

Circulation Research Compendium on Hypertension

The Epidemiology of Blood Pressure and Its Worldwide Management
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Giuseppe Mancia, Guest Editor

Hypertension Renin–Angiotensin–Aldosterone System Alterations

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Abstract: Blockers of the renin–angiotensin–aldosterone system (RAAS), that is, renin inhibitors, angiotensin (Ang)-converting enzyme (ACE) inhibitors, Ang II type 1 receptor antagonists, and mineralocorticoid receptor antagonists, are a cornerstone in the treatment of hypertension. How exactly they exert their effect, in particular in patients with low circulating RAAS activity, also taking into consideration the so-called Ang II/aldosterone escape that often occurs after initial blockade, is still incompletely understood. Multiple studies have tried to find parameters that predict the response to RAAS blockade, allowing a personalized treatment approach. Consequently, the question should now be answered on what basis (eg, sex, ethnicity, age, salt intake, baseline renin, ACE or aldosterone, and genetic variance) a RAAS blocker can be chosen to treat an individual patient. Are all blockers equal? Does optimal blockade imply maximum RAAS blockade, for example, by combining ≥ 2 RAAS blockers or by simply increasing the dose of 1 blocker? Exciting recent investigations reveal a range of unanticipated extrarenal effects of aldosterone, as well as a detailed insight in the genetic causes of primary aldosteronism, and mineralocorticoid receptor blockers have now become an important treatment option for resistant hypertension. Finally, apart from the deleterious ACE-Ang II-Ang II type 1 receptor arm, animal studies support the existence of protective aminopeptidase A-Ang III-Ang II type 2 receptor and ACE2-Ang-(1 to 7)-Mas receptor arms, paving the way for multiple new treatment options. This review provides an update about all these aspects, critically discussing the many controversies and allowing the reader to obtain a full understanding of what we currently know about RAAS alterations in hypertension. (*Circ Res.* 2015;116:960-975. DOI: 10.1161/CIRCRESAHA.116.303587.)

Key Words: aldosterone ■ angiotensin ■ angiotensin-(1–7) ■ AT₂ receptor ■ gender ■ hyperkalemia ■ primary aldosteronism ■ prorenin ■ reactive oxygen species ■ remodeling ■ renin ■ resistant hypertension

Blockers of the renin–angiotensin–aldosterone system (RAAS), that is, renin inhibitors, ACE inhibitors, angiotensin (Ang) II type 1 (AT₁) receptor antagonists, and

mineralocorticoid receptor (MR) antagonists, are a cornerstone in the treatment of hypertension. At first sight, their mechanism of action seems simple: they reduce the

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Nonstandard Abbreviations and Acronyms

APA	aldosterone-producing adenoma
ARR	aldosterone-to-renin ratio
AT₁	angiotensin II type 1
AT₂	angiotensin II type 2
BP	blood pressure
EC	endothelial cell
FH	familial hyperaldosteronism
GPER	G-protein-coupled estrogen receptor-1
KO	knockout
MR	mineralocorticoid receptor
NO	nitric oxide
PA	primary aldosteronism
(P)RR	(pro)renin receptor
RAAS	renin–angiotensin–aldosterone system
SHR	spontaneously hypertensive rat
VSMC	vascular smooth muscle cell

formation or block the effects of Ang II and aldosterone, thereby preventing the deleterious cardiovascular effects of these 2 compounds. Logically, they should then be particularly applied in patients with high RAAS activity, as measured in blood plasma. However, it is now well accepted that they are also effective in patients with medium-to-low RAAS activity. Moreover, after an initial suppression/blockade of Ang II/aldosterone, the plasma levels of these 2 compounds often return to normal or even rise above pretreatment levels: the so-called Ang II/aldosterone escape.^{1,2} Yet, remarkably, the RAAS blocker effect remains, at least partially. These puzzling observations have led to the concept of a local RAAS in various organs, that is, the real site of action of RAAS blockers. According to some investigators, this local RAAS occurs entirely intracellular (intracrine RAAS).³ In addition, during RAAS blocker application, an upregulation occurs of multiple angiotensin metabolites, which may exert actions of their own and possibly even contribute to the beneficial effects of RAAS blockade. Examples of these protective (vasodilator) pathways include the angiotensinase A-Ang III-Ang II type 2 (AT₂) receptor pathway and the ACE2-Ang-(1–7)-Mas receptor pathway (Figure 1). Further knowledge in this area might lead to new drugs.

For a long time, it was thought that the more RAAS blockade, the better, also in view of the above described Ang II/aldosterone escape. However, dual RAAS blockade trials have now shown that this is not necessarily the case and that the consequences of too much RAAS suppression (hyperkalemia, renal dysfunction, and hypotension) may overrule the beneficial effects of this approach. A variety of RAAS differences exists between men and women, and between black and white people, with men and white people generally having higher renin levels. This does not necessarily translate into similarly elevated aldosterone levels, and in fact, patients with high aldosterone-to-renin ratios (ARR) can be identified, which respond particularly well to MR blockers. A wide range of

mutations has recently been identified that gives rise to selective aldosterone rises.

This review will critically discuss all the above aspects. What is a local RAAS? What are the local actions of Ang II in the vessel wall? What are the (genetic) determinants of a solid response to a RAAS blocker? Is there such a thing as too much RAAS blockade? Are all RAAS blockers equally good? Are the sex- and ethnicity-related RAAS differences clinically relevant? What about the recent developments in primary hyperaldosteronism and the extrarenal effects of aldosterone? Finally, can we expect new RAAS drugs?

What Is a Local RAAS?

Originally, when developing the concept of local RAAS, it was proposed that all components required to generate Ang II, and aldosterone locally are synthesized at multiple sites in the body, allowing their generation to occur independently from the classical sites of RAAS component synthesis: the kidney (renin), liver (angiotensinogen), and adrenal (aldosterone). In addition, a wide variety of nonclassical enzymes, in particular chymase, was suggested to contribute to Ang II generation as well.⁴ Some, if not all, RAAS components were even detected in cells, leading to the concept of an intracrine RAAS, involving the intracellular generation of Ang II acting on intracellular (nuclear) receptors.³

Finally, the confusing observation that humans have large amounts of prorenin, the inactive precursor of renin, has led to a search for prorenin receptors that bind and activate prorenin locally, thus offering an explanation of why we have so much prorenin (its concentrations are ≤ 100 times of renin): it would then function as a regulator of tissue Ang generation. One such candidate, the so-called (pro)renin receptor ((P)RR), which binds both renin and prorenin, has received much attention during the last decade.⁵ Unfortunately, the concentrations of renin/prorenin (together denoted here as (pro)renin) that are required to result in receptor binding are far above the normal (patho)physiological levels, and transgenic rodents overexpressing either the (P)RR or prorenin did not reveal any evidence for (pro)renin–(P)RR interaction *in vivo*, that is, their Ang II levels were unaltered.⁶ Moreover, (P)RR knockout (KO), unlike renin KO, is lethal.⁷ This may relate to (P)RR's association with vacuolar H⁺-ATPase, a crucial enzyme found in virtually every cell type that is important for the acidification of intracellular compartments. Therefore, (P)RR research is now focusing on its functions beyond the RAAS because the (P)RR may not be a part of the RAAS after all, except perhaps in organs where (pro)renin is synthesized locally (allowing high local concentrations that result in receptor activation).

Similarly, the view of chymase as a major Ang I-II converting enzyme is most likely an *in vitro* artifact related to the measurement of Ang II formation in tissue homogenates (particularly from the human heart), where chymase is no longer in its intracellular storage sites.⁴ Careful measurements of Ang II in ACE KO mice did not support the concept that chymase is an Ang I-II converting enzyme *in vivo*.⁸ In fact, renin and angiotensin measurements are hampered by multiple technical difficulties, particularly in tissues, and because many of

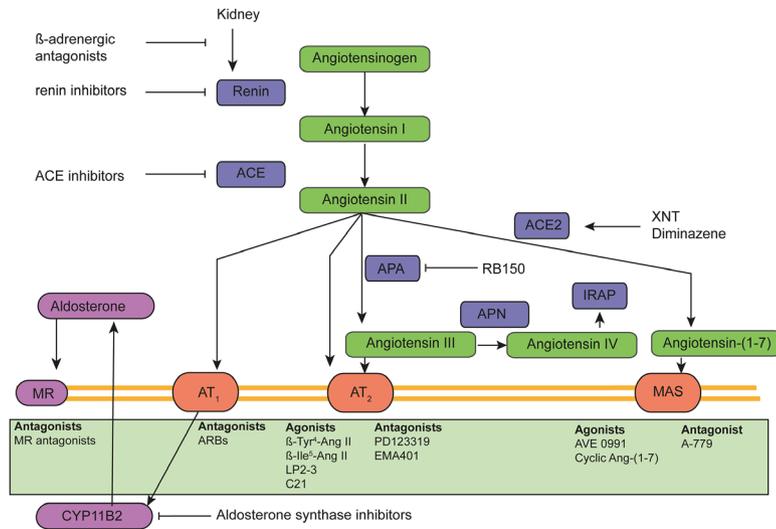


Figure 1. New and existing drugs interfering with the renin-angiotensin (Ang) system cascade. Classically, interference occurs at the level of renin, angiotensin-converting enzyme (ACE), the Ang II type 1 (AT₁) receptor (R), or the mineralocorticoid receptor (MR), with renin inhibitors, ACE inhibitors AT₁ receptor blockers (ARBs) or MR antagonists. Novel enzyme inhibitors now target aminopeptidase A (APA), which generates Ang III (=Ang-[2-8]) from Ang II (=Ang-1-8)), or aldosterone synthase (CYP11B2). Activators of ACE2 (XNT and diminazene), which generates Ang-(1-7) from Ang II, were recently found to act equally well in ACE2 knockout (KO) animals, thus questioning their mechanism of action. Numerous agonists for both the AT₂ receptor and Mas receptor are being developed. Aminopeptidase N (APN) degrades Ang III to Ang IV (=Ang-(3-8)), which may act on the AT₄ receptor, also known as insulin-regulated aminopeptidase (IRAP).

the original conclusions on tissue RAAS were based on such nonideal measurements, they need to be viewed with care. For instance, the original observations that renin and Ang II are unaltered after a bilateral nephrectomy turned out not to be true.⁹ Moreover, there is no intracellular Ang II in AT receptor KO mice.¹⁰ Clearly therefore, there is only one renin source in the body (the kidney), angiotensin generation occurs extracellularly (in blood, interstitial fluid, and on the cell surface), and any Ang II present in cells accumulated there after its internalization after AT receptor binding. Selective KO of renal angiotensinogen revealed that the concept of renal angiotensinogen contributing to renal Ang II production was not true: all renal Ang II generation depended on hepatic angiotensinogen.¹¹ Although similar conclusions have been reached in the heart,¹² local angiotensinogen synthesis has been claimed in the vessel wall,¹³ and adipocytes are generally believed to generate angiotensinogen.¹⁴ Surprisingly, adipocyte angiotensinogen deficiency did not affect plasma angiotensinogen levels, but greatly reduced circulating Ang II under high fat diet conditions.¹⁴ Additional studies, inducing selective KO of adipocyte angiotensinogen, hepatic angiotensinogen, or both are required to fully understand the contribution of adipocyte angiotensinogen to Ang II production. Also with regard to aldosterone, the original reports on its generation in heart, vessel wall, and kidney were not confirmed on careful re-examination of the measurements after adrenalectomy and in isolated organs.¹⁵⁻¹⁷

Summarizing, the current view is that Ang II generation in tissues does occur (in fact, >90% of tissue Ang II is synthesized locally and not taken up from plasma^{18,19}), but depends on renal renin and largely, if not completely, on hepatic angiotensinogen. Both diffuse into the interstitium, allowing local Ang II generation to take place in that compartment with the help of membrane-bound, ubiquitously present ACE (Figure 2). This Ang II rapidly binds to AT receptors, and such binding is followed by internalization, explaining why tissue Ang II levels are often high and correlate closely with tissue AT receptor density.²⁰ Aldosterone is exclusively adrenal-derived. To what degree prorenin has a role, beyond the (P)RR, remains to be determined.

Local Effects of Ang II in the Vessel Wall

When focusing on the vessel wall, it is well-established that activation of AT₁ receptors induces vasoconstriction, endothelial dysfunction, inflammation, growth, and remodeling, whereas AT₂ receptors are believed to counteract these effects (Figure 3). However, the latter is not a uniform finding,

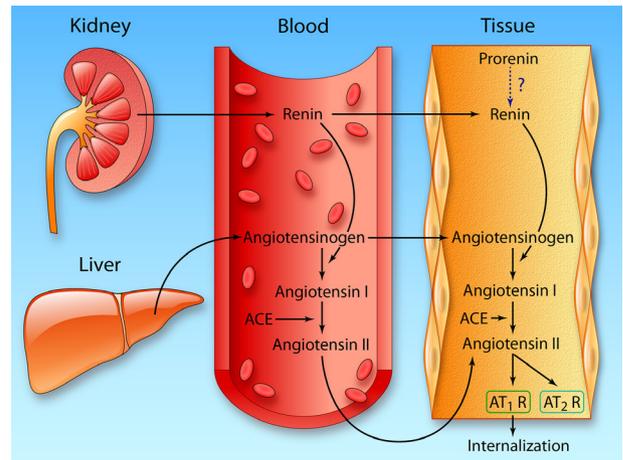


Figure 2. Circulating versus tissue renin-angiotensin (Ang) system. Circulating renin is kidney-derived, and circulating angiotensinogen originates in the liver. Angiotensin-converting enzyme (ACE) is located on endothelial cells. Ang II generated in the circulation will diffuse to tissues to bind to its main receptor (the Ang II type 1 receptor, AT₁R) to exert effects. In addition, circulating renin and angiotensinogen might also diffuse to tissue sites (eg, the interstitial space) and generate, with the help of tissue ACE, Ang II locally. In a limited number of tissues, renin's precursor prorenin is produced locally. To what degree such prorenin, for example, following its conversion to renin, contributes to local angiotensin production remains unknown. Although local production has also been claimed for angiotensinogen, in particular in the kidney, current evidence does not support a functional role for kidney-derived angiotensinogen because the renal Ang II levels in renal angiotensinogen knockout (KO) mice are identical to those in wild-type mice.¹¹ Locally generated Ang II rapidly binds to AT₁ and AT₂ receptors, the former being followed by internalization. This explains the intracellular presence of Ang II, as well as the high tissue levels of Ang II in high AT₁ receptor-density organs like the adrenal (Illustrated Credit: Ben Smith).

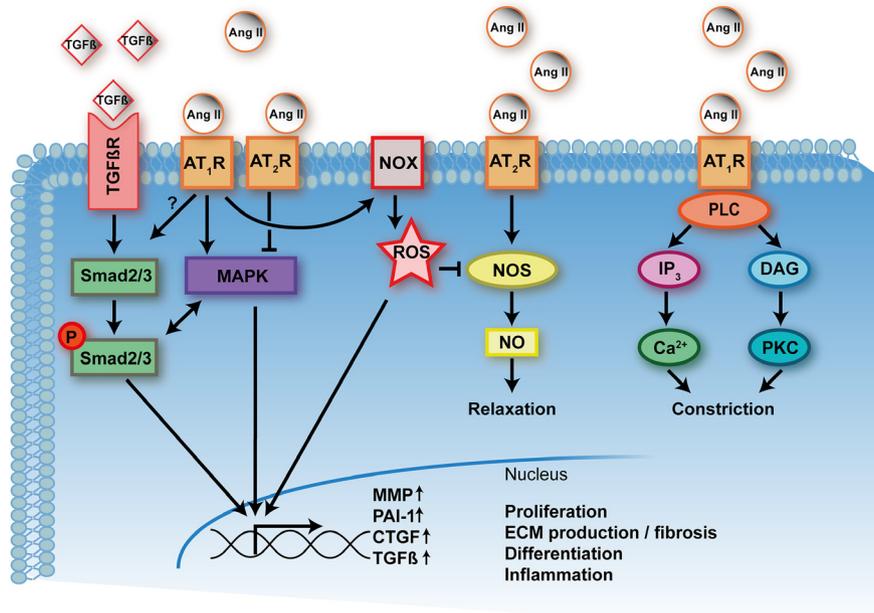


Figure 3. Effects of angiotensin (Ang) II, via its Ang II type 1 and 2 (AT₁ and AT₂) receptors (AT₁R, AT₂R) on vascular remodeling and constriction/vasodilation. Transforming growth factor-β (TGF-β) signaling by the TGF-β receptor (via the Smad2/3 pathway) and mitogen-activated protein kinase (MAPK) activation after AT₁ receptor stimulation jointly regulate the transcription of target genes (eg, matrix metalloproteinase, MMP; plasminogen-activator inhibitor-1, PAI-1; connective tissue growth factor, CTGF) that result in proliferation, extracellular matrix production/fibrosis, differentiation, and inflammation. AT₁ receptor stimulation additionally upregulates NAD(P)H oxidase (NOX), thereby increasing reactive oxygen species (ROS) formation, which also regulates the transcription of the above-mentioned target genes. AT₂ receptor stimulation inhibits this pathway by blocking MAPK. AT₂ receptors also induce vasorelaxation by activating NO synthase (NOS). This may counteract the constrictor effects of AT₁ receptor stimulation (mediated by the inositol trisphosphate [IP₃]-Ca²⁺ and diacylglycerol [DAG]-protein kinase C [PKC] pathways). Under pathological condition, ROS uncouple NOS, thereby diminishing NO production and potentially facilitating ROS formation by NOS.

and under certain conditions, for example, in the spontaneously hypertensive rat (SHR), AT₂ receptors may become AT₁ receptor-like.^{21,22} The mechanism behind this phenotype change is unclear, but most likely involves a difference in location (endothelial cell (EC) versus vascular smooth

muscle cell (VSMC)) and heterodimerization with AT₁ receptors. Therefore, whether upregulation of AT₂ receptors under pathological conditions is always beneficial remains unknown.²³ Similar opposing findings with regard to AT₂ receptor function have been made in the heart.²⁴ Of interest,

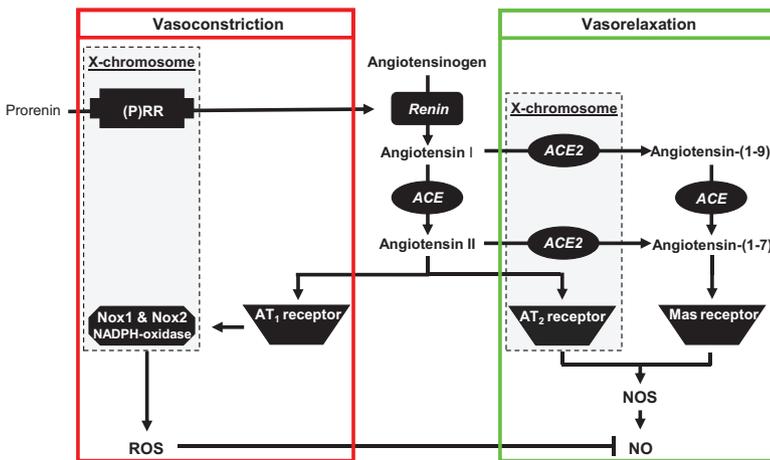


Figure 4. Vasoconstrictor-vasorelaxant balance of the renin-angiotensin-aldosterone system (RAAS) in relation to sex hormone status in men and pre- and postmenopausal women. The figure highlights X-chromosome-located RAAS genes, including the (pro)renin receptor ((P)RR) gene. The current view is that its relationship with the RAAS may be limited to (pro)renin-synthesizing organs, where (pro)renin is sufficiently high to result in significant receptor binding.

	Men	Premenopausal women	Postmenopausal women
Sex chromosomes	XY	XX	XX
Androgens	+++++	+	+
Estrogens	+	+++++	+
Net effect	Vasoconstriction	Vasorelaxation	Vasoconstriction

post myocardial infarction, a moderate cardiac AT₂ receptor overexpression in transgenic mice protected against maladaptive remodeling and dysfunction, whereas a massive, 9-fold overexpression did not yield such positive effects.²⁵ Thus, also the degree of overexpression may determine AT₂ receptor function.

Wide attention has been paid to the fact that increased vascular Ang II levels increase NAD(P)H oxidase activity in EC, adventitial cells, and VSMC, thereby stimulating reactive oxygen species formation in the vessel wall.²⁶ Reactive oxygen species products like superoxide and H₂O₂ subsequently activate multiple signaling pathways, involving mitogen-activated protein kinases, tyrosine kinases, phosphatases, calcium channels, and redox-sensitive transcription factors,^{26,27} together resulting in cell growth, expression of proinflammatory genes (eg, transforming growth factor- β), and the production of extracellular matrix proteins, like collagen, elastin, fibrillin, fibronectin, and proteoglycans. The latter production usually involves a phenotype switch in VSMC, from contractile to proliferative/synthetic. In addition, there is an imbalance between apoptosis and growth.

Recent evidence supports a role for the Ang II-transforming growth factor- β axis in aneurysm development.^{28,29} Infusion of Ang II in atherosclerotic apolipoprotein E- or LDL receptor KO mice provides an experimental model for the most common type of aneurysm, the abdominal aortic aneurysm. Thoracic aortic aneurysms are less common and often have a genetic background, involving mutations in the above-mentioned extracellular matrix proteins and transforming growth factor- β . A well-known example is Marfan's syndrome. Losartan was shown to be effective in adults with this syndrome,³⁰ and animal data suggest that this effect involves AT₂ receptor stimulation rather than AT₁ receptor blockade.³¹ Both the transforming growth factor- β -induced canonical (pSmad2/3) and noncanonical (mitogen-activated protein kinases) signaling pathways are upregulated in thoracic aortic aneurysms mouse models, and their suppression may underlie the effectiveness of AT₁ receptor blockade in these models.^{31,32} As a consequence of these exciting new findings, multiple trials now investigate the effectiveness of RAAS blockers in Marfan's syndrome.²⁹ An important issue will be to what degree ACE inhibition (which does not result in AT₂ receptor stimulation) differs from AT₁ receptor antagonism. One of these trials was recently published.³³ Involving 608 Marfan patients (age 6 months to 25 years), it did not show superiority of losartan versus the β -adrenergic antagonist atenolol. Beta-adrenergic antagonists are the current standard therapy in Marfan patients. Here it should be realized that such drugs also suppress renin release. The investigators applied a relatively high dose of atenolol and a low dose of losartan, and there was no placebo group. Moreover, treatment was started in most cases at an advanced stage of the disease. Thus, on the basis of this study, it can be concluded that losartan is a safe alternative for a β -adrenergic antagonist, but not yet whether (at the appropriate dose and perhaps when given at an earlier stage of development) it might be better.

Sex-Related Aspects

Physiologically, the 2 major differences between men and women are (1) different levels of sex hormones (testosterone versus estrogen) and (2) the sex chromosome complement (XY versus XX). The combination of a different hormonal milieu and different genes located on the sex chromosomes results in a transcriptome with a sex-specific and sex-biased expression. This leads to a marked sexual dimorphism in anatomy, physiology, and metabolism, but also extends to sex differences in blood pressure (BP), sensitivity to Ang II, and severity of cardiovascular disease.³⁴

Sex Hormones

Premenopausal women have a lower BP in comparison to age-matched men (\approx 10 mm Hg for systolic BP and \approx 5 mm Hg for diastolic BP). Because this sexual dimorphism in BP manifests itself during adolescence and disappears after the menopause, it is logical to assume a role for sex hormones. Testosterone binds to the androgen receptor, whereas estrogen (17 β -estradiol) stimulates the estrogen receptor α and β , as well as the G-protein-coupled estrogen receptor-1 (GPER). These receptors mediate both genomic and nongenomic effects. The former involve interaction of the hormone-receptor complex with nuclear DNA, modulating the transcription of sex hormone-responsive genes (taking hours), whereas the latter involves signaling cascades resulting in effects within seconds-minutes. In the case of estrogen, this results in endothelium-dependent and -independent dilator effects through nitric oxide (NO), cGMP, cAMP, and K⁺-channels.³⁵ Testosterone is believed to counteract such endothelium-dependent vasorelaxation and to exert direct constrictor effects³⁵; the BP-lowering effects after castration confirmed this view.³⁶

In addition, sex hormones affect RAAS components and modulate Ang II sensitivity. Indeed, estrogens increase angiotensinogen, ACE2, AT₂ receptor density, and endothelial NO synthase, whereas they decrease renin, ACE, AT₁ receptor density, and the NADPH oxidase subunits Nox1 and Nox2 (Figure 4).^{37,38} These alterations are suggestive for an upregulation of ACE2-derived angiotensin-(1-7) formation, enhanced AT₂ receptor stimulation and NO release, combined with reduced reactive oxygen species formation; in other words, they favor the vasodilator arm of the RAAS (see also below). Indeed, low doses of Ang II even decreased BP in female (but not male) rats,³⁹ and higher doses exerted larger BP-increasing effects in males than in females, whereas gonadectomy reversed these effects.⁴⁰ Testosterone increases renin, ACE, and AT₁ receptors and downregulates AT₂ receptors, thereby favouring the constrictor arm. There are no clear differences in aldosterone levels between men and women.⁴¹ In postmenopausal women, the balance will shift toward the vasoconstrictor arm, unless they receive hormone replacement therapy.³⁷

Sex Chromosomes

In most mammals, males are heterogametic, possessing 1 X and 1 Y chromosome, whereas females are homogametic with 2 X chromosomes. This characteristic plays a fundamental role in the sexual dimorphism through variances in gene expression. Evolutionary, sex chromosomes have evolved out of

a pair of matched autosomes, which eventually lost the ability to recombine because of an accumulation of male-specific functions on one chromosome and degradation of nonrecombining regions.⁴² Genes mapped to the Y chromosome play an important role in sex development, testosterone production, and fertility.

A limited number of studies suggest that sex chromosomes influence BP and regulate RAAS genes. Introgression of the Y_{SHR} chromosome from the SHR strain on a normotensive WKY background resulted in a ≈ 20 mmHg BP difference versus rats, where the normotensive Y_{WKY} chromosome was introgressed on the SHR background.⁴³ The four core genotype mouse model involves the deletion of the sex-determining region Y (Sry) from the Y chromosome and the insertion of the Sry transgene onto an autosome, thereby resulting in XY-Sry males.⁴⁴ Crossing these mice to normal XX females will result in 4 genotypes, XX gonadal males and females, as well as XY gonadal males and females. Interestingly, Ang II induced a larger BP rise in gonadectomized 4 core genotype mice with an XX genotype than in their XY counterparts independent of prior sex hormone status and gonadal phenotype.⁴⁵ It is tempting to speculate that this mechanism underlies the relatively rapid increase in BP observed in postmenopausal women.⁴⁶ The Sry gene family is known to upregulate angiotensinogen, renin, and ACE, whereas it downregulates ACE2 *in vitro*.³⁶ In addition, the (P)RR, ACE2, Nox1, Nox2, and the AT_2 receptor are mapped to the X chromosome (Figure 4). Although dosage-compensation takes place in women by an epigenetic mechanism called X-inactivation to prevent a lethal dose of X-mapped genes, several genes (15% to 20%) have been reported to escape X-inactivation⁴⁷ and contribute to sex differences because of a higher expression in XX than XY cells.⁴⁸ Such escape also applies to the (P)RR,^{49,50} but the physiological relevance of this observation is still unknown.

Consequences for Treatment?

It is well-accepted that premenopausal women are protected from the development of cardiovascular disease in comparison to age-matched men.⁵¹ Obviously, having a higher BP for ≤ 4 to 5 decades, even when modest, will have consequences. As discussed earlier, animal data support a major role for the RAAS in sex-related differences. Yet, there are no sex-specific recommendations for antihypertensive therapy, nor is there currently any evidence that men and women respond differently to RAAS blockers. One retrospective study in patients with heart failure claimed a higher efficacy of ACE inhibitors in males and of AT_1 receptor blockers in females.⁵² Although this potentially supports the importance of AT_2 receptor stimulation in women, large prospective studies are warranted to confirm such claims.

Determinants of RAAS Blocker Response and the Degree of Blockade

Prediction of RAAS Blocker Response

Although sex, as discussed earlier, is not an established determinant of RAAS blocker response, multiple attempts have been made to predict the response to a RAAS blocker

on the basis of alternative parameters. Genetic variation has been evaluated, usually by studying single nucleotide polymorphisms in RAAS genes in a retrospective manner in large clinical trials.^{53,54} Emphasis has been on the ACE insertion/deletion polymorphism.⁵⁵ Unfortunately, the effects were small and difficult to replicate, and, given the nonexistence of large prospective studies to further evaluate these findings, at this stage, there is no useful genetic information that can be applied to the individual patient to help choosing a specific RAAS blocker.

Along the same lines, it has been argued that patients with high RAAS activity (like patients with bad RAAS gene variants) should preferably be treated with RAAS blockers. Such patients should then be selected on the basis of their high renin, ACE, and aldosterone levels. Ang II levels might also be useful, but given the technical difficulties to measure this RAAS component, this is currently not feasible. The background of this concept is that patients with high baseline RAAS activity have a higher risk to develop cardiovascular disease. Indeed, retrospective analyses of patient populations in clinical trials in whom baseline renin measurements were available support that high renin levels are indicative of future cardiovascular disease and death, particularly in patients with kidney dysfunction, and hypertension.⁵⁶⁻⁵⁹ Remarkably, this relationship was not affected by the use of RAAS blockers, which, through interference with the negative feedback loop between Ang II and renin, increase renin release. Obviously, renin measurements, when based on activity, will be disturbed by the use of renin inhibitors, and thus during such treatment only measurements of plasma renin concentration, and not plasma renin activity, will give an indication of the true renin levels. In addition, salt intake affects renin secretion, with patients on a low salt diet displaying higher renin levels. Preferably therefore, when considering pretreatment renin levels as a treatment determinant, salt intake should be taken into account.

Laragh and Sealey distinguish a low renin, sodium volume-dependent form of essential hypertension and a medium-to-high renin form of hypertension.⁶⁰ The former occurs whenever body sodium content increases beyond the point where plasma RAAS activity is turned off, whereas the latter occurs when too much renin is secreted relative to the body sodium content. Antihypertensive treatment should then be aimed at reducing either body salt and volume content (diuretics, calcium antagonists) or RAAS activity (RAAS blockers and β -adrenergic antagonists; the latter suppress renin release). Retrospective analyses of BP trials confirmed this concept.^{61,62} In addition, Gupta et al observed that African Americans, who on average have lower renin levels compared with Caucasians,⁶³ responded less well to atenolol.⁶⁴ Yet, others observed either no role for baseline renin,⁶⁵ or at most a weak trend.⁶⁶⁻⁶⁸ Moreover, the BP decreases for a given baseline renin level (uncorrected for salt intake) varied >40 mmHg. In addition, a uniform definition of high renin (plasma renin activity/plasma renin concentration) is not available and clearly complicated by the intake of salt, sex, ethnicity, and the use RAAS-affecting drugs.^{61,65,67} Thus, although in general it is probably true that patients with high renin levels respond better to RAAS blockers (for instance, patients pretreated with a diuretic, which activates the RAAS),

the variation in renin is such (not even taking into consideration the additional variation at the tissue level!) that it is of limited practical use for the individual patient. Unfortunately, the same is true for plasma ACE.⁶⁹ The use of aldosterone measurements will be discussed below.

Desired Degree of RAAS Blockade

Given the Ang II/aldosterone escape during RAAS blocker treatment, usually occurring within days-weeks after drug initiation,⁷⁰ for many years it was argued that the more blockade, the better, to keep the levels of these active components (or their activity) low. Nevertheless, early animal studies in SHR⁷¹ had already shown that dual RAAS blockade, particularly under low-salt conditions (when the RAAS is most needed) is lethal: it caused a major decrease in BP and severe renal failure, which were accompanied by massive rises (up to several 100-fold) in plasma renin and renal renin levels, thereby decreasing the angiotensinogen concentration in plasma. These deleterious effects of dual RAAS blockade were prevented by a high salt diet. Studies in human cardiac tissue, obtained from patients undergoing cardiac transplantation or severe heart failure patients at the time of left ventricular assist device implantation,^{72,73} both being treated with (high) RAAS blocker doses, revealed that also in the human heart renin levels may rise >100-fold, thereby decreasing cardiac angiotensinogen. Interestingly, after left ventricular assist device implantation, renin levels dropped 10-fold and cardiac angiotensinogen levels rose again, thereby allowing a rise in cardiac Ang II levels.⁷³ This illustrates the fact that at high renin levels, angiotensinogen depletion essentially renders Ang II generation impossible.

Taken together, these data illustrate that too much RAAS blockade annihilates the capacity of tissues to acutely generate Ang II when necessary. Particularly in the kidney, this may be crucial to preserve glomerular filtration. Recent data obtained in salt-depleted healthy volunteers exposed to increasing doses of a new renin inhibitor, VTP-27999, provide further evidence for this concept.⁷⁴ To fully appreciate these data, it should be mentioned that renin inhibitors selectively accumulate in the kidney, remaining present in renal tissue at high levels, even at days-weeks after stopping treatment, when plasma levels are undetectable.^{75,76} It was observed that at the highest dose of VTP-27999 tested (600 mg), the drug blocks the renal RAAS more effectively than the circulating RAAS. Indeed, when stopping drug intake after 10 days of dosing, the plasma renin concentration levels at 24 to 72 hours after the last dose exceeded the capacity of extrarenal VTP-27999 to fully block renin's enzymatic activity (Figure 5). Therefore, even though the intrarenal RAAS is still inhibited at these times, extrarenal RAAS activation now occurred, increasing the circulating concentrations of Ang II and aldosterone. These findings are reminiscent of the nephrocentric view of ACE inhibition noted 25 years ago in patients with congestive heart failure.⁷⁷ It was asked why the kidneys continue to release renin in such patients; the answer being that they do everything possible to preserve renal function and glomerular filtration, apparently at the expense of the hemodynamic burden on the heart. Exactly this happened in the VTP-27999 study, where the kidneys responded to excessive renal RAAS

suppression by releasing large quantities of renin, resulting in elevations of plasma renin activity, Ang II, and aldosterone. Such elevated Ang II levels were most likely responsible for the (nonsignificant) increase in heart rate observed in the subjects treated with 600 mg VTP-27999. Clearly therefore, renin inhibition has an upper limit and more is not always better.

The latter also applies to other types of excessive RAAS blockade (eg, when combining an ACE inhibitor and an AT₁ receptor antagonist): several large dual RAAS blockade studies (ONTARGET, ALTITUDE, NEPHRON-D)^{78–80} in a variety of patients all concluded that the adverse effects (hypotension, hyperkalemia, and renal dysfunction), all because of (renal) Ang II depletion, outweighed the beneficial effects. In reaching this conclusion, it should be realized that often these patients additionally took β -adrenergic antagonists and MR antagonists and thus were in reality not exposed to dual but to quadruple RAAS blockade. This led Nussberger and Bohlender to conclude that the goal should not be maximal but optimal RAAS blockade, guided by regularly measuring BP, serum potassium, and creatinine.⁸¹

Recent guidelines no longer recommend the combined use of ACE inhibitors, AT₁ receptor blockers, and renin inhibitors in hypertension.⁸² Most evidence is obviously available for the ACE inhibitors. As explained earlier, there is still discussion to what degree the AT₂ receptor stimulation during AT₁ receptor blockade is beneficial or harmful. Two recent meta-analyses show that ACE inhibitors reduce all-cause mortality and cardiovascular death in patients with hypertension and diabetes mellitus, whereas AT₁ receptor blockers do not.^{83,84} This may relate to the possibility that AT₂ receptor stimulation affects the incidence of myocardial infarction^{85,86} and induces apoptosis in intestinal epithelial cells, thereby inducing severe gastrointestinal problems.^{87,88} Additionally, AT₂ receptor stimulation activates the bradykinin axis,⁸⁹ although bradykinin accumulation will also occur after ACE inhibition. The exact contribution of bradykinin to the beneficial effects of RAAS blockade in humans remains to be determined. Nevertheless, based on these findings, it is clear that ACE inhibitors should be considered as first-line agents in patients with hypertension and diabetes mellitus.

Aldosterone

Aldosterone is a steroid hormone produced in the zona glomerulosa of the adrenal gland. Like Ang II, aldosterone is an effector hormone of the RAAS, principally involved in volume and BP regulation. Beyond BP, aldosterone has emerged as a cardiovascular risk factor promoting cardiovascular and renal inflammation, fibrosis, and remodeling. Furthermore, in cohort studies of nonhypertensive individuals, higher circulating aldosterone levels, but still within the physiological range, are a risk factor for the development of hypertension.⁹⁰ With regard to hypertension, the importance of aldosterone is largely related to primary aldosteronism and treatment-resistant hypertension. The mechanism of action of aldosterone was thought to be restricted to its renal genomic effects, causing sodium and water retention. More recently, evidence has accumulated for effects of aldosterone on EC and VSMC that may or may not be mediated by the MR.^{91–93} In the first part of this

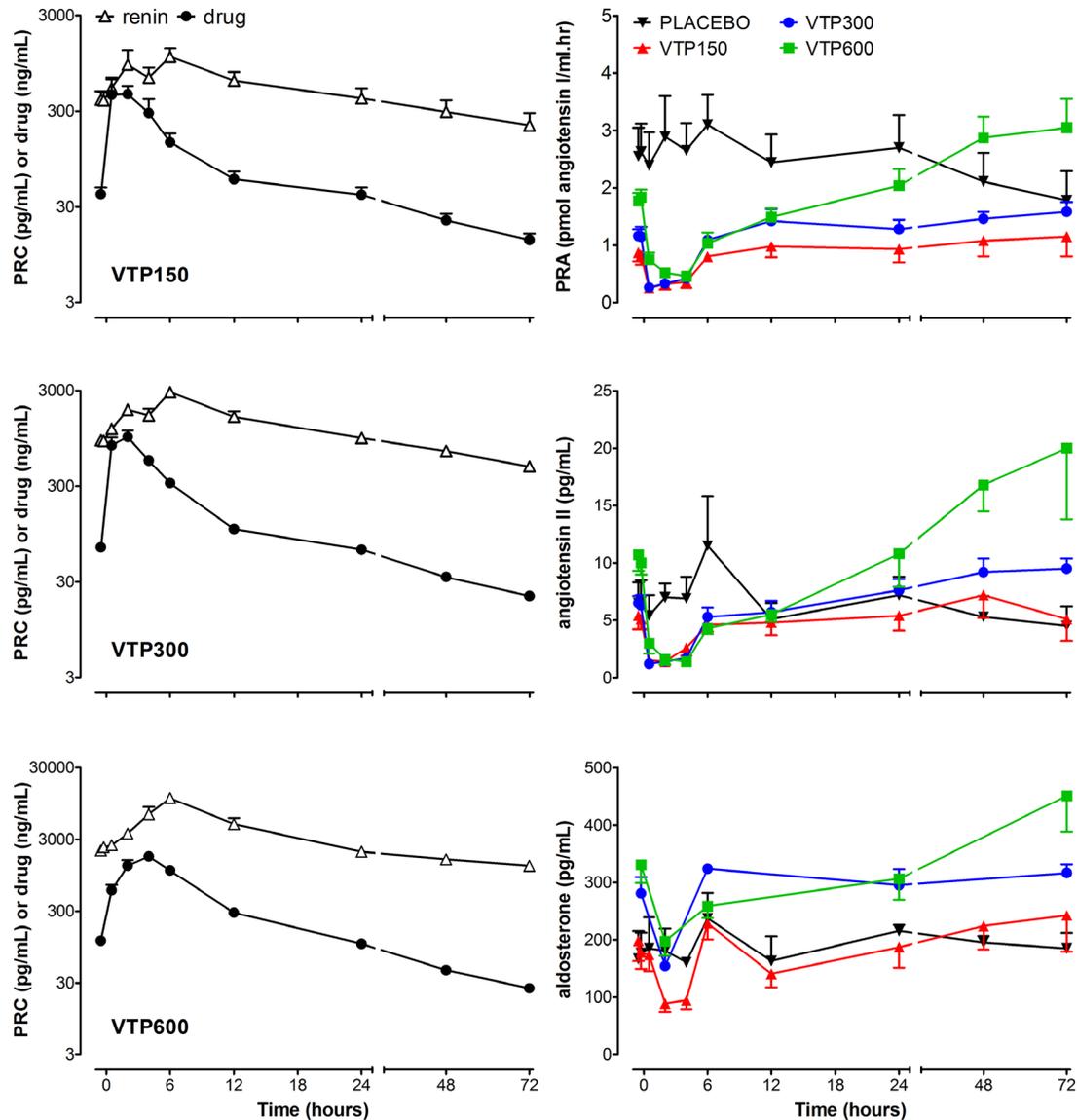


Figure 5. The consequences of too much renin inhibition (with the new renin inhibitor VTP-27999), as observed after stopping treatment. Normally, the decrease in plasma drug level and renin concentration after stopping run in parallel. However, after a high (600 mg) dose of VTP-27999 (which, like aliskiren, is known to accumulate in the kidney⁷⁶), plasma renin suppression lags behind the decline in plasma drug decrease, most likely because the renal renin–angiotensin–aldosterone system (RAAS) is still suppressed, thus keeping renin release at a (too) high level, which can no longer be suppressed by VTP-27999 in plasma. As a consequence, plasma renin activity, Ang II, and aldosterone rise above control levels. In other words, too much renin inhibition, by excessively blocking the renal RAAS, may effectively activate extrarenal RAAS activity. Data derived from Balcarek et al.⁷⁴

section, we focus on new insights in the potential vascular effects of aldosterone and the receptors involved. In the second part, new developments in the pathogenesis and etiology of primary aldosteronism and the role of aldosterone in resistant hypertension will be reviewed.

Aldosterone, Aldosterone Receptors, and Sodium Channels

Aldosterone is synthesized from cholesterol in the zona glomerulosa of the adrenal gland by a series of enzymatic reactions. The final steps of aldosterone synthesis are catalyzed by aldosterone synthase encoded by the gene *CYP11B2* located on chromosome 8q21-22. Classic stimulators of aldosterone biosynthesis are Ang II, extracellular potassium

concentration, and ACTH. Vascular endothelial growth factor has recently emerged as a stimulator of aldosterone production.⁹⁴ Stimulation by these factors results in activation of aldosterone synthase induced by an increase in intracellular calcium concentration.⁹⁵

Aldosterone classically works in a genomic way through the induction and modulation of gene transcription with the cytoplasmic/nuclear MR within the renal cortical collecting duct cells as its main target. After binding of aldosterone to the MR, causing dissociation of chaperones and formation of MR dimers, this complex translocates to the nucleus, resulting in increased expression of several intracellular kinases, including serum- and glucocorticoid-induced kinase 1, Kirsten Ras GTP-binding protein 2A, and WNK4. This process leads to

increased expression of the luminal located epithelial sodium channel, renal outer medullary K⁺-channels, and the basolaterally located Na⁺/K⁺-ATPase.⁹⁶ Increased renal epithelial sodium channel activity promotes renal Na⁺ reabsorption, resulting in volume expansion and a rise in BP.

Additionally to its expression in renal collecting duct cells, the MR is also expressed in ECs and VSMCs.^{92,93} Important new insight in the role of the VSMC-MR has been obtained by engineering a mouse with an inducible, selective deletion of VSMC-MR.⁹⁷ In these KO mice, BP at young age is similar as in wild-type control mice, but, despite intact renal MR receptors, the age-related rise in BP is attenuated. Furthermore, aged VSMC-MR-KO mice have a decreased vascular tone, and the aged vessels exhibit decreased contractile responses to thromboxane, Ang II, and calcium channel agonists.⁹⁷ Moreover, these mice have an attenuated increase in BP and superoxide production to Ang II infusion and a decrease in large-artery stiffness after aldosterone salt challenge compared with wild-type mice.⁹⁸

Several new molecular pathways activated by the interaction of aldosterone with the VSMC-MR and contributing to vascular remodeling have been described in the past several years.^{98–100} These pathways promote vascular inflammation, fibrosis and VSMC hypertrophy, and proliferation and may contribute to the development of large artery stiffness. Although the clinical implication of these pathways requires further research, it has already been shown in patients with familial hyperaldosteronism type I that cardiac and vascular damage may precede the development of hypertension.¹⁰¹

In ECs, aldosterone increases the expression of endothelial Na channels in a MR-dependent way that can be blocked by spironolactone.¹⁰² Increased endothelial Na channel activity in combination with a high plasma sodium leads to stiffening of the cortex of ECs caused by an increase in sodium influx. A direct consequence of this stiffening is a decrease in endothelial NO synthase-mediated NO release.¹⁰³ Thus, high aldosterone in combination with high salt intake may result in endothelial dysfunction, which may contribute to a rise in BP independent of the renal effects of aldosterone.

Besides its genomic effects mediated by stimulation of the MR receptors in the kidney and vasculature, rapid nongenomic effects of aldosterone have also been reported.⁹¹ These nongenomic effects of aldosterone may be mediated by GPER.^{91,104} GPER is a widely distributed receptor, also identified in EC and VSMC.¹⁰⁵ GPER in cultured EC and VSMC can be stimulated by estrogen but also by aldosterone at picomolar concentrations.⁹¹ Aldosterone-induced activation of aortic vascular ECs via GPER leads to vasodilation as well as to proapoptotic and antiproliferative effects.¹⁰⁴ These effects of aldosterone are blocked by the GPER receptor antagonist G15. Whether aldosterone also exerts effects on VSMCs through activation of the GPER is uncertain, as GPER expression is no longer present when aortic VSMCs are cultured.⁹¹ GPER seems to play a role in the potentiation of Ang II-induced vasoconstriction by aldosterone because this potentiation could be blocked by G15, but not by the MR-antagonist eplerenone.¹⁰⁶

Sporadic and Familial Primary Aldosteronism

Primary aldosteronism (PA) is characterized by excessive autonomous aldosterone secretion by the adrenal gland. The

consequent volume expansion and hypertension leads to renin suppression, and accordingly the ARR has been advocated as a screening test for PA.¹⁰⁷ Among hypertensive individuals, the prevalence of PA is high, ranging from 4.3% in a primary care setting to 9.0% of referred patients and to 20% of those with therapy-resistant hypertension.¹⁰⁸ We and others have shown that the sensitivity of the ARR as a screening test for PA is relatively poor, which may relate to the way patients were selected and to overestimation of the true renin concentration by the nowadays commonly used direct renin assay (because of codetection of prorenin) instead of plasma renin activity measurements.^{109,110}

PA can be divided in frequent sporadic and rare familial forms. Familial hyperaldosteronism (FH) type I, also known as glucocorticoid-remediable aldosteronism, is an autosomal dominant disease caused by a recombination between the CYP11B2 and CYP11B1 (the latter being responsible for cortisol synthesis) genes, creating a chimeric gene whereby the CYP11B1 promotor and CYP11B2 coding sequences are juxtaposed. In FH-I, aldosterone synthesis is regulated by ACTH rather than by Ang II. Administration of glucocorticoids (thereby suppressing ACTH) reduces aldosterone levels, and the lowest dose of glucocorticoids normalizing BP is the treatment of choice. The cause of FH-II has yet to be identified. FH-II is diagnosed if ≥2 members of one family are affected. Adenomas as well as bilateral hyperplasia may underlie FH-II. The first family with FH-III has been described in 2008.¹¹¹ The affected family members presented with severe hypertension and hypokalemia at young age and in contrast to FH-I aldosterone could not be suppressed by dexamethasone. FH-III appeared to be caused by a mutation in the KCNJ5-gene, encoding for the G-protein-activated inward rectifier potassium channel Kir3.4.¹¹² This mutation results in the loss of K⁺-selectivity and increased Na⁺ conductance, leading to membrane depolarization of the zona glomerulosa cell with subsequent opening of voltage-dependent calcium channels and activation of the calcium-signaling pathway (Figure 6). Several other mutations in the KCNJ5 gene causing FH-III, not always accompanied by a severe phenotype as described for the first cases, have been reported.¹¹³ In addition to the germline mutations causing FH-III, somatic KCNJ5 mutations, resulting in loss of the selectivity filter of Kir3.4 channel, have been identified in surgically removed aldosterone-producing adenomas (APAs). These mutations are present in ≤47% in APAs from Western populations and ≤65% from a Japanese population.⁹⁵ In addition, several other less frequently occurring somatic mutations in 2 members of the P-type ATPase gene family (ATP1A1 and ATP2B3) and in CACNA1D (encoding for the voltage-gated Ca²⁺ channel Ca_v1.3) have been identified (Figure 6).^{114,115} In adrenal glomerulosa cells, mutations in ATP1A1 result in inappropriate depolarization, mutations in ATP2B3 in decreased intracellular calcium clearance, and mutations in CACNA1D in increased Ca²⁺ influx. In 308 APAs, negative for KCNJ5 mutations, 5.2% somatic mutations in ATP1A1 and 1.6% mutations in ATP2B3 have been identified.⁹⁵ CACNA1D mutations may occur in ≤11% of APAs.¹¹⁶ Interestingly, KCNJ5 mutations are common in APAs resembling the cortisol-secreting cells of the zona fasciculata,

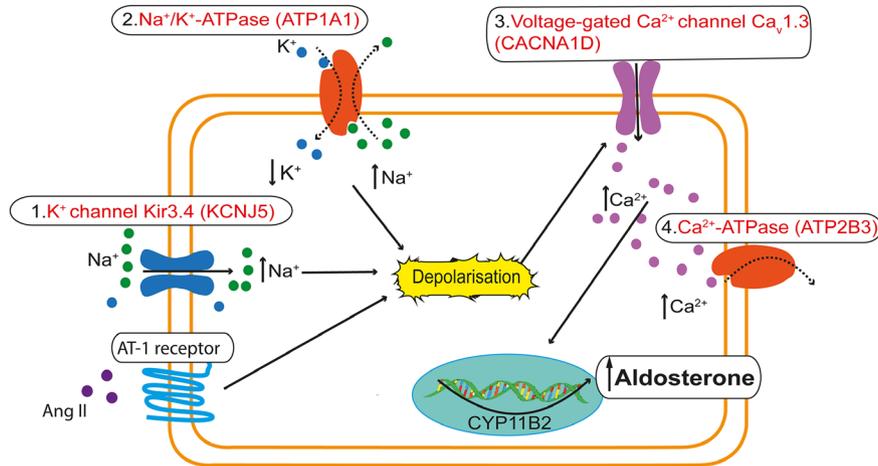


Figure 6. Mutations in ion channels (encoded by the genes KCN5, ATP1A1, CACNA1D, and ATP2B3) of the adrenal glomerulosa cell that have recently been linked to excessive aldosterone production. Normally, AT₁ receptor activation induces depolarization as a result of inactivation of the potassium channel Kir3.4 and Na⁺,K⁺-ATPase. Such depolarization triggers Ca²⁺-influx via voltage-gated Ca²⁺ channels (Ca_v1.3), and the resultant rise in intracellular Ca²⁺ activates the aldosterone synthase gene CYP11B2. Ca²⁺-ATPase subsequently removes Ca²⁺ from the cell. KCN5 mutations affect the selectivity of the Kir3.4, now also allowing Na⁺ conductance. Similarly, mutations in ATP1A1 result in loss of pump activity and strongly reduced affinity for potassium, thereby increasing intracellular Na⁺. Increased Na⁺ levels cause depolarization, even in the absence of AT₁ receptor stimulation. Mutations in CACNA1D facilitate Ca²⁺ influx, whereas mutations in ATP2B3 hamper its removal from the cell, thus both elevating intracellular Ca²⁺. This activates CYP11B2 transcription.

whereas mutations in P-type ATP-ases and CACNA1D have been found in small zona glomerulosa cell APAs.¹¹⁵ These genotype–phenotype correlations might hopefully be of clinical use in the near future.

Aldosterone and Resistant Hypertension

Resistant hypertension is defined as uncontrolled hypertension, despite therapy with 3 drugs, including a diuretic, or BP elevations requiring ≥ 4 drugs for control with an estimated prevalence of 10% to 15% of hypertensive patients treated.¹¹⁷ PA, because of its high prevalence, can underlie resistant hypertension, but also in patients without PA, BP control was lower in patients with an elevated ARR and higher aldosterone levels.¹¹⁸ That aldosterone plays a role in resistant hypertension is supported by trials showing that addition of MR blockers to usual antihypertensive treatment can sometimes produce pronounced BP reductions.¹¹⁹ In an open-label study, addition of spironolactone 25 to 100 mg per day to 175 patients with true resistant hypertension to existing antihypertensive therapy reduced ambulatory BP by 16/9 mmHg.¹²⁰ The mechanism of the BP-lowering effect of MR antagonists in resistant hypertension is incompletely understood because indices of aldosterone excess, such as low renin or high ARR or a low serum potassium, do not predict the response to aldosterone receptor blockers.¹²¹ Spironolactone-mediated inhibition of central sympathetic nervous system activity has been proposed as one of the mechanisms.¹²² Furthermore, reduction of vascular stiffness and improvement of endothelial function by blockade of vascular MRs may be involved.

Protective Arms of the RAAS: Can We Expect New RAAS Drugs?

All current RAAS blockers interfere with the renin–ACE–AT₁ receptor–aldosterone axis. Aldosterone synthase inhibitors are being considered an alternative for MR antagonists,¹²³

but such drugs would obviously also interfere with this pathway. Three new RAAS pathways have been discovered in the last 2 decades, which might be of interest for future drug development: (1) AT₂ receptor stimulation, (2) stimulation of ACE2–Ang-(1–7)–Mas receptor signaling, and (3) modulation of angiotensin III and IV signaling. In addition, drugs are being developed which block the RAAS plus an additional hormonal system, for example, combined AT₁ receptor blockers/neprilysin inhibitors, which prevent the degradation of natriuretic peptides (by neprilysin) and combined AT₁ receptor/endothelin-1 receptor antagonists. Their discussion is beyond the scope of this review.

AT₂ Receptor Stimulation

As discussed earlier, the AT₂ receptor is generally considered to have effects that are opposite to those of the AT₁ receptor. Its presumed endogenous ligands are Ang II, Ang III, Ang IV, and Ang-(1–7) in order of highest to lowest affinity (Figure 1).¹²⁴ Ang III seems to be the preferred agonist for AT₂ receptors.^{125–127} For reasons that are still unclear, AT₂ receptor-mediated vasodilation is best detected under partial AT₁ receptor blockade, and the same may apply to its natriuretic, antifibrotic, and anti-inflammatory effects in the kidney.^{125,128–130} AT₂ receptor KO mice display an increased BP, worsened pressure-natriuresis, increased baroreflex sensitivity, increased responsiveness to hypertensive stimuli (NO synthase inhibition), and decreased cardiac and vascular AT₁ receptor expression.^{131–133} AT₂ receptor overexpression shows the opposite.¹³⁴ It therefore seemed logical to develop specific AT₂ receptor agonists.

Currently, 4 candidate drugs for clinical development have been identified, namely the peptidergic agonists β -Tyr4-Ang II, β -Ile5-Ang II, and LP2-3 and the nonpeptide agonist Compound 21 (C21).^{130,135} β -Tyr4-Ang II and β -Ile5-Ang II are Ang II analogues in which the Tyr4 and Ile5 α -amino acid

residues have been replaced by β -amino acids, that is, amino acids containing an additional methylene group.¹³⁰ This results in an almost complete loss of AT₁ receptor affinity, a modest (\approx 5- to 15-fold) decrease in AT₂ receptor affinity and an increased stability. Both peptide agonists caused weak, AT₂ receptor-mediated, NO-dependent vasorelaxation in the mouse aorta. In addition, β -Ile5-Ang II lowered BP in SHR during coinfusion with candesartan.¹³⁰

LP2-3 is cyclic Ang-(1-7) (see below) with a D-lysine N-terminal extension.¹³⁵ Although it inhibits pathological remodeling of lung, cardiac, and vascular tissue in a model of hyperoxia-induced neonatal pulmonary dysplasia,¹³⁵ there is currently no proof of its claimed AT₂ receptor agonistic activity. Much more is known about C21, which is expected to enter the clinical phase of development this year.¹³⁶ Confusingly, despite the wealth of data on AT₂ receptor-mediated vasodilation, C21 has either no effect on BP or even increases BP.^{129,136,137} The latter may relate to the fact that, for instance in SHR, AT₂ receptors become AT₁ receptor-like (ie, constrictor), whereas at exceptionally high doses, C21 also activates AT₁ receptors.^{21,137} In vitro, C21 has a weak vasodilator effect, particularly observed during AT₁ receptor blockade, at concentrations above its K_d.¹³⁸ In fact, because such effects were also observed in vessels of AT₂ receptor KO mice, it has been proposed that C21 has pleiotropic effects; its ability to block vasoconstriction to nonangiotensin constrictors further confirmed this view.¹³⁷ In summary, although C21 does not seem to be an appropriate antihypertensive drug, the antifibrotic and anti-inflammatory effects of AT₂ receptor stimulation warrant alternative indications, including Marfan's syndrome.³¹

Because AT₂ receptors also stimulate neurite outgrowth, thereby facilitating pain,¹³⁹ a recent clinical trial has investigated to what degree AT₂ receptor antagonists might be applied in neuropathic pain. Indeed, already after 3 weeks, such drugs reduced pain in patients with postherpetic neuralgia.¹⁴⁰ Whether this outcome affects the future application of AT₂ receptor agonists cannot yet be said.

ACE2-Ang-(1-7)-Mas receptor Axis

Ang-(1-7), mainly produced from Ang II by ACE2 (Figure 1), opposes AT₁ receptor-mediated effects via its binding to the Mas receptor.¹⁴¹ Yet, it also binds AT₂ receptors and, at high concentrations, even acts as a partial AT₁ receptor agonist.¹⁴² A plethora of beneficial cardiovascular effects has been described for Ang-(1-7) in the past 25 years. These include protection against heart failure, natriuretic, antithrombotic, antihypertrophic, antifibrotic, and antiarrhythmic effects, attenuation of plaque formation, and amelioration of metabolic syndrome-related vascular dysfunction.^{129,143} It has weak vasodilator effects, which have not uniformly been confirmed. Ang-(1-7) also stimulates the production of endothelial progenitor cells (and tube formation thereof), but simultaneously inhibits tube formation by adult EC.^{129,143} The circumstances under which the BP-lowering effects of Ang-(1-7) have been investigated varied widely (species, models, cotreatment with RAAS blockers/NO synthase inhibitors, salt intake) so that even now it cannot be stated with certainty that Ang-(1-7) is an antihypertensive agent. Similarly, although ACE2 overexpression does lower BP, this may be simply because of its

capacity to degrade Ang II (rather than its generation of Ang-(1-7)).¹⁴⁴ Even more worryingly, the putative ACE2 activators 1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one (XNT) and diminazene were recently shown to act fully independently from either ACE2 or Ang-(1-7), thus questioning whether their in-vivo effects have anything to do with this pathway.¹⁴⁵

Despite these controversies, stimulation of the ACE2-Ang-(1-7)-Mas receptor axis might still be an interesting therapeutic option. In view of the rapid breakdown of Ang-(1-7) (as well as its AT₁ receptor agonistic properties at high concentrations), alternative strategies have been developed. These are oligosaccharide (hydroxypropyl β -cyclodextrin)-encapsulated Ang-(1-7), Ang-(1-7) peptide stabilization by thioether bridging (creating so-called cyclic Ang-(1-7)), NorLeu3-Ang-(1-7), the peptide drug CGEN856S, and the nonpeptide drug AVE0991.^{129,146} Cyclodextrin-encapsulated Ang-(1-7), AVE0091, and CGEN856S have shown BP-lowering effects in hypertensive animals, whereas the other agonists have not been tested yet in such a setting. Clearly, much more work is needed before these drugs can enter the clinic, although NorLeu3-Ang-(1-7) has been used clinically to treat foot ulcers in diabetic patients.¹⁴⁷

Blockade of Ang III and Ang IV

As discussed earlier, Ang III is believed to act as an AT₂ receptor agonist, for example, in the kidney and vessel wall. Yet, in the brain, it has been proposed to be the preferred AT₁ receptor agonist, thus causing hypertension.¹⁴⁸ On this basis, aminopeptidase A inhibitors (which block the conversion of Ang II to Ang III) are now being developed, which act exclusively in the brain. Indeed, RB150 (4,4'-dithio[bis(3S)-aminobutyl sulfonic acid]) is a prodrug that, after crossing the blood-brain barrier, is converted to the aminopeptidase A inhibitor EC33 ((3S)-3-amino-4-sulfanyl-butane-1-sulfonic acid).¹⁴⁹ RB150 can be delivered orally and has already shown antihypertensive and cardioprotective properties in animal models, so that it is now under evaluation in a phase Ib clinical study.¹⁵⁰

To what degree the aminopeptidase N product of Ang III, that is, Ang IV, has a function in BP regulation remains unclear. At high (micromolar) concentrations, it binds to AT₁ and AT₂ receptors, resulting in both constrictor and relaxant effects, the former possibly involving endothelin-1.^{151,152} However, the relevance of these observations, given its low (femtomolar) concentrations in vivo, is questionable. Instead, high potency effects of Ang IV may rather involve its binding to insulin-regulated aminopeptidase (IRAP), also known as the AT₄ receptor (please note that AT₃ receptors do not exist, the 4 refers to Ang IV).¹⁵³ Unfortunately, even this concept has recently been challenged,¹⁵⁴ leaving as a final option the observation that Ang IV binds with high affinity to AT₁ receptors that are constitutively active, that is, that already display activity without agonist binding.¹⁵⁵ Until today the physiological relevance of this phenomenon is unknown.

Conclusions

Tissue angiotensin generation depends on kidney-derived renin and hepatic angiotensinogen, occurs extracellular, and is followed by rapid AT receptor binding and internalization.

Locally generated Ang II affects the constrictor/relaxant balance, vascular remodeling, and inflammation. Tissue angiotensin generation does not necessarily run in parallel with angiotensin generation in the circulation, and this explains why the beneficial effects of RAAS blockers cannot be simply explained on the basis of changes in the circulating RAAS. Sex, ethnicity, salt intake, genetic variation, and the use of antihypertensive drugs determine the degree of RAAS activity, and although in general high RAAS activity (as evidenced by high plasma renin levels) would be supportive for the application of RAAS blockers, the interindividual RAAS component variation is such that it is impossible to exactly define high or low renin levels that warrant the choice for a certain RAAS blocker. Too much RAAS blockade yields effects that can be expected when removing Ang II/aldosterone (hypotension, hyperkalemia, renal dysfunction), and thus the goal should be to obtain optimal instead of maximal RAAS blockade, guided by regularly measuring BP, potassium, and creatinine. Aldosterone unexpectedly has a wide range of extrarenal effects, among others in EC and VSMC, and these are not necessarily all mediated via the MR. Together with the many mutations that have recently been discovered in genes encoding for proteins that control sodium, potassium, and calcium ion homeostasis in adrenal cells (with strong resultant effects on aldosterone synthesis), this explains the revived interest in drugs that block aldosterone, for example, in resistant hypertension. In addition, the discovery of the relaxant, protective arms of the RAAS, involving AT₂ and Mas receptor stimulation, may yield new drugs that can be applied in the future on top of the existing RAAS blockers in patients with cardiovascular and renal disorders.

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Disclosures

None.

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