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REVIEW

Blood pressure and renal hemodynamic effects of angiotensin fragments

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Angiotensin (Ang) II, the main effector peptide of the renin–Ang system, increases arterial blood pressure through Ang II type 1A (AT_{1a}) receptor-dependent arterial vasoconstriction and by decreasing renal salt and water excretion through extrarenal and intrarenal mechanisms. AT_2 receptors are assumed to oppose these responses mediated by AT_1 receptors, thereby attenuating the pressor effects of Ang II. Nevertheless, a possible role of AT_2 receptors in the regulation of renal hemodynamics and sodium homeostasis remains to be unclear. Several other Ang fragments such as Ang III, Ang IV, Ang-(1–7) and Ang A have also been shown to display biological activity. In this review, we focus on the effects of these Ang on blood pressure, renal hemodynamics and sodium water handling, and discuss the receptors involved in these actions.
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INTRODUCTION

The renin–angiotensin (Ang) system (RAS) plays a central role in the control of arterial blood pressure and sodium water homeostasis. Renin, a proteolytic enzyme secreted by the juxtaglomerular apparatus of the kidney, cleaves angiotensinogen at the N terminus to form the decapeptide, Ang I. The latter has no appreciable biological activity, but is converted by the dipeptidyl carboxypeptidase, Ang-converting enzyme (ACE), to the octapeptide Ang II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe)[1–3] (Figure 1). ACE is a membrane-bound enzyme on the surface of endothelial cells and is particularly abundant in the lung. ACE also inactivates bradykinin and a number of other peptides. Ang II, the main effector peptide of the RAS, is degraded by aminopeptidases A and N to Ang III and Ang IV, respectively (Figure 1). These metabolites have long been considered of little importance, but are now known to exert biological activity.[4–6] This is also the case for other Ang fragments: the heptapeptide Ang-(1–7), which is processed from Ang I by tissue endopeptidases,[7] and the octapeptide Ang A, which is generated from Ang II by enzymatic decarboxylation of Asp.[1,8]

The RAS was originally regarded as a circulating system; however, the existence of 'local' or 'tissue' RAS has been identified in most organs.[9] ACE is indeed also present in other vascular tissues and organs, including the heart, brain and kidney. Therefore, Ang II can be formed locally in different vascular beds and can exert biological effects independent of blood-borne Ang II. In the kidney, most of the intrarenal Ang II is locally generated, rather than derived from circulating Ang I or Ang II.[10,11] Ang II and Ang III concentrations in renal interstitial fluid are roughly 1000-fold higher than that in the plasma.[12]

In this review, we focus on the effects of different Ang peptides on blood pressure, renal hemodynamics and sodium excretion, and discuss the receptors involved in these effects (Table 1).

ANGIOTENSIN II

AT_1 and AT_2 receptors

The biological functions of Ang II are mediated by at least two pharmacologically distinct receptors, the Ang II type 1 (AT_1) and Ang II type 2 (AT_2) receptors (Figure 2).[13] Both are seven-trans-membrane glycoproteins (G-protein-coupled receptors) with 30% sequence similarity,[13,14] and both are expressed in the kidney.[15–18]

AT_1 receptors are abundantly expressed in cells of the renal glomeruli, tubules, vasculature and interstitial space.[18] In rodents, two subtypes of the AT_1 receptor have been identified, AT_{1a} and AT_{1b} receptors,[19] which share 95% sequence homology and exhibit similar ligand binding affinities and signal-transduction properties, but differ in their tissue expression. In the kidney, AT_{1a} mRNA is present in mesangial and juxtaglomerular cells, proximal tubules, vasa recta and interstitial cells, whereas AT_{1b} mRNA is found only in mesangial and juxtaglomerular cells, and in the renal pelvis. AT_{1a} receptors are more abundant in the kidney than AT_{1b}.[20] AT_{1a} receptors are also expressed in the liver, adrenal gland, ovary, heart, aorta, lung, testis, brain, adipose tissue and vascular smooth muscle, whereas AT_{1b} receptors are confined to the adrenal gland, brain and testis.[21]

AT_2 receptors are highly expressed during fetal development and in newborn mammalian kidneys, with very little expression in the adult mammalian kidney.[22] This has led to the suggestion that AT_2 receptors may be involved in development, differentiation and/or growth.[23–25] In the adult kidney, AT_2 receptors are mainly localized in the

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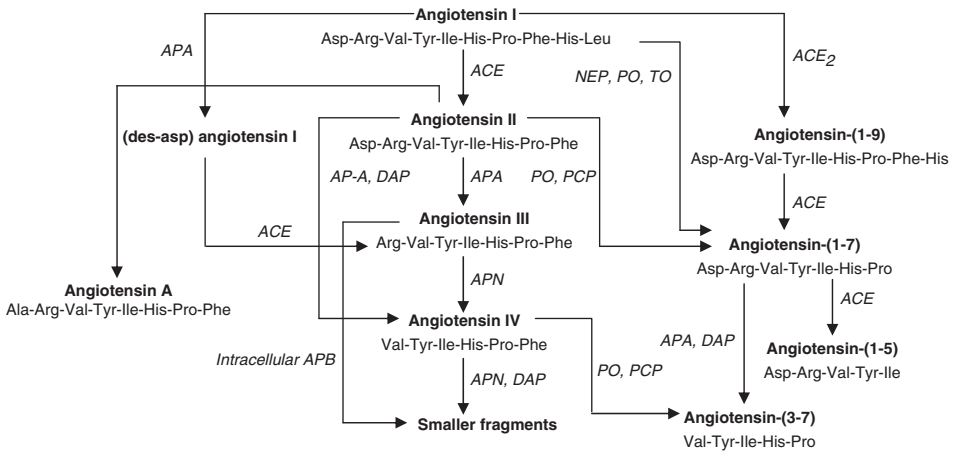

Figure 1 Overview of the chemical structures and the enzymes involved in the synthesis of Ang II and its different fragments. ACE, angiotensin-converting enzyme; ACE_2, human angiotensin-converting enzyme homologue; Ang, angiotensin; APA, APB, APN, aminopeptidase A, B and N; DAP, dipeptidyl aminopeptidases; PO, propyl oligopeptidase; PCP, carboxypeptidase; NEP, neprilysin; TO, thimet oligopeptidase.

Table 1 Main effects of Ang fragments in different animal models

	Rodents	*Dogs*	*Human*
Ang II	AT_1 receptor-mediated increased BP, RVR, decreased RBF; increased aldosterone secretion and increased sodium reabsorption AT_2 receptor-mediated vasodilation and hypotensive effect (conflicting results) AT_2 receptor-mediated natriuresis	Sodium retention at low doses and pressure natriuresis at high doses	AT_1 receptor-mediated increased BP, RVR, decreased RBF; increased aldosterone secretion and increased sodium reabsorption AT_2 receptor-mediated vasodilation (few studies)
Ang III	AT_1 receptor-mediated increased BP, RVR, decreased RBF; increased aldosterone secretion AT_2 receptor-mediated natriuresis (SHR)	Increased aldosterone secretion	Increased aldosterone secretion
Ang IV	AT_4 receptor-mediated increased RBF in some studies (not confirmed by others) AT_1 receptor dependent increased BP, RVR, decreased RBF	No effect on BP, sodium excretion or aldosterone secretion	No effect on BP, sodium excretion or aldosterone secretion
Ang-(1–7)	Mas receptor-mediated decreased BP Conflicting data on effects renal hemodynamics and sodium excretion	No effect on BP, sodium excretion or aldosterone secretion (increased sodium excretion in another study)	No effect on BP, sodium excretion or aldosterone secretion
Ang A	AT_1 receptor-mediated increased BP, RVR, decreased RBF;		

Abbreviations: Ang, angiotensin; AT_1, Ang II type 1A; BP, blood pressure; RBF, renal blood flow; RVR, renal vascular resistance; SHR, spontaneously hypertensive rats.

glomerular mesangial cells[22,26,27] or the adventitia of the preglomerular arcuate and interlobular arteries.[28] AT_2 receptors are upregulated in the adult kidney in response to sodium depletion[27] or kidney damage[29] in obese Zucker rats[30] and spontaneously hypertensive rats (SHRs).[31] In other pathological conditions, such as stroke, an increased AT_2 receptor gene expression in the infarcted cortex was reported.[32]

Few studies have investigated the functional expression of AT_2 receptors in human beings. They are expressed in human skin[33,34] and in the coronary circulation.[35] In the adult human renal cortex, AT_2 receptor mRNA is mainly expressed in interlobular arteries.[36]

Effects of Ang II on blood pressure and sodium–volume homeostasis

Ang II increases arterial pressure via two principal effects. The first, vasoconstriction, occurs very rapidly, within seconds, and very intensely in the arterioles and to a considerably lesser extent in the veins. The second is the effect on the kidneys to decrease the excretion of both salt and water. This increases the extracellular fluid volume, which then increases arterial pressure slowly over a period of hours and days. This long-term effect, acting through the extracellular fluid volume mechanism, is even more powerful than the acute vasoconstrictor mechanism in increasing blood pressure.

Ang II causes the kidneys to retain salt and water through extrarenal and intrarenal mechanisms.[37] Ang II increases sympathetic nerve stimulation, which increases renal tubular sodium reabsorption directly or indirectly through renal vasoconstriction. Moreover, Ang II stimulates the synthesis and secretion of aldosterone from the adrenal cortex, and aldosterone in turn increases salt and water reabsorption by the distal tubule.[38,39] Within the kidney, Ang II increases predominantly proximal tubular sodium reabsorption. It also induces renal microvascular constriction, in particular of the

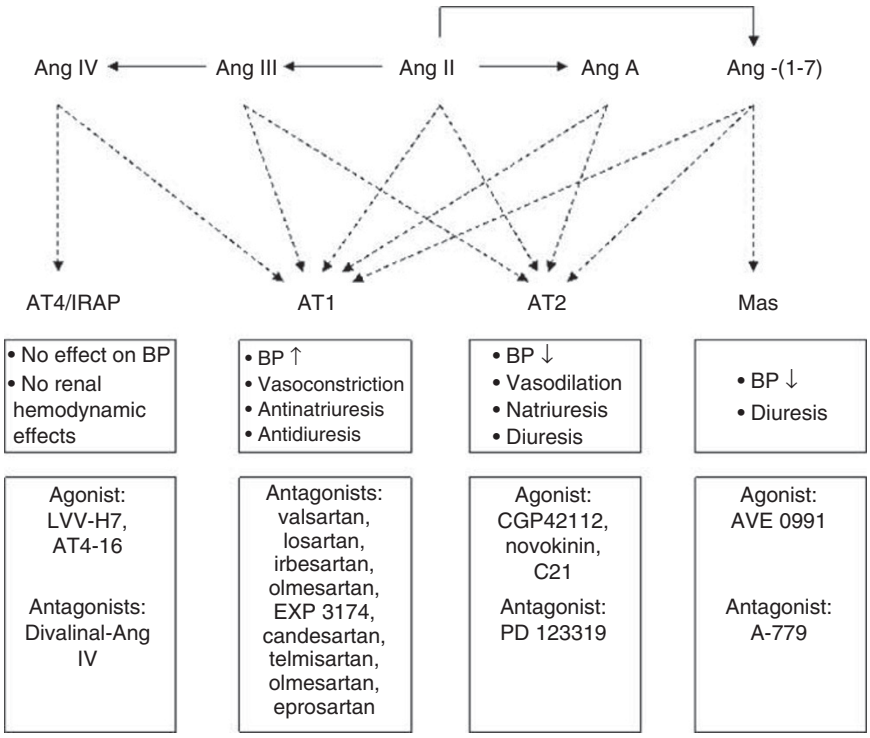

Figure 2 Angiotensin fragments and their actions on the different receptors.

efferent arterioles. This helps maintaining the glomerular filtration rate and tends to increase sodium reabsorption by altering peritubular capillary physical forces.[40]

During hypovolemia (for example, hemorrhagic shock) and in sodium-deficient states, intrarenal Ang II levels are elevated and, in turn, increase both renal sodium and water reabsorption, thereby playing an important physiological role in maintaining circulating volume and blood pressure.[37,41]

AT_1 receptor-mediated blood pressure effects of Ang II

The role of Ang II in the regulation of blood pressure has been extensively investigated using different animal models. In anesthetized rats, systemic (intravenous, i.v.) or local (intrarenal, i.r.) administration of Ang II led to pressor effects, which could be blocked by AT_1 receptor antagonists.[13,42–45] Gene-targeting studies further pointed out that these effects are mainly mediated by AT_{1a} receptors.[46,47] Knock out (−/−) of the *AT_{1a}* receptor gene decreased baseline blood pressure[46,48] and abolished the pressor response to Ang II infusion,[46,49] whereas deletion of *AT_{1b}* genes did not alter baseline blood pressure, nor the pressor response to Ang II.[46,50] Ang II induced dose-dependent increases in blood pressure in AT_{1a} (−/−) mice pretreated with an ACE inhibitor, which were inhibited by AT_1 receptor blockade.[51] Therefore, the AT_{1b} receptor may contribute to the cardiovascular effects of Ang II in the absence of the AT_{1a} receptor, and presumably is subsidiary to the major AT_{1a} subtype in normal animals. This notion is supported by data obtained in mice with a double AT_{1a} (−/−) and AT_{1b} (−/−), which have a more severe phenotype with a significant lower body weight, kidney weight, blood pressure and heart rate than mice lacking only the AT_{1a} receptor.[46,52]

AT_1 receptors are expressed in many organ systems, including the circulatory system, the central nervous system and the urinary system, and so on,[53] which are presumed to play key functions in blood pressure homeostasis. Interestingly, a cross-transplantation study between genetically matched AT_1 (−/−) and wild-type mice revealed that, for the development of Ang II-induced hypertension and cardiac hypertrophy, renal AT_1 receptors are required.[54]

In man, Ang II also exerts vasoconstriction, sodium retention and aldosterone secretion through AT_1 receptor stimulation, and drugs inhibiting Ang II formation or selectively blocking the AT_1 receptor are highly effective antihypertensive agents.[55,56]

AT_1 receptor-mediated renal effects of Ang II

Systemic administration[57] and i.r. administration[58–61] of Ang II produced dose-dependent decreases in total renal blood flow (RBF) and renal cortical blood flow (CBF), and increases in renal vascular resistance and renal cortical vascular resistance, which were blocked by AT_1 receptor antagonists.[47,62] Within the microvasculature, Ang II constricted both the afferent and efferent arterioles,[63,64] although the efferent arterioles are much more sensitive.[40] Blockade of the AT_1 receptor elicited increases in RBF, indicating a role of Ang II in maintaining renal vascular tone.[57]

I.r. infusion of Ang II in dogs at rates chosen to increase renal arterial concentration by only 10–15 pg ml, a level that is still within the physiological concentrations of Ang II, markedly reduced sodium excretion,[40] indicating that physiological levels of Ang II are capable of inducing antinatriuretic effects. Olsen *et al.*[65] compared the responses with various rates of Ang II infusion in normal dogs in which renal perfusion pressure was permitted to increase and in the same dogs when renal arterial pressure was servo-controlled during Ang II infusion. These experiments showed that, in normal dogs, low doses (5–45 ng kg^{-1} min^{-1}, i.v.) of Ang II decreased sodium excretion, whereas infusion rates of 135–1215 ng kg^{-1} min^{-1} i.v. caused natriuresis and diuresis related to the increase in blood pressure. This phenomenon is commonly referred to as 'pressure natriuresis'.[66–69] In contrast, when renal arterial pressure was servo-controlled, Ang II

infusion at all infusion rates (5–1215 ng kg^{-1} min^{-1}, i.v.) in the same dogs decreased urinary sodium excretion.[65] Therefore, in animal experiments, depending on the dose of the Ang II as well as the magnitude of the blood pressure responses, both antinatriuresis and natriuresis can occur. Consistent with this notion, micropuncture and microperfusion studies showed that Ang II modulated proximal tubular sodium reabsorption effects in a dose-dependent and biphasic manner[70–73] through apical sodium channels.[74] The physiological significance of this pressure natriuresis is uncertain, although it is probably involved in certain pathological conditions such as malignant hypertension.[40]

AT_2 receptor-mediated renal and blood pressure effects of Ang II?

The function of the AT_2 receptor is less well understood, possibly because the AT_2 receptor has a low degree of expression compared with that of the AT_1 receptor. In general, it is assumed that AT_2 receptors oppose the responses mediated by AT_1 receptors.[35,75,76] Although the AT_1 receptor is involved in pressor, vasoconstrictor and antinatriuretic effects, the AT_2 receptor appears to mediate depressor effects, vasodilation and natriuresis.[77,78] AT_2 receptor stimulation could increase the production of NO and cGMP either by increasing bradykinin production and bradykinin B2 receptor stimulation or by direct activation of NO production.[79] AT_2 receptor-mediated vasodilation has been shown in small resistance arteries of the mesenteric, uterine, adrenal, coronary and peripheral circulations in a wide variety of animal models and, in human beings, in large capacitance vessels such as the aorta and in the fetal circulation.[79] AT_2 receptor-mediated vasodilation in the renal vasculature has so far not been shown. AT_2 receptors were reported to be upregulated and to contribute to Ang II-induced vasodilation in resistance arteries of hypertensive type 2 diabetes patients treated with AT_1 receptor blockers.[80] AT_2 (−/−) mice have elevated basal blood pressure and exaggerated pressor responses to exogenous Ang II as compared with wild-type litter mates, which is line with the hypothesis that AT_2 receptors counteract AT_1 receptor-mediated responses.[46,75,76,81,82] Moreover, AT_2 (−/−) mice have increased prostaglandin E2 and prostacyclin levels, suggesting that these vasodilator prostanoids might be important in preventing hypertension in this model.[83] Alternatively, the enhanced pressor response to Ang II in AT_2 (−/−) mice could also be due to the upregulation of AT_1 receptors triggered by AT_2 receptor deficiency.[84,85] AT_2 receptor-mediated vasodilator and depressor actions of Ang II appear more easily when AT_1 receptors are blocked by AT_1 receptor antagonists.[79,86,87] This might be due to the predominance of the AT_1 receptor over the AT_2 receptor expression in blood vessels.[79] It was suggested that hypotensive responses to AT_1 receptor blockade are mediated, at least in part, by AT_2 receptor activation. The vasodilator effects mediated by AT_2 receptors are also facilitated when the RAS is activated by dietary Na^+ restriction,[79] or when AT_2 receptors are upregulated as in SHR.[31,86–89]

AT_2 receptor-mediated inhibition of Na^+ transport in rabbit proximal tubule cells has been shown in an *in vitro* study.[90] AT_2 (−/−) mice were shown to display antinatriuretic hypersensitivity to exogenous Ang II and a shift to the right (less sensitive) in their pressure–natriuresis curves.[82,91] Direct renal interstitial microinfusion of a selective AT_1 receptor antagonist did not influence systemic hemodynamics and did not induce any hormonal changes, but induced a natriuretic response that was abolished by intrarenal co-infusion of the AT_2 receptor antagonist PD-123319 (ref. 92), suggesting that the natriuretic response to AT_1 receptor blockade is mediated by AT_2 receptor activation.

Effect of Ang II on medullary RBF

Ang II elicits a paradoxical medullary vasodilatation in normotensive animals, mediated by a secondary activation of vasodilator paracrine agents such as prostaglandins, kinins and NO, rather than to a direct action via AT_2 receptors.[42,93,94] Sarkis *et al.*[95] observed a biphasic medullary blood flow following i.v. injection of Ang II in normotensive rats characterized by an initial rapid and short-lasting (<1 min) decrease (vasoconstrictor component), followed by a marked and longer-lasting (>2 min) increase (AT_1 receptor-dependent vasodilator component) in medullary blood flow. The vasodilator component was mainly due to the release of prostaglandins and, to a lesser extent, of nitric oxide (NO) and kinins.[95] In SHR and Lyon genetically hypertensive rats, the vasoconstrictor component was more pronounced, and/or the vasodilator component was attenuated after stimulation with Ang II.[95,96]

ANGIOTENSIN III

Ang III is generated from Ang II by the enzyme aminopeptidase A (Figure 3),[97] or from Ang I, which can be converted directly to Ang III by ACE.[98] Like Ang II, Ang III displays similar affinity for AT_1 and AT_2 receptors (Figure 2),[99] but it is more sensitive to aminopeptidase N.[100]

Ang III reduces RBF after systemic administration.[101] I.v. Ang III or Ang II achieving the same plasma concentrations in conscious dogs had equipotent AT_1 receptor-dependent effects on blood pressure and sodium excretion, but the metabolic clearance rate of Ang III was five times that of Ang II.[102] This study supports earlier conclusions that Ang II is the main effector of the 'circulating RAS'. However, data obtained in SHR point to Ang III as an important effector peptide. Ang III- and Ang II-induced dose-dependent increases in renal perfusion pressure were both enhanced in SHR compared with WKY rats,[103] but kidneys of SHR displayed higher activity of aminopeptidase A, the principal enzyme that hydrolyzes Ang II to Ang III,[104] suggesting that Ang III could contribute to the enhanced renal response to Ang II in the SHR. Moreover, Ang III, but not Ang II, induced natriuresis through AT_2 receptor activation during AT_1 receptor blockade in SHR.[92] This natriuresis was augmented by the blockade of aminopeptidase N, an enzyme metabolizing Ang III to Ang IV,[105] suggesting an important role of Ang III in sodium excretion. Further research is required to clarify a possible role of

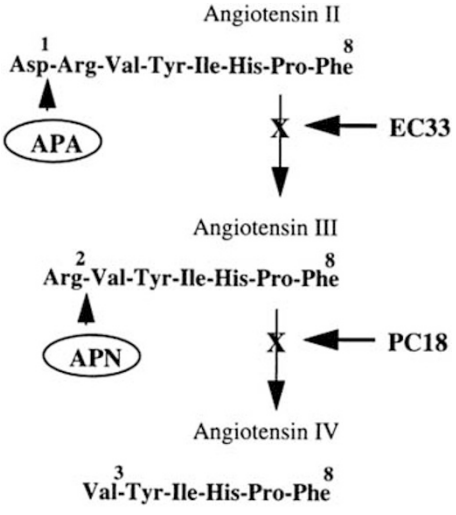

Figure 3 Metabolic conversion of angiotensin (Ang) II, via Ang III–IV. EC33 and PC18 are selective inhibitors of aminopeptidase A (APA) and aminopeptidase (APN), respectively.

Ang III in the regulation of the renal hemodynamics and sodium excretion.

Ang III is also a potent stimulator of aldosterone secretion. In the rat[57,106] and in anesthetized dogs,[107,108] infusion of Ang III stimulated aldosterone release similarly to Ang II, although it appeared less potent. On the other hand, in a study in conscious dogs during double blockade of the RAS (combined ACE inhibition and aldosterone receptor blockade), Ang III was significantly more potent than Ang II in increasing aldosterone release[55,109] and also produced a very potent antinatriuretic effect already at a dose not producing an increase in blood pressure.[109] In healthy human beings under acute combined ACE inhibition and aldosterone receptor antagonism, Ang III infusion increased blood pressure and markedly elevated plasma aldosterone without affecting renal sodium excretion. These changes were observed in the absence of a measurable increase in Ang immunoreactivity, suggesting that Ang III is more potent than Ang II in stimulating aldosterone secretion.[109] On the basis of these experiments, these two groups of investigators raised the question of a specific receptor for Ang III, which has so far, however, never been established.[55,109]

ANGIOTENSIN IV

Ang IV is formed by removing the first NH_2-terminal amino acid (Arg^2) from Ang III by aminopeptidase N and/or aminopeptidase B (Figure 3).[14,110–113] Ang IV is then quickly further cleaved into smaller inactive peptide fragments.[114] Ang IV has low affinity for AT_1 and AT_2 receptors (EC_{50} within the micromolar range).[99,115] The plasma clearance of Ang III is substantially higher than that of Ang II.[55,109] Yet, Ang IV displays certain biological effects already at nanomolar concentrations, which are not blocked by AT_1 and AT_2 receptor antagonists (Figure 2). This, together with the discovery of high-affinity binding sites for [^{125}I]Ang IV in the central nervous, vascular and renal systems,[16,116,117] has led to the concept of a novel Ang receptor subtype: the 'AT_4 receptor',[14] which was convincingly shown to be 'insulin-regulated aminopeptidase (IRAP)', a membrane-anchored zinc-dependent metallopeptidase.[118]

Autoradiographic and radioligand binding experiments have localized AT_4 binding sites on microvilli and cell bodies of rat proximal convoluted and straight tubules,[17] cultured rat mesangial cells,[111] cultured opossum proximal tubule cells,[119] apical and basolateral membranes of rabbit cortical tubules,[119] cultured rabbit[120] and human collecting duct cells[121] and cultured human proximal tubule epithelial cells.[122] In the brain, the AT_4 binding sites are prominent in structures important to cognitive processing and sensory and motor functions.[123] The distribution of AT_4 binding sites in the human brain has been shown to be clearly different from that of AT_1 receptors.[124] AT_4 binding sites are also present in other peripheral tissues, including vascular smooth muscle cells, bladder, heart, spleen, prostate, adrenal gland and colon.[14]

Blood pressure and renal responses to Ang IV

In anesthetized rats, i.v. Ang IV infusion at picomolar to nanomolar concentration caused dose-dependent increases in blood pressure, although with less potency than Ang II. This pressor response was completely abolished by AT_1 receptor blockade.[44,47,62,125]

Studies on the effects of Ang IV in the kidney have yielded conflicting results. Infusion of Ang IV directly in renal arteries was reported to increase renal CBF in anesthetized rats as measured by laser Doppler flowmetry,[60,126] an effect blocked by the specific 'AT_4 receptor' antagonist, Divalinal-Ang IV, but not by selective AT_1 and AT_2 receptor antagonists. L-NAME, an NO synthase inhibitor, also blocked this vasodilator response.[60] In the same line, studies on the pulmonary and cerebral vasculature also suggested that Ang IV produced an endothelium-dependent vasodilatation associated with increased endothelial NO and cGMP production.[127–129] In addition, Ang IV was reported to promote the release of vasodilating prostaglandins.[130] Taken together, the 'AT_4 receptor' was suggested to mediate renal NO and/or prostaglandin-dependent vasodilation. In contrast, systemic and intrarenal administration of Ang IV reduced RBF in rats when measured using pulsed Doppler flow probes placed around the renal artery, and this response was prevented by AT_1 receptor blockade.[44,131,132] Infusion of LVV-H7, a more stable ligand with a high affinity ($K_i \sim 73$ nM) for the AT_4 receptor, but no affinity for AT_1 receptors,[133] failed to alter RBF.[132] One study in anesthetized rats reported that intrarenal Ang IV produced an AT_1 receptor-dependent biphasic response with an immediate dose-dependent vasoconstriction, followed by a prolonged vasodilation.[59]

It was suggested that these conflicting results might be explained by differences in the methods used to assess flow and the site of measurement.[126] Reports of AT_1-dependent, Ang IV-induced decreases in flow measured total RBF, whereas the studies that observed increases in flow attributed to IRAP/AT_4 receptor stimulation, involving laser Doppler methods, which measured superficial CBF. It was argued that Ang IV could induce selective shunting of blood to surface nephrons,[134] with only small concomitant overall changes in flow.[126] However, simultaneously monitored RBF and CBF responses after i.v. administration of Ang IV, Ang II and the dopamine receptor agonist fenoldopam showed a high correlation between RBF and CBF measurements, excluding the above possibility.[62] Using simultaneous monitoring of RBF and CBF in rats, Ang IV elicited total and cortical renal vasoconstrictor effects after systemic administration through stimulation of AT_1 receptors, but with lower potency than Ang II.[62] Direct intrarenal infusion of Ang IV also induced dose-dependent AT_1 receptor-mediated pressor and renal vasoconstrictor effects. Ang IV significantly reduced RBF and CBF also when administered intrarenally at subpressor doses. These results are in line with other recent studies showing that Ang IV can induce renal cortical vasoconstrictor effects through AT_1 receptor-activated signaling at nanomolar concentrations.[44] In the same line, AT_1 receptor-mediated vasoconstrictor effects of Ang IV were shown in rat renal interlobular arteries and in afferent and efferent arterioles of isolated perfused hydronephrotic kidneys, also at concentrations several fold higher than those required for Ang II.[135]

Our experiments in rats with Ang IV and with more selective 'AT_4-ligands', LVV-H7 and AT4–16, did not reveal any putative AT_4/IRAP-mediated vasodilator response neither after systemic nor after renal administration, and also not after AT_1 receptor blockade.[62]

Using transgenic mice, we showed pressor and renal vasoconstrictor responses to Ang IV to be mediated by the AT_{1a} receptor subtype.[47] The responses were indeed almost completely absent in AT_{1a} (−/−) mice, whereas responses in AT_{1b} (−/−) mice were comparable to those in corresponding wild-type mice. IRAP/AT_4 receptor (−/−) mice had comparable baseline blood pressure and CBF, and comparable responses to Ang IV as their corresponding wild-type mice, confirming that the putative IRAP/AT_4 receptor is not involved in the pressor and renal hemodynamic effects of Ang IV.[47]

Ang IV has been reported to increase whole kidney urinary sodium and water excretion independent of sympathetic innervation in one study.[122] In line with this natriuretic effect, Ang IV has been shown to produce a dose-dependent inhibition of tubular sodium reabsorption.[17] Pretreatment with the specific AT_4 receptor antagonist, Divalinal-Ang IV, blocked this effect.[126] Taken together, these results

supported the hypothesis that Ang IV may act as a natriuretic agent via the 'AT_4 receptor'.[126] However, we did not confirm an AT_4-mediated natriuretic response to Ang IV in rats, although we were able to document a natriuretic response to fenoldopam, which was used as a positive control.[62,125]

In both conscious dogs and in normal human beings, i.v. infusion of low-dose Ang IV during acute double blockade of the RAS did not modify blood pressure and sodium excretion and did not increase plasma aldosterone concentration.[55,109]

ANGIOTENSIN-(1–7)

Ang-(1–7) is generated directly from Ang II by ACE2, another isoform of ACE, or from Ang I, via Ang-(1–9), a pathway that utilizes both ACE2 and ACE.[136] Ang-(1–7) may also be generated from Ang I by various protease enzymes, including neprilysin, thimet oligopeptidase and prolyl oligopeptidase (Figure 1). Ang-(1–7) has a shorter half-life than Ang II; it can be catabolized by ACE into the biologically inactive pentapeptide, Ang-(1–5), or by aminopeptidases into inactive fragments.[100,137] The G-protein-coupled Mas receptor was reported to mediate some of the effects of Ang-(1–7).[138] Alternatively, other effects of Ang-(1–7) appeared to be mediated by AT_1 and AT_2 receptors as they were inhibited by the AT_1 and AT_2 receptor-selective antagonists (Figure 2),[139] although radioligand binding assays suggested that these were low-affinity interactions.[140]

Some studies suggest that Ang-(1–7) may have a hypotensive activity.[139,141] Untreated essential hypertensive patients exhibited lower urinary concentrations of Ang-(1–7) than normotensive controls.[141,142] Ang-(1–7) inhibited Ang II-induced pressor responses in SHR, an effect reduced by blockade of the Mas receptor, cyclooxygenase inhibition or NOS inhibition, suggesting a role for Mas receptor-mediated release of prostaglandins and NO in this blood pressure-lowering effect of Ang-(1–7).[143] In conscious SHR treated with the AT_1 receptor antagonist candesartan, Ang-(1–7) evoked a depressor response via activation of the AT_2 receptor, involving the bradykinin-NO cascade.[139,144] Hypertensive animals treated with ACE inhibitors had a 25- to 50-fold increase of circulating levels of Ang-(1–7), suggesting that Ang-(1–7) might contribute to the antihypertensive effects produced by ACE inhibitors.[145–147]

The role of Ang-(1–7) in the regulation of kidney function is not well understood and conflicting data were reported. Some groups failed to detect effects of Ang-(1–7) on RBF in rats,[145,148] but others observed a renal vasodilator response, and reported afferent arteriolar dilatation mediated by NO.[149] In contrast, in isolated perfused hydronephrotic rat kidneys, Ang-(1–7) at high concentrations activated the AT_1 receptor, thereby inducing renal microvascular constriction in small interlobular arteries, afferent arterioles and efferent arterioles.[135]

The data on the role of Ang-(1–7) in the regulation of salt and water excretion are also difficult to reconcile. In anesthetized rats, administration of Ang-(1–7) increased urinary flow rate and sodium excretion, an effect abolished by the Ang-(1–7) receptor antagonist A-779 (ref. 146). However, the increase in urinary sodium and water excretion after intrarenal infusion of Ang-(1–7) in dogs was reduced by AT_1, but not AT_2 receptor blockade, suggesting a role for Ang-(1–7)-mediated signaling via the AT_1 receptor.[150] In contrast, in water-loaded rats, infusion of Ang-(1–7) decreased urine volume, an effect reversed by Mas receptor blockade.[140] In conscious dogs and in normal human beings, i.v. infusion of a low dose of Ang-(1–7) during acute double blockade of the RAS did not modify blood pressure and sodium excretion and did not increase plasma aldosterone concentration.[55,109] Other studies in man have shown a slight pressor response after infusion of a higher dose of Ang-(1–7) in healthy volunteers,[151] whereas infusion into the brachial artery of patients with heart failure treated with an ACE inhibitor did not induce significant forearm blood flow changes.[152]

In conclusion, Ang-(1–7) mediates its effects by binding to kidney Mas receptors, although some actions may occur via AT_1 or AT_2 receptors. The role of this peptide in the physiological and pathophysiological regulation of blood pressure and renal function awaits clarification.

ANGIOTENSIN A

Ang A, a newly discovered Ang-derived peptide, was detected in plasma of healthy human beings and, in increased concentrations, in patients with renal failure.[8] In the presence of mononuclear leukocytes, Ang A appears to be generated from Ang II by enzymatic decarboxylation of Asp^1 (ref. 8). Jankowski *et al.*[8] claimed that Ang A is a partial agonist with the same affinity for the AT_1 receptor as Ang II, but a higher affinity for the AT_2 receptor (Figure 2). More recent *in vitro* binding studies of Ang A in CHO-hAT_1 and to CHO-AT_2 cells did not confirm this, and showed that Ang A has similar binding affinity for the AT_1 receptors and the AT_2 receptors as Ang II. The reason for this discrepancy may be due to the different experimental conditions. Moreover, in experiments with AT_1-mediated IP accumulation, Ang A produced a similar maximal effect as Ang II, indicating that it is a full AT_1 receptor agonist.[153]

Jankowski *et al.*[8] also claimed that Ang A may modulate the harmful effects of Ang II because of more pronounced effects at the AT_2 receptor.[8] However, in the isolated perfused rat kidney, Ang A induced dose-dependent vasoconstriction, which was abolished by AT_1 receptor blockade, but not by the AT_2 receptor antagonist PD123319 (ref. 8). In the same line, Ang A induced, although with lower potency than Ang II, pressor and renal vasoconstrictor responses (with maximal responses of the same magnitude as Ang II) in normotensive rats and SHR, which were abolished by AT_1 receptor blockade, but not modified by AT_2 receptor blockade.[153,154] Furthermore, by using transgenic mice, we showed that the AT_{1a} receptor subtype mediates these pressor and renal vascular effects of Ang A. No putative AT_2 receptor-mediated vasodilator effects of Ang A were detected in normotensive rats, SHR and mice, and also not under conditions of AT_1 receptor blockade.[153]

Overall, in contrast to earlier observations, Ang A is not a partial agonist with greater affinity for AT_2 receptors than Ang II, but displays similar *in vitro* and *in vivo* properties as Ang II. This is in line with the hypothesis that the N-terminal aspartate residue of Ang II does not play an important function in binding to and stimulating these receptors.[99,155] There are no published data on the effects of Ang A on sodium handling and aldosterone secretion nor on effects in human beings, but based on the above findings, it can be speculated that exogenous Ang A administration would produce effects similar to Ang II.

A well-validated nano-liquid chromatography-tandem mass spectrometry method,[156] which has limits of quantification for Ang fragments in the low pM range, failed to detect Ang A in the plasma of rats, suggesting that the endogenous plasma levels of Ang A in rats are very low compared with plasma concentrations of Ang II. This is in line with the reportedly lower plasma concentrations of Ang A *vs.* Ang II in healthy subjects and end-stage renal failure patients.[8]

CONCLUSION

Ang II, the main effector peptide of the RAS, increases arterial pressure through arterioral vasoconstriction and by decreasing renal salt and

water excretion through extrarenal and intrarenal mechanisms. Both effects are largely mediated through stimulation of AT_1 receptors, resulting in renal cortical vasoconstriction (but vasodilation of the medullary vessels) and sodium reabsorption. The inhibition of these deleterious AT_1 receptor-mediated effects through ACE inhibitors or Ang receptor blockers (and more recently the direct renin inhibitor aliskiren) largely explains as to why the two former classes of antihypertensive drugs have become the 'gold standard' of antihypertensive therapy.[157]

Although it is generally assumed that AT_2 receptors oppose the responses mediated by AT_1 receptors, and therefore may attenuate the pressor effects of Ang II, a possible role of AT_2 receptors in the regulation of renal hemodynamics and sodium homeostasis remains to be unclear. The recent availability of Compound 21, the first orally active selective AT_2 receptor agonist, opens the possibility to explore whether AT_2 receptor stimulation could be a valuable concept for an innovative antihypertensive therapy.[56,158,159] However, so far controversial results have been reported regarding its effect on blood pressure, depending on the species and the experimental conditions, and it is therefore difficult to predict the effects of long-term AT_2 receptor stimulation in human beings.[159] The current knowledge does rather suggest that AT_2 receptor stimulation will not become another antihypertensive strategy. However, there appears to be other more immediate indications for the clinical drug development of Compound 21, such as heart failure, nephroprotection, stroke and anti-inflammation.[158] Nevertheless, the availability of this new selective compound will certainly help to better understand the physiological and pathophysiological role of the AT_2 receptor, and the complex interactions between the two Ang receptor subtypes.

Ang III also increases blood pressure and reduces RBF through activation of AT_1 receptors, but is more rapidly cleared from the plasma than Ang II. Ang III may be a relatively more important regulator of renal hemodynamics and sodium homeostasis in SHR. The observation that Ang III is more potent than Ang II in stimulating aldosterone secretion may suggest the possibility of the existence of a specific receptor for Ang III, which, however, awaits identification.

Ang IV has AT_1 receptor-dependent pressor renal vasoconstrictor effects, but with lower potency than Ang II. The possibility of a role of the IRAP/AT_4 receptor in the regulation of renal hemodynamics and renal sodium handling suggested by earlier reports has not been confirmed by more recent research. Current evidence does not suggest that this peptide plays a significant role in the control of blood pressure or renal function.

Ang-(1–7) may have a hypotensive activity through interaction with the Mas receptor, resulting in prostaglandin and NO release. Studies on the possible role of this peptide in the regulation of renal hemodynamics and sodium excretion have yielded conflicting results. Overall, the currently available evidence does not suggest that this peptide plays a major role in the regulation of blood pressure and renal function, although it may act as a modulator of Ang II-mediated effects under certain conditions.

Studies on the effects of Ang A, a recently discovered novel human Ang-derived peptide, where only the N-terminal amino acid is different from Ang II, have also yielded conflicting results. One group of investigators suggested a role for Ang A as a naturally occurring peptide counteracting the Ang II-mediated vasoconstrictor effects via a predominant AT_2 receptor-mediated effect. This was, however, not confirmed by more recent research, indicating that Ang A displays similar *in vitro* and *in vivo* properties as Ang II. These findings, together with the very low plasma concentrations of Ang A, do not provide evidence for a physiological role of Ang A as a naturally occurring peptide, possibly counteracting the Ang II-mediated vasoconstrictor effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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