access to the effective agonist aldosterone.

NAD(P)H: nicotinamide adenine dinucleotide (NAD) is a coenzyme involved in redox reactions in cells

(P)RR: (pro)renin receptor

Based on current evidence, the exact mechanisms involved in MR activation by ROS are unclear. As a consequence, the activated MR further increases ROS levels, and together with Ang II, elicits fibrosis and hypertrophy in vascular smooth muscle cells and cardiomyocytes in a vicious cycle (25). Furthermore, it influences electrical coupling in the heart and induces arrhythmias (24). These effects are mostly conveyed by the classical genomic actions of the MR binding to specific promoter regions and activating genes. However, fast nongenomic actions by aldosterone, which may also employ MR, have been described to be involved in its cardiovascular effects (25, 26). The signaling pathways are partly identical with those of Ang II including ERK1/2 phosphorylation, ROS generation, and EGFR transactivation. Most of these effects are blocked by MR antagonists, such as spironolactone and eplerenone, explaining their surprising effectiveness in cardiovascular diseases (17, 18).

ACE2–Angiotensin-(1–7)–Mas System

The local generation and action of Ang II depends not only on the classical RAAS proteins. Recently, several new components have been described. A homolog of ACE, ACE2, was discovered and shown to release the C-terminal phenylalanine from Ang II, leaving behind Ang-(1–7) (**Figure 2**) (27–29). This peptide is a ligand of the G-protein–coupled receptor Mas (30), which is mainly expressed in brain and testis but also in kidney, heart, and vessels (31, 32). Mas can heterodimerize with the AT1 receptor (33) and thereby antagonize it (34). Ang-(1–7) interacting with Mas elicits numerous protective actions such as vasodilatation and nitric oxide (NO) generation (35). Thus, ACE2 is pivotal for the physiological effect of the RAAS in each tissue. The local activity of the enzyme determines the relative levels of the vasoconstrictory and pro-oxidative peptide Ang II and its vasodilatory and antioxidative metabolite Ang-(1–7) at their receptors. Consequently, the ACE2–Ang-(1–7)–Mas axis counterregulates the cardiovascular actions of the classical RAAS in target organs (35).

(Pro)renin Receptor

Recently, a protein was discovered, that binds and activates renin in tissues: the (pro)renin receptor [(P)RR] (36). It also binds the normally inactive precursor prorenin and renders it active

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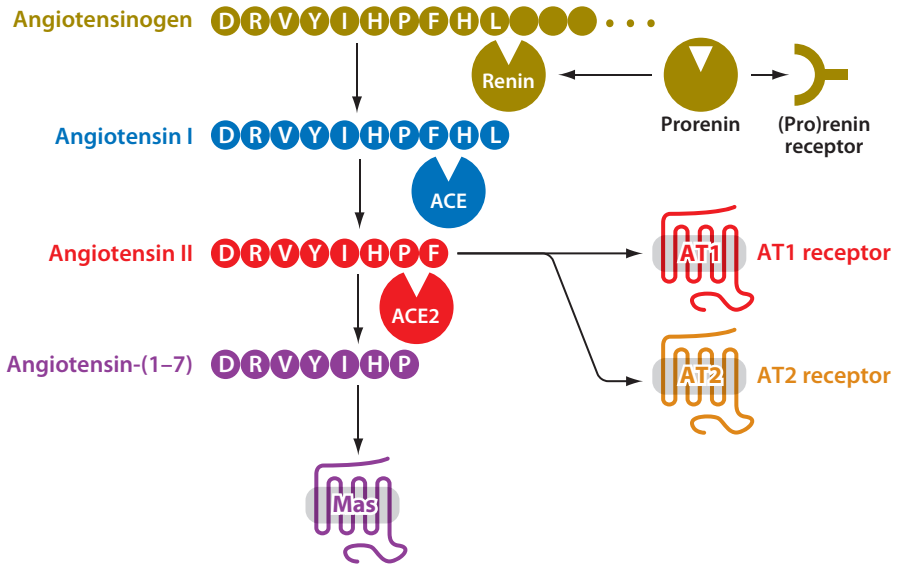

Figure 2

New components of the RAAS. Besides the AT1 receptor, Ang II can also interact with the AT2 receptor, and it can be metabolized by ACE2 to Ang-(1–7), which is a ligand for the receptor Mas. Both pathways mostly counteract the effects elicited by the AT1 receptor. Furthermore, renin and its precursor prorenin can bind to the (pro)renin receptor, (P)RR, which retains them in tissues, amplifies their Ang-II generation capacity, and signals into the cell after binding.

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without cleaving off the profragment. By retaining (pro)renin in tissues and boosting local Ang II production, it can be considered to be a powerful amplifier of tissue RAAS. Moreover, this protein exhibits intracellular signaling properties upon (pro)renin binding, such as ERK1/2 phosphorylation and PLZF activation (36, 37). It is not yet clear whether this signaling is conveyed by the protein itself or by another receptor to which a soluble form of the (P)RR may bind (38). The physiological role of this protein is not yet solved because it is mainly localized in intracellular vesicles and exerts essential but yet undefined functions in cellular physiology (39).

TISSUE RAAS

Brain

The brain is separated from the circulation by the specialized endothelium of its blood vessels, the blood-brain barrier, which is impermeable for all RAAS components. Therefore, Ang II needs to be generated locally to be active on most of the receptors found on neurons and astrocytes of the central nervous system (CNS). Most angiotensinogen in the brain comes from astroglial cells (40–42), and large amounts of this precursor are found in the cerebrospinal fluid (43). Whereas ACE is also prominently present throughout the brain (44), the discussion of local generation of renin in this organ is still controversial (45). In addition, uptake from the circulation through the blood-brain barrier is unlikely. The CNS contains large amounts of enzymes such as cathepsins (46) and tonin (47), which can also generate angiotensins from angiotensinogen. However, the relevance of these alternative enzymatic pathways for Ang II generation in the brain is also elusive.

CNS: central nervous system

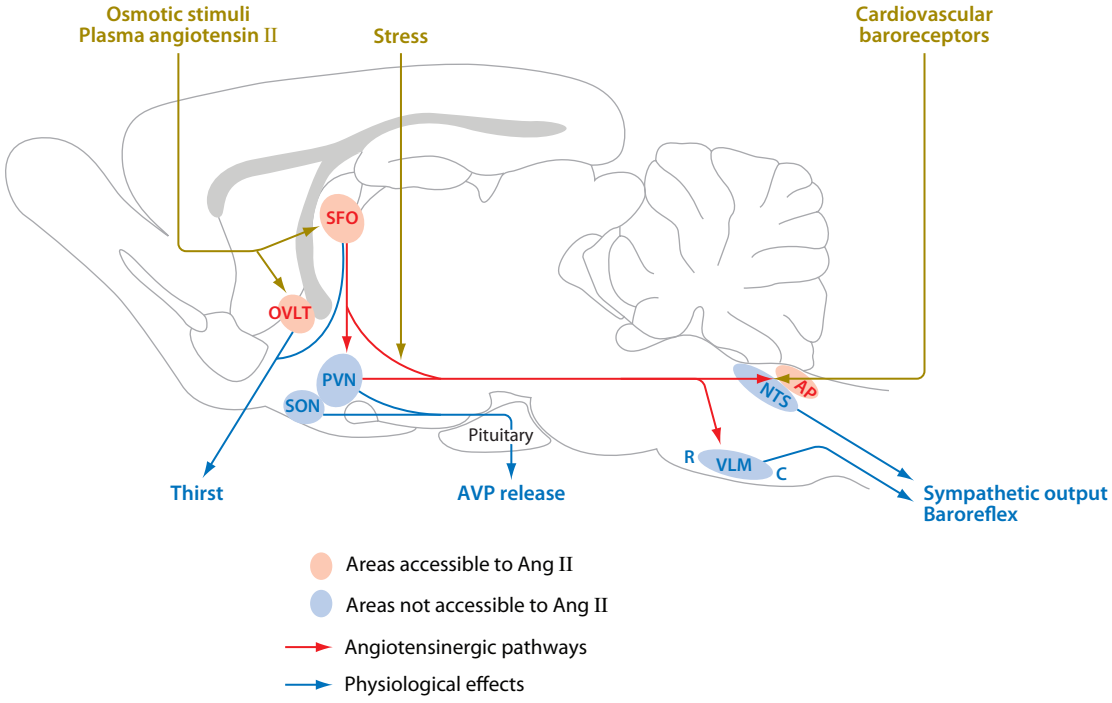

Figure 3

In the brain, some areas (*red*), such as the subfornical organ (SFO), the organum vasculosum of the lamina terminals (OVLT), and the area postrema (AP), contain AT1 receptors that are accessible to circulating Ang II. Other areas (*blue*), such as the supraoptic (SON) and the paraventricular nucleus (PVN) of the hypothalamus, the rostral (R) and caudal (C) ventrolateral medulla (VLM), and the nucleus tractus solitarii (NTS), also contain AT1 receptors that cannot be reached by systemic Ang II owing to the blood-brain barrier. These regions are only accessible to Ang II synthesized locally in the brain. The angiotensin-sensitive areas are influenced by outside effectors including plasma Ang II (*red*) and interconnected by angiotensinergic pathways (red arrows) that elicit the indicated physiological effects (blue arrows).

There are two possible modes of brain RAAS operation (48). The first is based on volume transmission: Angiotensinogen may be produced and released by astroglial cells and enzymatically converted in the extracellular space to active angiotensins that act on specific receptors localized on neurons or glia. The second mode is wiring transmission: Ang II is generated inside neurons from absorbed angiotensinogen, stored in vesicles, and released at nerve endings to act at receptors situated on postsynaptic cells.

Vasopressin: arginine-vasopressin (AVP) is a peptide hormone generated in the hypothalamus, stored and secreted in the pituitary, that acts on the distal convoluted tubules and collecting tubules in the kidney to enhance water reabsorption

Both the AT1 and AT2 receptors are abundantly expressed in the CNS (44, 49–52). The AT1 receptor has been found in brain areas involved in blood pressure and fluid homeostasis, such as the paraventricular and the supraoptic nucleus of the hypothalamus, the nucleus tractus solitarii, the rostral ventral lateral medulla, the subfornical organ, and the area postrema (**Figure 3**). The latter two areas are exceptional owing to their fenestrated endothelium, which allows them to sense the concentrations of substances in the plasma, in particular, the circulating Ang II. The AT2 receptor is highly expressed in the lateral septum, several thalamic nuclei, the subthalamic nucleus, the locus coeruleus, and the inferior olive, but its central function is still elusive.

The brain RAAS is involved in the modulation of cardiovascular and fluid-electrolyte homeostasis by influencing the autonomic nervous system, the hypothalamus-pituitary axis, vasopressin release, and baroreflex sensitivity, and stimulating thirst and salt appetite (53–58). Altogether, brain

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RAAS activation mostly leads to an increased blood volume and blood pressure. Furthermore, Ang II is also implicated in higher brain functions, including cognition, memory, pain perception, sexual behavior, and stress (58, 59).

Transgenic animal models with genetic alterations in the brain expression of RAAS components were instrumental in validating the local actions of Ang II. Transgenic mice with increased Ang II generation in the brain became hypertensive (60, 61). In these models, the high blood pressure could be reduced by intracerebroventricular (icv) injection of AT1 antagonists. Vasopressin mediates this effect, at least partially, because intravenous injection of a V1 receptor antagonist attenuated the hypertensive phenotype. Concordantly, transgenic animals with specifically reduced Ang II synthesis in the brain show opposite phenotypes. A transgenic rat with markedly blunted RAAS activity in the brain by the glial-specific expression of an antisense RNA against angiotensinogen is hypotensive and exhibits reduced vasopressin levels in the circulation. This demonstrates the central involvement of Ang II in vasopressin secretion (62). Furthermore, these rats show decreased activity of the sympathetic nervous system, leading to increased baroreflex sensitivity (63, 64). Mice expressing human angiotensinogen in the whole brain, except the subfornical organ after local injection of an adenovirus that deleted the angiotensinogen transgene, had a blunted pressor response to icv human renin infusion (65). The same is true for transgenic mice overexpressing the Ang-II-degrading enzyme ACE2 in this brain area by lentiviral gene transfer (66). When the local expression of angiotensinogen in the subfornical region was ablated in transgenic animals carrying human renin and human angiotensinogen, water intake decreased (67). Thus, the subfornical organ is of pivotal importance for the central pressor and dipsogenic effects of Ang II. It is one of the areas that senses Ang II in the circulation by its fenestrated endothelium.

These results from transgenic rat and mouse models indicate that the effects of peripheral Ang II on drinking and blood pressure control are mediated by locally generated Ang II. For example, after sensing Ang II in the plasma, neurons of the subfornical organ activate by Ang-II release AT1-receptor-bearing areas in the hypothalamus and brain stem responsible for vasopressin secretion and sympathetic control. Thus, there is strong evidence that the peripheral RAAS is depending on the brain RAAS for its central actions.

Ang II is locally generated in the brain, with major implications for cardiovascular and fluid homeostasis and notoriously underestimated pathophysiological relevance in the development of hypertension. Furthermore, all new components of the RAAS are expressed in the brain, such as ACE2 (68), Mas (31), and (P)RR (36). Although the local cardiovascular function of the (P)RR in the CNS has not been studied yet, Ang-(1–7) and Mas have been shown to enhance baroreflex sensitivity and influence blood pressure in different directions, depending on the brain area studied (69). Besides these cardiovascular actions, Mas affects behavior and electrophysiology of the hippocampus (70).

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Kidney

In the kidney, Ang II is formed from systemically delivered angiotensinogen and Ang I, as well as from angiotensinogen locally formed in proximal tubular cells (**Figure 4**) (71–73). Moreover, the kidney is the site of the highest level of production of renin, the rate-limiting enzyme in the RAAS cascade (74). It is secreted from juxtaglomerular cells first into the renal interstitium and from there into the circulation. Furthermore, renin and its mRNA were found in proximal tubular cells, where renin is not involved in the regulation of blood Ang II levels (75). ACE is present on endothelial cells of the renal vasculature and on the proximal tubular brush-border membrane (76). AT1 receptors are located on luminal and basolateral membranes of tubules,

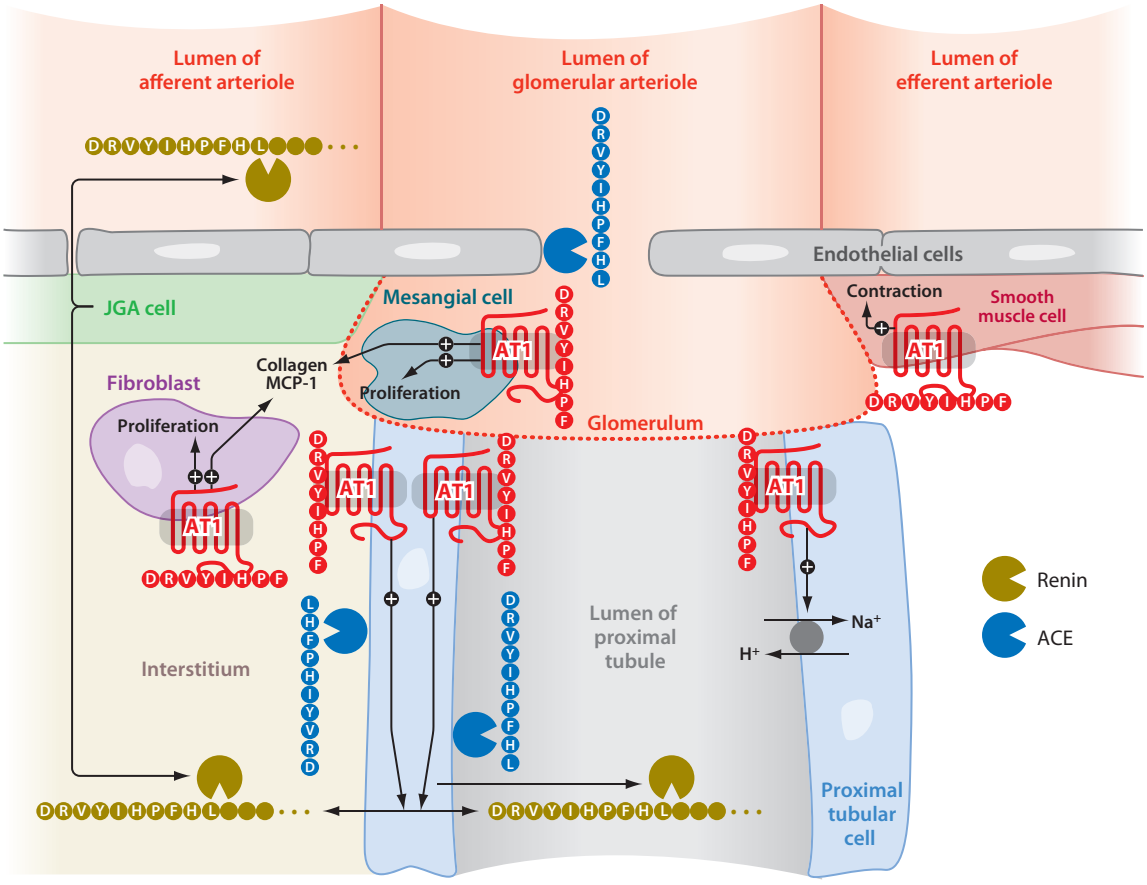

Figure 4

In the kidney, Ang II is formed from systemically delivered angiotensinogen and Ang I, as well as from angiotensinogen locally formed in proximal tubular cells. Renin is secreted from juxtaglomerular (JGA) cells into the renal interstitium and into the circulation. Moreover, renin production has been reported to take place also in proximal tubular cells. ACE is located in endothelial cells of the renal vasculature and on the proximal tubular brush-border membrane. These components interact in the renal interstitium to synthesize Ang II. In the proximal tubular lumen, Ang II is generated from angiotensinogen and ACE from tubular cells as well as from Ang I in the glomerular filtrate. AT1 receptors present on the luminal membranes of tubules are activated and increase sodium reabsorption by activation of the sodium-hydrogen exchanger. AT1 receptors present on mesangial cells and interstitial fibroblasts stimulate the proliferation of these cells and the synthesis of fibrosis-related proteins such as collagen as well as cytokines and chemoattractants for leukocytes such as monocyte chemoattractant protein (MCP) 1. Ang II via AT1 receptors constricts the efferent arteriole more intensively than the afferent, thereby increasing glomerular filtration pressure.

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mesangial cells, fibroblasts, and smooth muscle cells of the renal vessels (52, 77). Because intrarenal Ang II generation is under positive feedback control by Ang II itself, the peptide reaches higher concentrations in the kidney and the proximal tubular fluid than in the circulation, providing evidence for the functional importance of locally generated Ang II in the kidney (78–80).

Ang II plays an essential role in kidney development (81). Knockout mice lacking Ang II or AT1 receptors develop thickened vascular walls of renal vessels owing to smooth muscle proliferation and obstructive nephropathy owing to a dysmorphology of the pelvis. Therefore, ACE inhibitors, AT1 antagonists, and renin inhibitors are contraindicated during pregnancy.

In the adult kidney, Ang II participates in the regulation of renal function (**Figure 4**) (82, 83). Intrarenal blockade of Ang II by AT1 antagonists increases renal blood flow, glomerular filtration rate, and fractional sodium excretion. Ang II regulates renal blood flow by differential constriction of the afferent and efferent glomerular arterioles and sodium retention by direct effects on tubular epithelial cells in the nephron. Intraluminal application of AT1 blockers or ACE inhibitors reduces proximal tubular sodium reabsorption rates by blocking the sodium-hydrogen exchanger. Other studies demonstrated that Ang II is also important for the regulation of distal reabsorption (84), indicating that Ang II is active all along the nephron.

Accordingly, transgenic mice generating more Ang II exclusively in the kidney without spillover into the circulation, either by a transgenic human RAAS (85) or by local overexpression of rat angiotensinogen (86), develop high blood pressure and renal injury. This seems to be caused by an increase in Ang II in the tubular lumen, changing tubular function and leading to blood pressure elevation. Other transgenic mouse experiments have shown that AT1 receptors in the kidney are relevant for baseline blood pressure regulation and hypertension induced by Ang-II infusion (87, 88). Binephrectomized mice transplanted with one kidney lacking AT1 receptors hardly reacted to Ang II infusion, whereas mice lacking AT1 receptors in all tissues except a transplanted kidney developed the same increased blood pressure levels as wild-type (transplanted) mice.

Moreover, dysregulation of the intrarenal RAAS also contributes to target organ damage observed in hypertension and diabetes (89). Accordingly, inhibitors of the RAAS have been very successful in protecting the kidney from tubulointerstitial injury characterized by fibrosis and monocyte infiltration. However, the mechanisms involved are not yet completely understood. Ang II has been shown to act as a growth factor in the kidney, in particular for mesangial cells and interstitial fibroblasts. It induces the generation of extracellular matrix proteins such as collagen and fibronectin, probably via transforming growth factor β (TGFβ) (90, 91). Accordingly, we showed that mice lacking intrarenal angiotensinogen synthesis develop less hypertensive damage in the kidney (92). Furthermore, the double-transgenic rat model expressing the human renin and angiotensinogen genes (93) revealed that Ang II elicits an inflammatory and immunological response that contributes to interstitial fibrosis, glomerulosclerosis, albuminuria, and, finally, renal failure (94, 95). It promotes inflammatory processes characterized by the infiltration of monocytes, neutrophils, and other cells of the immune system by NF-κB activation and by inducing the expression of cytokines.

The (P)RR has also been postulated to be involved in the pathogenesis of hypertensive and diabetic kidney damage. This is the conclusion from experiments showing that transgenic rats with generalized overexpression of the (P)RR develop glomerulosclerosis (96). Furthermore, a (handle-region) peptide inhibiting the interaction of (pro)renin with (P)RR blunted renal damage induced by diabetes and hypertension (97, 98). However, these data need confirmation because groups using other renal damage models have recently shown the same peptide to be inactive (99).

All components of the ACE2–Ang-(1–7)–Mas system are found in the kidney, and a permanent increase in Ang-(1–7) in transgenic rats exhibits weak antidiuretic effects (100). Mas-deficient mice exhibit hyperfiltration and microalbuminuria (101), and mice lacking ACE2 develop glomerulosclerosis and albuminuria at one year of age and accelerated nephropathy in diabetes (102–104). Moreover, ACE2 inhibition worsens glomerular injury in diabetes (104, 105). ACE2 may act by reducing local Ang II concentrations or by increasing Ang-(1–7), both of which would favorably change renal hemodynamics (106).

The kidney is the major target for the classical action of aldosterone: sodium retention (2). It acts on epithelial cells in the distal convoluted tubule and reorganizes the activity of ion pumps and channels accordingly. In particular, the epithelial sodium channel, ENaC, is regulated by

TGFβ: transforming growth factor β is a secreted protein that interacts with TGFβ receptors that are serine-threonine kinases activating SMAD transcription factors

Albuminuria: appearance of serum albumin in the urine, which can normally not pass the glomerular filter, indicating glomerular damage

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aldosterone at multiple levels. However, aldosterone also acts on other renal cells and amplifies the deleterious actions of Ang II.

This research all contributes to the conclusion that a local RAAS in the kidney exists and significantly contributes to renal function and nephropathology.

Vascular Wall

Angiotensinogen mRNA, protein, and the local generation of Ang II have been detected in the vessel wall (**Figure 5**) (107, 108). The expression of renin is hardly detectable there, but uptake of the protein from the circulation has been described (108). By directly activating AT1 receptors on vascular smooth muscle cells (52), Ang II enhances intracellular calcium and induces constriction, thereby increasing vascular tone and blood pressure. Furthermore, Ang II elicits the production of ROS in vascular smooth muscle. ROS are involved in vascular hypertrophy and inflammation

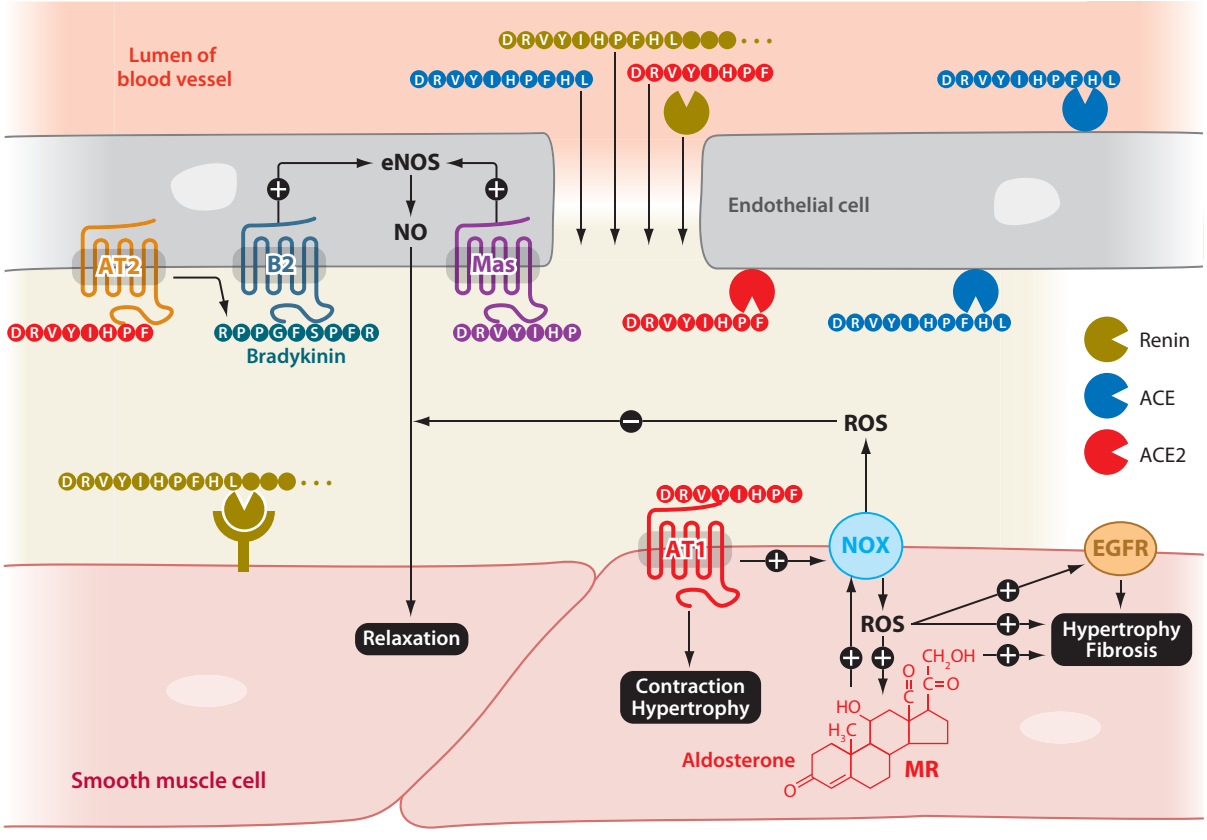

Figure 5

In the vascular wall, renin and angiotensinogen are primarily taken up from the plasma, and renin may be retained in the tissue by the (P)RR. ACE on endothelial cells generates Ang II, which interacts with AT1 receptors on smooth muscle cells to induce contraction. Ang II also elicits the generation of ROS by NAD(P)H oxidases (NOX), which in turn induce hypertrophy directly or after transactivation of the EGF receptor (EGFR) or the mineralocorticoid receptor (MR). On endothelial cells, Ang II binds to the AT2 receptor, which reacts by increasing bradykinin and activating the kinin B2 receptor. Furthermore, ACE2 metabolizes the peptide further to Ang-(1–7), which binds to its receptor Mas on the same cells. Mas and the B2 receptor both stimulate endothelial nitric oxide synthase (eNOS) to generate NO, which diffuses to the smooth muscle cells, where it induces relaxation.

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by inducing multiple kinase and transcription factor pathways and inactivating nitric oxide (NO) (109). Upregulation of ROS and downregulation of NO are hallmarks of endothelial dysfunction, which often accompanies or even causes hypertension.

There is evidence that the AT2 receptor exerts opposite effects to those of AT1 in the vascular wall. It activates the kallikrein-kinin system (KKS) and stimulates NO generation (110). Accordingly, mice deficient for AT2 are prone to hypertension (111), and animals overexpressing this receptor in vascular smooth muscle cells are protected from it (112).

However, not only smooth muscle and endothelial cells may mediate Ang-II actions in the vasculature. T-lymphocyte–deficient mice did not develop hypertension, endothelial dysfunction, or vascular damage after low-dose Ang II infusion. Thus, this cell type may also be involved in the actions of the peptide on vascular tone (113). These data may provoke a totally new concept of the vascular RAAS, but they need independent confirmation.

Recently, the issue became even more complicated with the appearance of new players in the RAAS, such as the (P)RR, ACE2, Ang-(1–7), and Mas, all present in the vascular wall (32). There, uptake of renin from the circulation may be carried out by the (P)RR. Accordingly, transgenic rats overexpressing this protein in smooth muscle cells accumulate prorenin in the vessel wall and develop elevated blood pressure (114, 115).

Ang-(1–7) is generated in the vascular wall from Ang II by ACE2 and interacts with Mas on endothelial cells (**Figure 5**) (32, 116). This interaction improves endothelial function and reduces blood pressure by decreasing ROS and increasing NO levels, as we recently showed using Mas-deficient mice (117, 118). Ang-(1–7) effects are mediated through stimulation of Akt-phosphorylation and eNOS activity (116, 119). Accordingly, when ACE2 is overexpressed in the vessels of spontaneously hypertensive rats, which are partially deficient for this enzyme, endothelial function improves and blood pressure significantly falls (120). Thus, the ACE2–Ang-(1–7)–Mas system counteracts the classical RAAS in the vessel wall.

Interestingly, Ang II can release NO and dilate vessels when AT1 is overexpressed in endothelial cells of transgenic mice (121). Thus, the net cardiovascular effect of angiotensin peptides in the vascular wall depends on the relative expression of classical and novel components of the RAAS in endothelial and smooth muscle cells.

Heart

For more than 20 years, it has been known that the heart can produce Ang II (**Figure 6**) (122, 123). Angiotensinogen is expressed in all parts of the heart and in cultured cardiac myocytes and fibroblasts (124). Although renin and its mRNA have been found in atria, ventricles, and isolated cardiomyocytes (125–127), the amount of renin mRNA in the heart is marginal, and, therefore, the heart itself is probably not a major source of cardiac renin. Accordingly, in binephrectomized animals cardiac renin activity vanished (128). (P)RR or other renin-binding proteins are probably also responsible for the uptake of the enzyme from the circulation into the heart (129). Furthermore, mast cells that invade the heart, in particular after myocardial infarction, can express and release renin from their granules (130).

ACE is produced in cardiac fibroblasts and coronary endothelial cells (131, 132). However, a second enzyme exists in particular in the human heart, the mast-cell derived chymase, which can also convert Ang I to Ang II (133) and is not inhibited by ACE inhibitors. However, because nearly all Ang II generation in the intact cardiac vessels is blunted by ACE inhibition (134), the relative role of chymase in the generation of the active peptide remains unclear.

Cardiac myocytes and fibroblasts, as well as sympathetic nerve endings in the heart, express AT1 receptors (52, 135, 136). By interacting with these receptors, Ang II exerts positive inotropic

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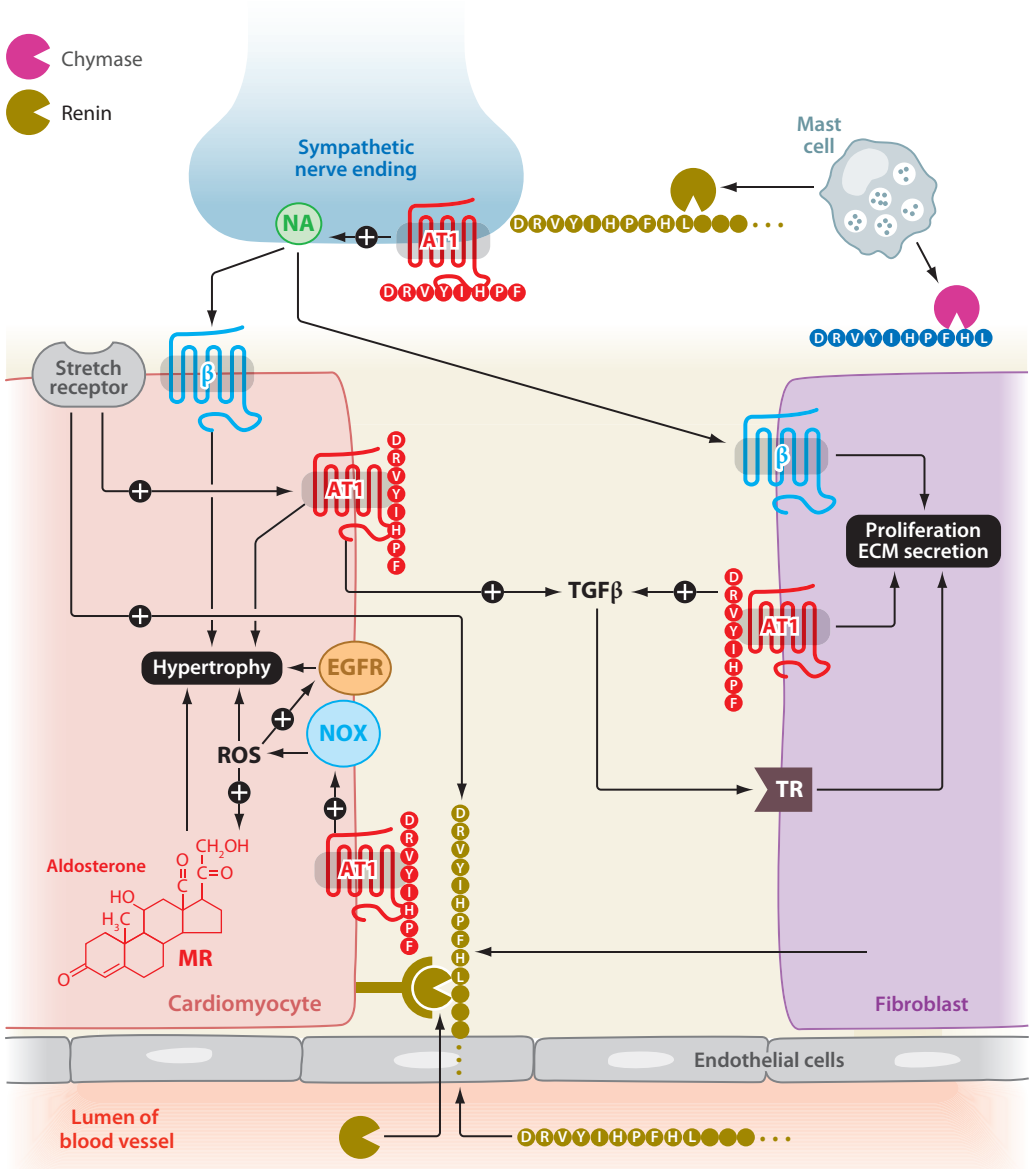

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Figure 6

In the heart, renin is primarily taken up from the plasma and retained in the tissue by the (P)RR but can also be produced by mast cells. Angiotensinogen is generated locally in cardiomyocytes and fibroblasts but can also come from circulating sources. ACE is available in abundance, although Ang II may also be generated by mast-cell-derived heart chymase. Ang II interacts with AT1 receptors on cardiac myocytes and fibroblasts, eliciting hypertrophy and extracellular matrix (ECM) secretion, respectively. It also elicits the generation of ROS by NAD(P)H oxidases, which in turn increase hypertrophy directly or after transactivation of the EGFR. Moreover, aldosterone binds to the MR in the presence of ROS and further aggravates the hypertrophic response. Ang II stimulates the release of norepinephrine (NA) from sympathetic nerve endings and TGFβ from cardiac cells that further enhance its effects by α- and β-adrenergic and TGFβ- (TR) receptors, respectively. Mechanical stretch elicits an increase in local angiotensinogen expression and thereby Ang-II generation and can directly activate AT1 receptors.

actions (137). A direct regulation of calcium homeostasis and transmembranous conductance in cardiomyocytes (138), and a facilitation of norepinephrine release from sympathetic nerve endings (139), mediate this effect.

Moreover, Ang II employs the AT1 (140) to induce cardiac hypertrophy and fibrosis, whereas AT2 receptors may have opposite effects (3, 141). Besides such direct effects, mediators such as endothelin (142) or norepinephrine (143) may be involved in the actions of Ang II on cardiomyocyte growth. Ang II also elicits the generation of ROS, which in turn induces cardiac hypertrophy, partially by transactivation of the EGFR (10). In addition, ROS may activate MR bound mainly by glucocorticoids in the heart (see above) and thereby increase hypertrophy and fibrosis, an effect that can be ameliorated by MR antagonists.

There is a longstanding debate about the importance of mechanical stretch for cardiac hypertrophy and the regulation of the local RAAS in the heart. Stretch induces the release of Ang II in the myocardium, as well as from cardiomyocytes in culture (144, 145), but can activate AT1 receptors in the absence of the ligand (146). Transgenic animal models have been generated to tackle the question of whether mechanical stretch needs the cardiac RAAS to induce hypertrophy. Indeed, we could show that mice lacking local angiotensinogen generation in the heart are at least partly protected from hypertension-induced cardiac hypertrophy and fibrosis (92). However, mice totally lacking Ang II or AT1 receptors develop cardiac hypertrophy after volume or pressure overload (147). Cardiomyocytes isolated from angiotensinogen-deficient mice exhibit a hypertrophic response to mechanical stretch. However, in contrast to control cells, AT1 antagonists do not block this effect (148). Furthermore, in the kidney-transplantation experiments that employ AT1-receptor-deficient mice mentioned above, the extent of cardiac hypertrophy correlated only with the blood pressure of transplanted mice and not with the presence or absence of AT1 receptors in the heart (88). These results indicate that there are pathways of growth induction by stretch in cardiomyocytes for which the cardiac RAAS may be only a modifier.

Transgenic experiments designed to answer the opposite question, whether Ang II alone is able to induce cardiac hypertrophy without hemodynamic changes, gave inconsistent results (149). When angiotensinogen, ACE, or a protein releasing Ang II were overexpressed exclusively in the heart of transgenic rats or mice, these animals did not exhibit cardiac hypertrophy as long as they were not hypertensive, despite augmented cardiac Ang II concentrations (150–152). Nevertheless, in some cases increased fibrosis and an amplified hypertrophic response to pressure overload were reported (150, 151). Most but not all transgenic rat and mouse models overexpressing the AT1 receptor on cardiomyocytes showed the same phenotype (153, 154). However, some animal models with cardiac AT1 overexpression developed cardiac hypertrophy but only when the interaction domain of the transgenic AT1 receptor with the EGFR was intact (155–158). Thus, locally produced Ang II alone is probably not sufficient to induce cardiac hypertrophy, but it may enhance this process after induction by stretch and EGFR.

Besides hypertrophy, Ang II also induces cardiac fibrosis, that is, the proliferation of cardiac fibroblasts and the excessive deposition of extracellular matrix in the cardiac interstitium (159). Fibrosis is of major pathophysiological relevance because the resulting increase in ventricular stiffness causes diastolic dysfunction and, ultimately, heart failure. Experiments with chimeric mice carrying AT1-deficient cardiac cells, which are surrounded by normal tissue, revealed that the activation of fibroblasts by Ang II depends on the interaction of the peptide with neighboring cardiomyocytes (160). Probably, cardiomyocytes need to release a paracrine factor, for example, TGFβ (161), which then conveys the mitogenic effect of Ang II on fibroblasts. Nevertheless, direct growth-promoting actions of Ang II on cultured cardiac fibroblasts have also been repeatedly demonstrated (159).

Numerous studies have recently been performed to clarify the role of the ACE2–Ang-(1–7)–Mas system in the heart. Mice lacking ACE2 or Mas exhibit reduced ventricular contractile

function (102, 162, 163). Permanent increase of Ang-(1–7) in the plasma of transgenic rats overexpressing an artificial carrier protein protects the animals from cardiac hypertrophy (164). A similar phenotype is observed when Ang-(1–7) is overexpressed, specifically in the heart of transgenic mice (165) and rats (M. Bader, unpublished results), using the same peptide-releasing system. By interacting with Mas, the peptide increases NO production and Akt phosphorylation in cardiomyocytes (166) and reduces the expression of collagen and growth factors in cardiac fibroblasts (167).

Whereas general (168) or cardiac overexpresssion of ACE2 in adult rats by lentiviral gene transfer exerts protective actions (169), transgenic mice with cardiac ACE2 overexpression surprisingly developed arrhythmias and early death (170). In these mice, however, ACE2 is already expressed in embryos and may metabolize substrates other than angiotensins, leading to developmental abnormalities. Apelins are candidates for the observed phenotype because they are ACE2 substrates (29) and are crucial for cardiac development, at least in zebrafish (171). Taken together, the ACE2–Ang-(1–7)–Mas system is present in the heart and exerts protective actions, at least in adults.

This research shows that there is a local RAAS in the heart, which is involved in the crosstalk between fibroblasts and cardiomyoytes and thereby in the pathogenesis of cardiac hypertrophy and fibrosis. Consequently, ACE inhibitors, AT1, and MR antagonists prevent or decrease cardiac hypertrophy in humans and experimental animal models, even in concentrations that do not influence systemic hemodynamics. These drugs also attenuate cardiac remodeling after myocardial infarction and improve the functional status of the heart, thereby reducing mortality (172, 173). Consequently, most clinical trials with patients suffering from left ventricular dysfunction after myocardial infarction confirmed a strong cardioprotective action of ACE inhibitors, AT1, and MR antagonists (17, 18, 172, 174–180).

THERAPEUTIC IMPLICATIONS

Existing Drugs

ACE inhibitors. Pharmacological intervention in RAAS began in the late 1960s with the discovery that the venom of the Brazilian snake *Bothrops jararaca* contains a substance that inhibits ACE (181). In the first clinical trials, this substance proved to be a potent antihypertensive agent, but it had the disadvantage that it could only be taken by injection. By modeling the active site of ACE and designing drugs potentially binding to this site, the first orally available ACE inhibitor, captopril, was discovered (182). In the meantime, more than a dozen -prils have been developed and marketed: captopril, enalapril, lisinopril, perindopril, cilazapril, benazepril, quinapril, fosinopril, ramipril, moexipril, imidapril, and trandolapril. ACE inhibitors are first-choice antihypertensive drugs. Furthermore, a multitude of large-scale clinical studies have proven a strong beneficial effect of these drugs on morbidity and mortality in congestive heart failure (e.g., after myocardial infarction) and chronic renal diseases (e.g., caused by diabetes or hypertension) (175, 183). Because ACE is a major kinin-degrading enzyme (184), ACE inhibitors also increase kinin concentrations. Furthermore, it has been shown that these drugs potentiate kinin effects by modulating a direct interaction between the ACE protein and the kinin B2 receptor, which is independent from the enzymatic activity of ACE (185). Kinin potentiation may be involved in the beneficial actions of ACE inhibition because kinins are known to exert cardio- and renoprotective actions. However, it is also the major reason for the adverse side effects of ACE inhibitors, namely cough and angioedema.

AT1 antagonists. A second group of drugs that interfere with RAAS are specific antagonists for the AT1 receptor. The first example of this class was losartan (186), which was followed by

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several other –sartans: telmisartan, candesartan, valsartan, eprosartan, irbesartan, olmesartan, and zolasartan (187). These drugs exert a more complete angiotensin blockade because alternative pathways of angiotensin generation not affected by ACE inhibitors and employing cathepsins, tonin, or chymase become ineffective by AT1 antagonism. They are also more specific for the RAAS than ACE inhibitors because other peptide systems are not affected. However, the compensatory increase in renin concentration after AT1 blockade leads to an accumulation of Ang II, which activates the AT2 receptor. It is yet unknown whether this AT2 stimulation often followed by kinin generation is involved in the action of AT1 antagonists.

Renin inhibitors. The most logical point to interfere pharmacologically with the RAAS is the rate-limiting enzyme renin. Intervention at this step is more specific than ACE inhibition and AT1 antagonism because very little angiotensin peptide can be generated, and no other peptide system would be directly affected. Renin inhibitors were developed decades ago, but the first one, aliskiren, came on the market only recently (188). Because the human renin protein is different from rodent enzymes and interacts with only primate or human angiotensinogen, double transgenic rats with the human RAAS were instrumental in developing these drugs (189). The first trials show that this new class of drugs seems to be at least as effective as established inhibitors of the RAAS (190–193).

Vasopeptidase inhibitors. Inhibition of two related enzymes, ACE and neutral endopeptidase 24.11 (NEP), may exert potentiated beneficial actions. NEP degrades vasodilatory peptides such as kinins, natriuretic peptides, and adrenomedullin; therefore its inhibition should complement the vasodilatory action of ACE inhibition (187, 194). Because ACE and NEP are similar in structure, it was possible to develop inhibitors with dual specificity for both enzymes, such as omapatrilat, fosidotrilat, sampatrilat, and gemopatrilat. In the first clinical trials, the -patrilats have proven to be even more effective than ACE inhibitors in blood pressure reduction and in improving congestive heart failure (195). However, the side effects increased, mainly owing to accumulation of kinins; therefore, the safety profile of these substances has to be improved. Pure NEP inhibitors in combination with AT1 receptor blockers may be a safer alternative (196). In any case, NEP inhibition has to be regarded with caution owing to the multiple peptide systems influenced by the treatment. As one example, NEP-deficient mice are more susceptible to Alzheimer's disease (197).

MR antagonists. As described above, MR is a potent inducer of fibrosis in the heart, and therefore antagonists such as spironolactone and eplerenone have been very successfully used in clinical trials to treat congestive heart failure (17, 18). Spironolactone causes more unwanted side effects because it is less specific for the MR than eplerenone and inhibits the androgen receptor, causing unwanted side effects (198).

Vaccination. An old concept has been resuscitated recently, which is the use of vaccination to treat hypertension (199). A multicenter study of a vaccine that induces antibodies against Ang II has yielded promising results in terms of blood pressure reduction (200). However, the specificity and controllability of the treatment needs to be evaluated.

Novel Drug Targets Based on Tissue RAASs

The pathophysiological importance of tissue RAASs has obvious therapeutic implications. Several novel drug targets have appeared, based on the findings summarized above.

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AT2 agonists. The AT2 receptor, which has been described to be beneficial in heart and vessels, is one of the new drug targets (3). A first agonistic compound, compound 21, has shown promising results in preclinical studies (201).

Dual-action antagonists for AT1 and endothelin ETA receptors. Endothelin via its ETA receptor has similar effects as Ang II in heart, vessels, and kidney, and there is an intensive interplay between the actions of the two peptide systems in these organs. Therefore, inhibitors have been developed that block both receptors. The first data from animal models look promising, and clinical trials are pending (187, 202).

Activators of the ACE2–Ang-(1–7)–Mas system. Another obvious target is the ACE2–Ang-(1–7)–Mas system, which counteracts Ang II in cardiovascular organs. In this respect, activators of ACE2, such as XNT, have been developed and successfully tested in animal models (203, 204). Furthermore, Mas agonists such as Ang-(1–7) itself packaged in an oral formulation (205) or analogs of it, such as AVE0991, have been synthesized and also yielded promising results in animal models of cardiovascular diseases (206). However, the efficiency of these drugs awaits validation in humans.

(P)RR inhibitors. For heart and vessels, the (P)RR or other renin-binding proteins are pivotal for the activation of the local RAAS, because the initiating enzyme of the cascade, (pro)renin, is largely missing in these organs and has to be taken up from the circulation. Thus, targeting renin uptake would block most tissue RAASs at their initial step. The handle-region peptide is a prototype of such drugs (207), which are being developed in several labs. (P)RR inhibitors should also exert additional protective actions by blocking the signaling function of (P)RR. However because the (patho)physiological role of the (P)RR and the mechanisms of action of the handle region peptide are not yet completely resolved, the suitability of such drugs needs confirmation.

Aldosterone synthase inhibitors. Although glucocorticoids may be agonists on the MR in cardiovascular target organs (see above), aldosterone synthase inhibitors such as FAD286 have been developed and tested in animal models. Clear data show that they are effective in reducing cardiac and renal damage, even in the presence of normal glucocorticoid levels (20, 208). This provides further evidence for aldosterone and not glucocorticoids to be the major pathophysiological agonist at the MR in cardiovascular tissues, and provides the opportunity to inhibit the RAAS at another level to treat cardiovascular diseases.

CONCLUSIONS AND PERSPECTIVES

Local RAASs exist in organs relevant for cardiovascular control such as heart, kidney, brain, and vessels. In general, they mediate and enhance the effects of the circulating RAAS in respective organs. Local RAASs have important physiological functions in cardiovascular homeostasis but are also of central relevance for the etiology of hypertension and hypertensive and diabetic end-organ damage. This explains the particular effectiveness of drugs interfering with the RAAS in cardiovascular diseases.

Novel therapeutic strategies should target these tissue-based RAASs. Studies on the brain RAAS predict that RAAS inhibitors that cross the blood-brain barrier should be more efficient than others, but clinical trials confirming this notion are pending. Furthermore, novel classes of drugs targeting locally active RAAS components, such as aldosterone synthesis blockers, (P)RR antagonists, ACE2 activators, and Mas- and AT2-agonists are being evaluated and may augment our

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toolbox to beneficially interfere with the RAAS. This toolbox has already recently been extended by the implementation of MR antagonists, renin and vasopeptidase inhibitors, and vaccination.

SUMMARY POINTS

1. The classical endocrine RAAS is a central regulator of cardiovascular homeostasis, but its actions are often mediated by local RAASs in tissues.
2. New components of the RAAS have recently been described, including the ACE2–Ang-(1–7)–Mas system, which mainly counteracts angiotensin II effects, and the (pro)renin receptor, which retains and activates (pro)renin in tissues.
3. In most areas of the brain, only locally generated angiotensins can bind to respective receptors because the blood brain barrier deters plasma peptides from entering.
4. In the brain, locally generated angiotensin II mediates the actions of circulating angiotensin II by stimulating vasopressin secretion, sympathetic activity, and thirst, and by modulating the baroreceptor reflex.
5. In the vascular wall, the local activity of ACE2 determines the relative amounts of the vasoconstrictive and reactive-oxygen generating peptide angiotensin II and the vasodilatory and NO-generating peptide angiotensin-(1–7).
6. In the kidney, angiotensin II is locally generated in the interstitium and in the tubular lumen. The peptide is involved in epithelial transport processes and in the development of diabetic and hypertensive nephropathy.
7. In the heart, angiotensin II is locally synthesized and participates in the cross talk between cardiomyocytes and fibroblasts, leading to cardiac hypertrophy and fibrosis.
8. Inhibitors of the classical RAAS have been in clinical use for more than 30 years and are extremely successful for the treatment of cardiovascular diseases, such as hypertension and heart failure as well as diabetic nephropathy.

FUTURE ISSUES

1. Physiological roles of the ACE2–Ang-(1–7)–Mas system and of the (pro)renin receptor need to be further clarified.
2. Mechanisms by which ROS, glucocorticoids, and aldosterone regulate mineralocorticoid receptor activity in extrarenal tissues need to be characterized. Moreover, mediators of the fast nongenomic actions of aldosterone have to be found.
3. Enzymes involved in the generation of angiotensins in the brain, besides renin and ACE, as well as the site of their synthesis, intracellular vesicles, or extracellular space, need to be elucidated.
4. In the vascular wall, the role of cells of the immune system in angiotensin II actions is of highest interest.
5. In the heart, the relative importance of cardiomyocytes and fibroblasts in angiotensin II actions awaits clarification.

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6. Based on findings about local RAASs, drugs that specifically target these systems, in particular the one in the brain, should be selected or developed for therapy.
7. Newly discovered components of the RAAS are suitable drug targets that should be validated.

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