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The renin angiotensin aldosterone system and its suppression

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Abbreviations

11β-HSD2 11β-hydroxysteroid dehydrogenase type 2

ACE angiotensin converting enzyme

ACEI angiotensin converting enzyme inhibitor

ARB angiotensin II type-1 receptor blocker

AngI angiotensin I

AngII angiotensin II

AT_1R angiotensin II type-1 receptor

AT_2R angiotensin II type-2 receptor

CHF congestive heart failure

CKD chronic kidney disease

DCM dilated cardiomyopathy

DRI direct renin inhibitor

GFR glomerular filtration rate

HCM hypertrophic cardiomyopathy

HFpEF heart failure with preserved ejection fraction

HFrEF heart failure with reduced ejection fraction

HTN systemic hypertension

MMVD myxomatous mitral valve disease

MR mineralocorticoid receptor

MRA mineralocorticoid receptor antagonist

RAAS renin angiotensin aldosterone system

UAldo:C urine aldosterone to creatinine ratio

UP:C urine protein to creatinine ratio

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Abstract

Chronic activation of the renin-angiotensin-aldosterone system (RAAS) promotes and perpetuates the syndromes of congestive heart failure (CHF), systemic hypertension (HTN), and chronic kidney disease (CKD). Excessive circulating and tissue angiotensin II (AngII) and aldosterone levels lead to a pro-fibrotic, - inflammatory, and -hypertrophic milieu that causes remodeling and dysfunction in cardiovascular and renal tissues. Our understanding the RAAS's role in this pathologic remodeling has grown over the past few decades and numerous medical therapies aimed at suppressing the RAAS have been developed. Despite this, morbidity from these diseases remains high. Continued investigation into the complexities of the RAAS should help us modulate (suppress or enhance) components of this system and improve quality of life and survival. This review focuses on updates in our understanding of the RAAS and the

pathophysiology of AngII and aldosterone excess, reviewing what is known about its suppression in cardiovascular and renal diseases, especially in the cat and dog.

Introduction

Although RAAS activation can be compensatory in the early stages of cardiovascular and renal disease, long-term activation is maladaptive. The relative increase in plasma renin activity and blood aldosterone and norepinephrine concentrations in human patients are therefore considered markers of, and contributors to, the hemodynamic and anatomic derangements of this syndrome.[1-5] The adverse effects of chronic exposure to high concentrations of AngII and aldosterone are outlined in Table 1.[6-14] Suppression of RAAS is a key strategy in the therapy of chronic cardiovascular and renal disease and is achieved by the administration of angiotensin converting enzyme inhibitors (ACEI), AngII type-1 receptor blockers (ARB), and mineralocorticoid receptor antagonists (MRA), alone or in combination. The more we learn about this system, however, the broader and more complex it becomes. Redundancies that bypass our therapeutic blockade or inadvertent suppression of beneficial components of this system might reduce the efficacy of our RAAS suppressive therapies. Angiotensin II and aldosterone levels occasionally rise despite pharmacologic RAAS suppression, and the underlying mechanisms are still not well understood.[15-32] The RAAS is also continuously adapting to the individual's metabolic state, underlying disease process, and therapy. Some drugs and treatment strategies used to treat cardiovascular disease, including furosemide, amlodipine, hydralazine

and dietary sodium restriction, also stimulate the RAAS (Supplemental Figure 1).[5,33-39] Continued research into this complex system is necessary to improve medical therapies for cardiovascular and renal diseases, allowing us to more adeptly modulate this system and improve clinical outcomes.

Circulating and tissue renin angiotensin aldosterone systems

Renin is synthesized as preprorenin in the juxtaglomerular epithelioid cells, cleaved to prorenin, and either released as prorenin or further processed to form active renin, which is stored in granules. Renin granules are then released in a controlled manner, making renin the rate-limiting step of the renin-angiotensin-aldosterone cascade in most species. Conversely, angiotensinogen is constitutively released from the liver (Figure 1), and is usually present in excess when compared to renin. Increased renin synthesis and release occur in situations of low systemic blood pressure, hypovolemia, sodium deprivation, and sympathetic stimulation. Short-term activation is beneficial, resulting in improved cardiac output and blood pressure. In the circulation, renin metabolizes angiotensinogen, liberating angiotensin I (AngI). Angiotensin converting enzyme (ACE), which is released from endothelial cells, converts AngI to AngII. AngII acts at 2 receptors, the angiotensin type-1 and type-2 receptors (AT_1R and AT_2R). AngII's actions at the AT_1R leads to increased sodium retention, vasoconstriction (including preferential constriction of the efferent arteriole of the kidney), stimulation of thirst and desire for salt, enhanced sympathetic nervous system activity, and aldosterone release from the adrenal

gland's zona glomerulosa. The interaction of AngII at this receptor mediates much of the pathologic changes associated with chronic RAAS activation summarized in Table 1. Actions of AT_2R-stimulation are counter-regulatory to those of the AT_1R, where stimulation leads to anti-inflammatory, anti-fibrotic, and vasodilatory effects. The AT_2R is the dominant receptor type in the fetus and plays a key role in development, but is less relevant in the normal adult. The AT_2R, however, might be up-regulated in certain disease states.[40-42]

Aldosterone, the terminal hormone of RAAS, exerts 90% of the mineralocorticoid activity of adrenal secretions, and is a key regulator of sodium, potassium, and body fluid balance.[43,44] Angiotensin II and increased extracellular K^+ concentration, the strongest secretagogues for aldosterone, increase expression of the CYP11B2 gene, which encodes aldosterone synthase.[45] Acting via the mineralocorticoid receptor (MR), aldosterone modulates the expression of ion channels, pumps, and exchangers in epithelial tissues (kidney, colon, and salivary and sweat glands). This ultimately leads to an increase in transepithelial Na^+ and water reabsorption and K^+ excretion. Mineralocorticoid receptors are also found in non-epithelial tissues such as the retina, brain, myocardium, vascular smooth muscle cells, macrophages, fibroblasts, and adpiocytes.[46-50] Aldosterone's effects are therefore wide spread, extending well beyond its role as a 'renal hormone'. Specifically, aldosterone is thought to mediate inflammation and affect energy metabolism in non-epithelial tissues.[50]

The unbound MR is primarily located in the cytoplasm and when bound by ligand it is shuttled to the nucleus where it acts as a transcription factor.[51] Some actions of aldosterone are non-genomic and occur relatively rapidly. These actions are likely mediated by several mechanisms: activation of the small fraction of cell membrane localized MR and MR interaction with other receptors such as the G-protein coupled estrogen receptor, AT_1R, and epidermal growth factor receptor.[52] Aldosterone is the primary physiological ligand of the MR, though glucocorticoids, such as cortisol have a similar receptor affinity. In epithelial cells and vascular smooth muscle cells, the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) alters the glucocorticoids and prevents their binding to the MR. Thus, more abundant glucocorticoids are prevented from out-competing mineralocorticoids for the MR. In tissues such as the myocardium where 11β-HSD2 is scarce, cortisol binding to the MR is likely important.[53]

Angiotensin peptides and aldosterone are also produced in tissues such as brain, blood vessels, kidneys, and the heart.[40,54-57] Locally produced RAAS hormones play important roles in normal cardiovascular function and electrolyte-fluid homeostasis, yet also mediate pathologic remodeling in the tissues. In vitro work on cultured vascular smooth muscle cells and cardiomyocytes has shown that aldosterone up-regulates components of the RAAS, including ACE activity and AngII-stimulated signal transduction, leading to increased local (tissue) activity of the RAAS.[58-62] These local or tissue RAAS components are also modulated by mechanical stretch of the myocardium and vessels, adipocyte secretions, presence of reactive

oxygen species (ROS), and inflammation.[63,64] Although the amount of aldosterone produced in the tissues is normally < 1% of the amount produced by the adrenal glands[56,65], locally produced aldosterone likely plays a role in pathologic remodeling (hypertrophy and fibrosis), and tissue RAAS management will likely be part of future pharmacotherapeutic strategy.[11,12,40]

Recently, attention has turned toward the prorenin receptor ((P)RR), which binds both renin and prorenin, and is a multifunctional receptor found in several locations within the kidney, as well as the heart, adipose tissue, and brain.[66] The diverse roles of this receptor include maintenance of intra- and extracellular pH, central regulation of blood pressure, and sodium homeostasis in the kidney. The role this receptor plays in the pathophysiology of cardiovascular and renal diseases remains unanswered, yet it is known that the (P)RR binds prorenin and renders it active in the tissues, enabling the *local* generation of angiotensin peptides.[54,66,67]

The quest to find novel/alternative pathways of angiotensin generation and metabolism led to the discovery of angiotensin (1,12) and (1,25), which are found in cardiovascular and renal tissues and serve as precursors for angiotensin peptides such as AngII (Figure 2).[68,69] Chymase catalyzes the formation of AngII from both angiotensin (1,12) and AngI, allowing ACE-independent formation of AngII in the tissue, and this pathway is likely the primary generator of tissue AngII.[69-71] In fact, chymase has been labeled the 'most efficient AngII-forming enzyme' and is released from mast cells, cardiac fibroblasts, and vascular endothelial cells during acute and

chronic tissue injury and remodeling.[72-74] Chymase activation results in pro-fibrotic, anti-fibrotic, or pro-inflammatory phenotypes, with the exact phenotype or combination of phenotypes depending upon the tissue type and the nature and timing of the stress.[75] Chymase is an important player in AngII formation (and a pro-fibrotic phenotype) in cardiac disease.[76,77] Chymase is also hypothesized to exacerbate cell death and mitochondrial injury after cardiac ischemia/reperfusion.[78] Additionally, mast cell activation, and increased release of mediators such as chymase has been implicated in the pathophysiology of cardiometabolic disease, such as diabetes mellitus and obesity.[79] The phenotype of chymase activation differs in canine models of hemodynamic overload and might contribute to both extracellular matrix degradation and fibrosis formation, contributing to, or counter-balancing ventricular dilation.[80-84]

The metabolism/degradation of angiotensin peptides is of interest as these pathways can reduce levels of AngI and AngII and result in peptides that are 'active' and, in some cases, counter-regulatory to RAAS activation. A counter-regulatory pathway, the ACE2-Angiotensin(1,7)-Mas axis, is currently being investigated, as it appears to elicit protective actions, including vasodilation and increased nitric oxide synthesis.[40,54] The generation of Angiotensin(1,7) [Ang(1,7)] in the heart and brain arises from both ACE2 processing of AngII, whereas in the circulation and kidney, Ang(1,7) arises from processing of angiotensin I by endopeptidases, such as neprilysin.[54,85] Angiotensin(1,7) and its metabolite Ala^1-Ang(1,7) bind the Mas and Mas-related G protein-coupled receptor and elicit the effects noted above.[86] The expression of Mas

receptors has been most thoroughly explored in rats and mice, where it is expressed in brain, testis, kidney, heart, and vessels, with expression patterns changing with age.[87] Current research is exploring the role of this counter-regulatory pathway in the pathophysiology of cardiovascular and renal disease.

The actions of other newly discovered angiotensin peptides are also being studied and might reveal pharmacologic targets for both up- and down-regulation. For example, angiotensin I can also be metabolized by ACE2 to form to Ang(1,9), which appears to bind at the AT_2R.[88] Also, AngII is metabolized by aminopeptidase A to form angiotensin III, which like its parent peptide, is capable of binding both the AT_1R and AT_2R.[89] Angiotensin III can then be metabolized to angiotensin IV via aminopeptidase N. Both of these angiotensin peptides lead to increased atrial stretch-induced atrial natriuretic peptide secretion in animal models, via the AT_2R and the insulin-regulated aminopeptidase, respectively.[90,91] For a more comprehensive review of tissue RAAS, novel RAAS components, and the production of aldosterone in tissues, the reader is referred elsewhere.[40,42,54,55]

Chronobiology of the RAAS

RAAS peptides and antidiuretic hormones oscillate in a circadian (approximately 24 hours) periodicity.[92-94] This leads to day-night variation in urine flow rate, urinary electrolyte excretion, and blood pressure. The circadian 'clock' begins at the cellular level where transcriptional and

translational regulators induce expression of multiple genes that ultimately dictate the metabolic rates and homeostasis of an organism.[95] Central clocks, residing in the brain, and local cellular clocks respond to environmental and metabolic signals, allowing the organism to adapt to changing circumstances. The time-variant fluctuations in RAAS hormones in dogs[92,96] is, similar to humans and urine flow and sodium excretion have diurnal peaks whereas urine osmolality and potassium excretion have nocturnal peaks. A more recent and detailed characterization of the chronobiology of these peptides show that, in dogs fed a once daily (A.M.) normal sodium meal, that renin activity and the urinary aldosterone to creatinine ratio (UAldo:C) decrease after feeding, then rise throughout the afternoon, peaking in the evening.[97] Systemic blood pressure oscillates in parallel with RAAS peptides, increasing in the first half of the night, decreasing as morning approaches, which is opposite to what is described in healthy adult humans.[98] The timing of feeding also affects the circadian rhythm of both the RAAS and systemic blood pressure suggesting that dietary sodium is a key mediator of this modulation.[97,99] An improved understanding of RAAS chronobiology has implications in both the characterization and quantification of RAAS activity and will likely inform the timing of drug administration.[100,101]

RAAS, Renal Hemodynamics and Tubular Function

The principal effects of AngII in the kidney include vasoconstriction of the interlobular artery and afferent and efferent arterioles (greatest effect on the latter), enhanced afferent arteriolar response to tubuloglomerular feedback, and constriction of the glomerular mesangium. The net

result of these changes is an increase in filtration fraction. RAAS activation enhances sodium reabsorption throughout the renal tubules with AngII effects greatest at the proximal tubule and loop of Henle and aldosterone effects greatest at the distal convoluted tubule and collecting duct. Changes in renal perfusion pressure can also elicit biophysical and paracrine effects, such as increased generation of ATP, nitric oxide, and ROS, and induction of cyclooxygenase enzymes that lead to a pressure natriuresis.[102-104] This allows the kidney to 'escape' from the sodium retaining effects of excess AngII (and increased aldosterone production).[105] Natriuretic peptides also counterbalance increases in blood volume and are released from both the atria and ventricles in the presence of increased stretch. Recent studies of the chronobiology of the RAAS, as well as studies of experimental sodium loading in dogs, suggest that the circulating and local RAAS might play a more important role than pressure natriuresis in physiological sodium homeostasis in this species.[106-108]

Assessment of RAAS Activity

Commonly evaluated components of the RAAS include plasma renin activity, direct plasma renin levels, plasma or serum aldosterone and AngII concentrations, plasma ACE activity, urine and serum Na^+/K^+ ratios, 24-hour urinary aldosterone excretion, and the UAldo:C. More recently, liquid chromatography – mass spectrometry (LC-MS)/MS has been used to create a comprehensive renin-angiotensin system Fingerprint® (Attoquant Diagnostics GmbH Vienna Austria) from both blood and tissue samples (Figure 2).[109,110] A comprehensive assessment is

beneficial, as looking at only 1 or 2 components of this system might be misleading. For example, assessment of only ACE activity during ACEI therapy might suggest very effective suppression, yet AngII and aldosterone does 'break through' this therapy in some patients.[15,17,31,106,111,112] Additional information regarding aldosterone metabolism and the assessment of RAAS activity in dogs and cats can be found in the supplemental section of this review.

Genetic profiling of specific populations and individuals will also likely play a role in the development of future RAAS modulation strategies. RAAS genotype evaluation has led to the discovery of polymorphisms such as the insertion or deletion of a base pair in intron 16 of the ACE gene in humans and dogs. These insertion and deletion genotypes impact baseline ACE activity and might influence an individual's response to ACEI therapy. A more individualized and comprehensive approach to future RAAS modulation should include a RAAS profile, RAAS genotype, metabolic profile, and diet survey.

Angiotensin II and Aldosterone Excess

Angiotensin II and Aldosterone as cardiovascular and renal toxins

There is a substantial body of evidence, that AngII and aldosterone likely acting in concert are "cardio-" and "nephrotoxic"(Table 1)[48,113,114]. This is based on direct (hormone infusion,

transgenic animal models) and indirect (RAAS suppression) evidence (Figure 3 and supplemental Table 1). These studies link the fibrosis that attends the pathologic remodeling caused by excess AngII and aldosterone to myocardial, vascular, and renal (especially glomerular) dysfunction. The mechanisms by which increased AngII and aldosterone levels lead to fibrosis are multifactorial and likely involve stimulation of fibroblasts, generation of ROS, inflammation, and upregulation of transcription factors such as nuclear factor kappa B, cytokines such as transforming growth factor beta and tumor necrosis factor alpha, and upregulation of molecules such as plasminogen activator inhibitor-1.[12,115-122] Ultimately, these changes lead to increased collagen gene expression and synthesis, as well as decreased fibrinolysis. Excess AngII and aldosterone also directly lead to vascular endothelial dysfunction via the vasoconstrictive effects of AngII, increased endothelin expression, inhibition of nitric oxide synthase, cyclooxygenase-2 activation, enhanced generation of ROS, and non-genomic effects, such as activation of protein kinase C.[11,123-127]

RAAS activation also contributes to the immune cell infiltration that contributes to the inflammation and fibrosis that attends renal ischemia, myocardial infarction, and systemic hypertension.[128-130] More specifically, the MR on macrophages has been implicated in polarization of the macrophage population from the anti-inflammatory M2 subtype to the inflammatory M1 subtype, perpetuating inflammation and tissue remodeling.[130-133] Antagonism or deletion of the macrophage MR is protective against cardiovascular remodeling, even in

situations where aldosterone levels are normal.[130-132] Oxidative stress also appears to amplify inflammation and directly activate the MR or allow glucocorticoids to activate the MR.[134,135] Finally, aldosterone excess decreases baroreceptor sensitivity in healthy humans and dogs with experimentally induced heart failure, the result being an undesirable increase in heart rate.[8,136,137] The mechanisms by which neurohormonal activation decreases baroreceptor sensitivity are not fully understood.[136,138] Decreased baroreceptor-heart rate sensitivity is a key feature of the heart failure syndrome[139] and is an independent predictor of poor prognosis in people with heart failure.[140,141]

The deleterious effects of excess aldosterone are augmented when the relationship between sodium and aldosterone is abnormal. In the aforementioned animal studies (Table 1, supplemental materials), sodium was variably unrestricted or given in excess. Under normal physiologic conditions, sodium supplementation leads to the inhibition of aldosterone release, allowing excess sodium to be excreted by the kidney. In the face of high or even normal total body sodium a high aldosterone level is therefore inappropriate. Further discussion of the interplay between aldosterone, sodium status, metabolic state, inflammation, and oxidative stress can be found in the supplemental materials.

Suppression of RAAS

ACE-inhibitors are widely used in the treatment of cardiovascular and renal diseases in multiple species. The ACEI used most commonly in veterinary medicine have been reviewed and are summarized in Supplemental Tables 2 and 3.[142] Most ACEI are administered as pro-drugs requiring esterification in the liver to become active. Drugs in this class inhibit ACE and not only decrease formation of AngII, but also decrease the degradation of the vasodilatory compound bradykinin. The pharmacokinetic properties of ACEI are complex and their disposition during repeated dosing cannot be characterized by a classical noncompartmental model.[143] The plasma concentration-time profile has initial and protracted elimination phases, due to clearance of free drug and release of drug from tissue-binding sites, respectively.[144] Using circulating plasma ACE activity as a surrogate for efficacy, dosages have been determined for several ACEI in animals (Supplemental Tables 2 and 3). Although circulating ACE activity is significantly suppressed at these recommended dosages, clinical trials are required to determine efficacy and if there are outcome differences between ACEI. Variable efficacy amongst ACEIs in reducing cardiovascular and renal endpoints might arise from differences in the structure of the active moieties, their lipophilicity and bioavailability, affinity for tissue-bound ACE, and elimination method. The clinical relevance of these differences, however, is not known. As ACE is anchored to the cell membrane such that its catalytic site faces the extracellular space,[145,146] differences in lipophilicity should be less important, except in the case of certain centrally-acting ACEI.[147] In humans, differences in the clinical efficacy of ACEI has been hypothesized to be associated with variation in tissue-ACE affinity[57] and this might hold true for

dogs and cats, too. Chymase has a greater catalytic efficiency than ACE and is thought to serve as the primary generator of AngII in the tissues however, and likely contributes to apparent inefficacy of ACEI.[74,148-150] Additional explanations for the sporadic failure of ACEI to prevent pathologic remodeling as a class, include inadequate aldosterone suppression (aldosterone breakthrough), under-dosing, and poor compliance. As previously discussed, novel approaches to target tissue RAAS management is likely to be part of future pharmacotherapeutic strategies.[11,12,40] The most common adverse effects of ACEI in man include cough or angioedema, or both, due to the accumulation of bradykinin, as well as increased prostacyclin and nitric oxide formation. These adverse effects are rarely, if at all, recognized in dogs and cats.

Angiotensin II receptor blockers prevent AngII from binding its AT_1 receptor and were developed due to the cough and angioedema adverse effects seen with ACE-inhibition in man. Theoretical benefits of ARBs include blockade of the actions of AngII at the AT_1R, regardless of the pathway of its formation (via ACE or non-ACE pathways, such as chymase). Also, increased circulating AngII, resulting from AT_1R blockade, might stimulate the AT_2R, thought to be counter-regulatory to the maladaptive actions mediated by the AT_1R.[151] Commonly used ARBs are summarized in Supplemental Table 4. As with ACEI, some ARBs are administered as pro-drugs that require metabolism to an active molecule. Whether ARBs are superior to ACEI in dogs and cats with either cardiovascular or renal disease is not known, though the early

experience with telmisartan in cats with hypertension, chronic kidney disease, and proteinuria suggests that this may be the case (see below). A recent meta analysis of studies[152-155] of dual (ACEI and ARB) therapy in patients with heart failure concluded that until further clinical trials are performed, dual therapy should not be routinely administered.[156] Controlled clinical trials in dogs and cats evaluating combination ACEI and ARB therapy have not been performed.

Spironolactone and eplerenone are synthetic steroidal mineralocorticoid receptor antagonists. MR antagonism in the distal renal tubule cells causes an increase in urinary Na^+ excretion and a decrease in K^+ excretion. In the normal dog, spironolactone decreases K^+ excretion, yet does not significantly increase urine Na^+ excretion and urine volume, even at the high dosage of 8mg/kg/day.[157] In dogs with chronic heart failure and chronic aldosterone excess, however, spironolactone likely increases urinary sodium excretion and urine volume. In the dog, spironolactone is quickly absorbed through the gastrointestinal tract into the plasma and is then converted to several active metabolites.[158,159] The bioavailability in the dog is highest when given with food, reaching 80 to 90%.[160] During an experimental model of hyperaldosteronism, the spironolactone dosage of 2mg/kg PO once daily restors the urinary Na^+/K^+ ratio to near normal, and this is likely the optimal dosage in dogs.[160] Because of spironolactone's ability to bind other steroid hormone receptors, adverse effects in people include decreased testosterone production, inhibition of testosterone binding its receptor, and increased estradiol levels, which can cause gynecomastia in men and menstrual abnormalities in women.[161-163] For this reason, the

more selective MRA, eplerenone, was developed. Spironolactone is relatively inexpensive and is the most frequently used MRA in veterinary medicine. Additional pharmacological therapies targeting the aldosterone/MR pathway are currently under active investigation. The third generation MRA (finerenone) holds promise, as it also modifies co-regulators of MR signaling.[164] Fourth generation MRA might provide even greater MR selectivity, targeting the MR in specific tissues and possibly modulating only specific transcriptional functions.

Direct renin inhibitors (DRI) such as aliskiren theoretically prevent the initiation of the renin-angiotensin cascade. First generation DRIs have not proved to be a panacea for cardiovascular and kidney diseases, however, and next generation DRIs have been developed and evaluation of their efficacy is underway.[165,166] There are currently no studies of DRIs in veterinary patients. First generation aldosterone synthase inhibitors unfortunately lack selectivity and decrease cortisol production.[167] For this reason, more specific, next generation aldosterone synthase inhibitors are being developed.[168]

Novel RAAS modulators include neprilysin inhibitors, recombinant human ACE2, and chymase inhibitors. Inhibition of the neutral endopeptidase, neprilysin, decreases the breakdown of endogenous vasoactive peptides, including natriuretic peptides, bradykinin and adrenomedullin. When the neprilysin inhibitor, sacubitril, was combined with an ACEI, the frequency of angioedema and cough in people was unacceptably high.[169] A new combination product

(Entresto®), therefore, pairs sacubitril and the ARB valsartan. This combination was evaluated in a large human clinical trial, where it was superior to ACE inhibition alone in reducing the risk of death and hospitalization for patients with heart failure with reduced ejection fraction (HFrEF).[170] This new drug is now a class I recommendation for treatment of HFrEF in humans.[171] In dogs with RAAS activation secondary to sodium restriction, Entresto® (sacubitril dosed at 15mg/kg PO q24h) led to a sustained reduction in circulating aldosterone levels when compared to dogs treated with either placebo or valsartan.[172] This combination has also been evaluated (sacubitril dosed at 20mg/kg PO q12h) in a small study of dogs with ACVIM stage B2 MMVD[173] (Figure 4) and was well tolerated during the 30-day trial.[174] Another novel therapeutic target is the ACE2 enzyme. This enzyme is considered counter regulatory to the vasoconstrictive and fluid retentive aspects of RAAS, as it catalyzes the conversion of AngII to the vasodilatory, anti-inflammatory, anti-fibrogenic peptide Ang(1,7).[175-178]Human recombinant ACE2 has been evaluated in 1 small study in people with acute and chronic heart failure where it was found to normalize AngII levels and increases levels of the beneficial Ang(1,7) metabolite.[110] Finally, based on the likely role of chymase in the local generation of angiotensin peptides, such as AngII, chymase inhibitors offer an additional approach to RAAS modulation in cardiovascular and renal diseases. Aside from sacubitril/valsartan, these novel RAAS modulators have not yet been evaluated in veterinary patients.

Cardiac disease

Studies in humans have shown the benefits of ACEI in the treatment of systolic heart failure, associated with moderate to severe[179] and mild to moderate symptoms.[180,181] Veterinary clinicians have had experience with enalapril, captopril, benazepril, lisinopril, imidapril, alacepril, and ramipril (Supplemental Tables 2 and 3). Of these, only enalapril and benazepril, and to a lesser degree imidapril and ramipril, have been extensively studied. The benefits of ACE-inhibition as part of the therapy of heart failure have been demonstrated in multiple, placebo- or drug-controlled clinical trials in dogs with heart failure (Figure 5).[182-186] The first, randomized placebo controlled clinical trial evaluating enalapril in dogs with CHF due to naturally occurring heart disease (either MMVD or dilated cardiomyopathy; DCM) showed an improvement in short-term clinical and hemodynamic variables (namely pulmonary capillary wedge pressure and pulmonary edema scores).[187] A subsequent study, found that enalapril was associated with an improvement in several clinical variables (including heart failure classification, pulmonary edema score, mobility, attitude, and cough frequency).[185] Improvement in all of these variables was significant in the DCM group, whereas only improvement in cough and mobility parameters reached significance in the MMVD group.[185] Two randomized, placebo controlled studies in dogs with CHF due to MMVD or DCM subsequently showed an improvement in survival time and delay in the time to worsening of heart failure.[183,184] This benefit was no longer statistically significant when only the DCM subgroup was analyzed, yet low numbers in the DCM groups in these studies make interpretation of this result difficult and larger studies of RAAS suppression in DCM are needed. Additional

studies have found that imidapril is non-inferior to enalapril and to benazepril.[182,186] In a U.S. FDA pivotal trial (double-blind, active-control), the outcome of dogs in CHF due to MMVD and DCM, was compared between those treated with enalapril plus standard therapy vs. those receiving standard therapy plus the inodilator, pimobendan (Vetmedin®).[188] There was no difference between treatment groups in the primary endpoint of treatment success or in the heart insufficiency and pulmonary edema scores. Total deaths due to CHF at study end (day 56) were identical between groups at 14%. A subsequent European study comparing pimobendan and the ACEI, benazepril, showed modest survival benefit in those receiving pimobendan.[189]

While the use of ACEI in canine CHF is well accepted, the data for ACEI are less robust for dogs with cardiac disease, prior to the onset of CHF (ACVIM Stages A and B; Figure 4). There have been 4 studies addressing this, 2 prospective, double blind, placebo-controlled trials (enalapril vs. placebo) and 2 retrospective studies (benazepril vs. no therapy). The MMVD studies are summarized in Figure 5. The first of these looked at dogs with MMVD where approximately half were ACVIM Stage B1 and half were Stage B2. Enalapril was dosed at 0.37 mg/kg/day.[190] Of these 229 dogs (all Cavalier King Charles Spaniels; CKCS), 43% and 42% in the treatment and placebo groups, respectively, reached the endpoint, onset of CHF. The second study[191], also a placebo-controlled, double-blind study in dogs with MMVD, included 23 breeds, with all 124 dogs shown by echocardiography and radiography to be in ACVIM Stage B2 cardiac disease. The treatment group received enalapril at an average dosage of 0.45 mg/kg/day.

The result was a significant (P<0.02) improvement in all-cause mortality.[191] A retrospective study of dogs with ACVIM Stage B1 MMVD, which had received either an ACEI (benazepril) or no treatment concluded that dogs receiving benazepril had a significantly longer survival time and fewer cardiac events, including cardiac death.[192] A retrospective study of 91 Doberman Pinchers with occult DCM, which had received either an ACEI (benazepril) or no treatment concluded that dogs receiving benazepril had a significantly longer median time to onset of overt DCM.[193]

In the aforementioned study[192], the CKCS did not benefit from RAAS suppression, when compared to other breeds. This raises the question of pharmacogenomic differences amongst dog breeds. Recently a single nucleotide polymorphism in intron 16 of the ACE gene in a group of 31 dogs (including 10 CKCS) has been described.[194] The ACE activity in dogs with the polymorphism was significantly lower than those without, yet both groups had significant suppression of ACE activity after 2 weeks of ACEI therapy. Six of the 10 CKCS carried the polymorphism, which provides support to the concept, held by some, that the RAAS of the CKCS may not truly represent the species as a whole. Further study is necessary to determine whether differences in RAAS phenotypes impact the response to RAAS suppressive therapy and the natural history of cardiac disease in this breed.

In dogs with experimentally created mitral regurgitation, captopril therapy led to a fall in total peripheral resistance index, decrease in regurgitant fraction, and increase in forward ejection fraction as compared to untreated controls.[195] Conversely, the use of either an ACEI or ARB was found to exacerbate the left ventricular dilation and extracellular matrix loss in other studies of experimentally created mitral regurgitation in dogs.[82,83] These acute to subacute models add valuable insight to the pathophysiology of left ventricular volume overload due to mitral regurgitation, yet further study is needed in patients with naturally occurring MMVD. Overall, the effect of ACE-inhibition in pre-clinical MMVD might be variable among dogs. Whereas improvement might result from reduced afterload, decreased size of the regurgitant orifice, and an antifibrotic effect in later stages, detrimental effects might arise from blockade of the inotropic effects of AngII and possible potentiation of extracellular matrix degradation and disruption of myocyte stability in the early stages of remodeling.[75]

The recently completed placebo-controlled, double blind EPIC trial enrolled dogs of various breeds with ACVIM stage B2 MMVD with radiographic and echocardiographic evidence of cardiomegaly. This study showed that the inodilator pimobendan significantly prolonged the pre-clinical period in this cohort of dogs.[196] Compared to the VETPROOF trial, the EPIC trial had more strictly defined cardiomegaly entry criteria and enrolled over 2.5 times as many dogs. The relatively narrow 95% confidence interval of the hazard ratio for the CHF component of the

primary endpoint in the EPIC trial supports the benefit of pimobendan in the cohort studied. A similar hazard analysis is not available for the VETPROOF data.

Three large human studies[197-199] have shown a clear benefit from the inclusion of broader RAAS suppressive therapy in the treatment of HFrEF with the addition of a MRA to standard therapy, including an ACEI or ARB. The ground breaking RALES (ischemic and non-ischemic heart disease, NYHA III-IV) and EPHESUS (post-myocardial infarction) studies evaluated human patients with severe HFrEF. The EMPHASIS-HF study (ischemic and non-ischemic heart disease) evaluated patients with mild to moderate HFrEF. In these studies, a MRA (spironolactone or eplerenone) was added to optimal heart failure therapy including RAAS suppression (either an ACEI or ARB) and beta blockade in the latter 2 studies. Each of these 3 studies showed a reduction in mortality and cardiac-associated hospitalization. These significantly positive results have led to the recommendation in recent guidelines that MRA be used as a standard therapy in the treatment of HFrEF.[200] Furthermore, the clinical benefit associated with addition of the MRA in these studies was seen in people with normal, low, and elevated blood aldosterone levels at study outset. The changes in patient neurohormonal profiles during chronic spironolactone therapy was monitored in a subset of patients in the RALES trial.[201] The patients in the spironolactone group had significantly increased circulating AngII and aldosterone levels at 3 and 6 months when compared to people receiving placebo. These increases were hypothesized to result from failure of an aldosterone-generated negative feedback

loop to reduce renin secretion from the juxtaglomerular apparatus, though local tissue production of AngII and aldosterone could also be contributing. As there was a survival benefit associated with spironolactone, the impact of these hormone increases are likely mitigated by antagonism of the MR. It is currently not known if these increased concentrations of circulating AngII and aldosterone secondary to receptor antagonism have any receptor-independent effects.

On average, plasma aldosterone concentrations in dogs with naturally occurring MMVD and DCM (both asymptomatic and with CHF) are higher than that of controls.[35,202] In dogs with heart failure due to MMVD, 1 study found that, on average, dogs treated with enalapril had an initial decline in plasma aldosterone concentrations, when first re-evaluated after 3 weeks of treatment. This was followed by an increase in average plasma aldosterone concentration, above baseline levels, 6 months after initiation of treatment.[37] Another study, however, showed a significant decrease in plasma aldosterone concentrations in all but 1 of 7 dogs with MMVD and CHF, after initiation of captopril.[35] Additionally, studies in dogs using pharmacologic RAAS activation and experimentally-induced heart failure have found rising aldosterone levels despite ACEI therapy.[38,203-207] Regardless of its frequency, chronic neurohormonal activation has consequences and has been linked to pathologic remodelling in dogs with naturally occurring heart disease.[208] Overall, these data add support for the adjunctive use of a MRA in dogs with heart disease. Although low-dosage spironolactone (0.5mg/kg PO q24h) did not significantly impact survival in dogs with advanced CHF due to either MMVD or DCM, a subsequent double-

blind, placebo-controlled field study in 212 dogs with MMVD and mild to moderate heart failure demonstrated a significant reduction in risk of morbidity and mortality due to heart disease with the addition of spironolactone (2mg/kg PO q24h) to 'standard therapy' (ACEI, furosemide ± digoxin).[209,210]

The role of spironolactone in the treatment of heart failure, due to ventricular diastolic dysfunction with preserved ejection fraction (HFpEF) is an area of on-going research. Pathological activation of the RAAS and, in particular, hyperaldosteronism, are implicated in the pathologic ventricular remodeling and diastolic myocardial dysfunction in hypertrophic cardiomyopathy (HCM) in humans.[122] Small studies in humans with HFpEF have shown that MR blockade, with spironolactone, improves diastolic function.[211,212] The addition of spironolactone to existing heart failure therapy was recently evaluated in a large, placebo-controlled, double blind study of people with symptomatic HFpEF (excluding infiltrative or hypertrophic cardiomyopathies). This study did not result in a significant survival benefit or reduction in hospitalization for heart failure. However, there were substantial differences in patient response among geographic areas, and post-hoc subgroup analyses raised concerns about disparities in actual use of spironolactone and heart failure severity (less severe) in the Russian/Georgian patients.[213-215] Overall, the incidence of hospitalization for heart failure was significantly lower in the spironolactone group as a whole and the use of spironolactone was associated with significant improvement in the quality of life score.[213,216] In a separate study of

patients with HFpEF, spironolactone was associated with improved exercise capacity.[217] A second clinical trial of spironolactone in the treatment of HFpEF is on-going (https://clinicaltrials.gov/ct2/show/NCT02901184?term=SPIRRIT&rank=1).

Plasma aldosterone concentration is elevated above normal in Maine coon cats with asymptomatic HCM.[218] Short-term (4 months) use of spironolactone (2 mg/kg PO q12h) in these asymptomatic cats did not, however, provide reduction in left ventricular mass or improvement in an echocardiographic parameters of diastolic function, and 4 of the 13 cats treated with spironolactone developed ulcerative facial dermatitis.[219] A double-blinded, placebo-controlled clinical trial has more recently evaluated the use of spironolactone (1.7 to 3.3 mg/kg PO q24h) in client-owned cats with heart failure due to cardiomyopathy, the majority being afflicted with HCM.[220] All cats (n=20) were also receiving furosemide and an ACEI. Significantly fewer cats receiving spironolactone reached the primary endpoint (cardiovascular death), compared to those receiving placebo. Also, no cat receiving spironolactone experienced ulcerative facial dermatitis. Because of a small sample size and disparities in disease severity between the 2 groups at baseline, the authors concluded that these results are promising, yet additional studies evaluating spironolactone in cats with HCM are warranted.

Systemic hypertension

Suppression of RAAS activity with ACEI, ARB and MRA, alone or in combination has been shown to improve blood pressure control in people with systemic hypertension.[221-223] Whereas in people, systemic hypertension (HTN) is usually primary (essential), in dogs and cats it is usually secondary to an underlying renal or endocrine disorder. In dogs, HTN secondary to chronic kidney disease (CKD), is often treated with RAAS suppression (ACEI or ARB).[224] Hyperadrenocorticism is a common endocrine disease in the dog and is often complicated by HTN, especially in dogs with untreated adrenocortical tumors.[225,226] The mechanisms involved in HTN in dogs with hyperadrenocorticism are multifactorial and the general approach to therapy is an ACEI alone or in combination with amlodipine. Dogs with adrenocortical tumors have significantly higher plasma aldosterone concentrations, when compared to normal dogs and dogs with pituitary-dependent hyperadrenocorticism[226], and may benefit from the addition of an MRA. The role of hyperaldosteronism in dogs with hypertension, secondary to pituitary dependent hyperadrenocorticism, is unclear[226-228], as HTN often persists despite medical therapy and control of the hyperadrenocorticism.[225] In these cases, the addition of an MRA should be considered.

In cats, HTN, is usually secondary to renal and/or endocrine disease (e.g. hyperthyroidism and various adrenal diseases). In this species, HTN is a common cause of left ventricular hypertrophy, diastolic dysfunction, and other target organ damage. Plasma aldosterone concentrations and the aldosterone to renin ratio in azotemic, hypertensive cats have been shown

to be significantly elevated, as compared to normotensive cats.[229] In the non-azotemic, hypertensive cats in this study, plasma aldosterone concentration was also elevated, but independent of plasma renin activity, which was actually depressed in this group. In this "low-renin" group, the cats' aldosterone concentrations and aldosterone to renin ratios were not supportive of hyperaldosteronism. In fact, their neurohormonal profile was similar to that of low-renin hypertension, which is diagnosed disproportionately in human patients of African ancestry. In humans, low-renin hypertension is most often due to either a low-renin essential hypertension or primary aldosteronism. In people, the MRA, eplerenone, and the ARB, losartan, are equivalent in lowering blood pressure in the high-renin patient, yet eplerenone is superior to losartan in the low-renin patient.[230] Despite this, the authors are unaware of studies using MRA therapy in cats with hypertension.

A recent study in normal laboratory cats showed that the ARBs, telmisartan and irbesartan, and the ACEI, benazepril, significantly attenuated an AngI-induced blood pressure response, whereas losartan did not.[231] The effect of telmisartan was significantly greater than that of the other 3 drugs, 90-minutes after oral administration. Telmisartan and benazepril were also compared 24-hours after their oral administration and telmisartan, again, led to a more significant attenuation of an AngI-induced blood pressure rise. A recent clinical field study showed that telmisartan was tolerated and lowered mean arterial blood pressure when compared to placebo in cats with HTN that was either idiopathic or secondary to chronic kidney disease (CKD) or

hyperthyroidism.[232] This study excluded cats with pretreatment blood pressures >200mmHg and further study is still needed in this cohort. Finally, circulating RAAS activation in cats is not always associated with the development of hypertension, as demonstrated in studies of cats with hyperthyroidism and CKD.[233,234] Amlodipine, a commonly used antihypertensive agent in both dogs and cats, is known to cause aldosterone levels to rise in normal dogs[38], yet this does not appear to be the case in cats with naturally occurring systemic hypertension.[229] In summary, the role of RAAS in feline hypertension appears to be complex and, as yet, not adequately defined.

Addition of a MRA is increasingly employed in humans with resistant hypertension, already receiving an ACEI and/or ARB.[221-223] A recently completed randomized, placebo controlled clinical trial found that spironolactone was superior to the addition of either a beta blocker or an alpha-1 blocker in patients with resistant systemic hypertension (persistent despite triple therapy with an ACE, ARB, and calcium channel blocker).[235] From this study, it appears that spironolactone is the best additional drug for such resistant systemic hypertension. MRA add-on therapy in dogs and cats with systemic hypertension has not yet been evaluated in veterinary patients.

Proteinuric kidney disease

Proteinuria, a marker of kidney injury, is likely a predictor of increased risk for disease progression, and might play a causal role in progression of glomerular disease.[236-238] Clinical

trials involving RAAS suppression in people with proteinuric kidney disease are summarized in Supplemental Table 5. In people, the combination of ACEI and ARB is usually more effective at reducing proteinuria in various forms of kidney disease than either therapy alone. The reduction in proteinuria does not, however, always translate to improved renal outcomes (need for dialysis, increasing creatinine, and fall in estimated GFR) and is sometimes associated with increased incidence of hyperkalemia and acute injury when compared to monotherapy.[239,240] The addition of a MRA to an ACE or ARB (alone or together) has also been shown to augment proteinuria reduction in people with either systemic hypertension, diabetic nephropathy, or chronic kidney disease with and without systemic hypertension.[241,242] The addition of an MRA to an ACEI or ARB, however, increases the risk of hyperkalemia in patients with chronic kidney disease.[113] The effect of finerenone on cardiovascular and renal outcomes in human patients with diabetic kidney disease is currently being evaluated in 2 large scale prospective randomized trials (https://clinicaltrials.gov/ct2/show/NCT02545049?term=figaro&cond=Diabetic+Kidney+Disease&rank=1 and https://clinicaltrials.gov/ct2/show/NCT02540993?term=finerenone&cond=Diabetic+Kidney+Disease&rank=1). Combination RAAS suppression and the addition of a MRA to standard pharmacotherapy, has not been studied dogs and cats with naturally occurring renal disease.

The benefit of RAAS suppressive therapy in canine proteinuric CKD, including idiopathic glomerulonephritis, has been demonstrated in experimental models and small clinical studies.[243-

[246] In uninephrectomized dogs, with experimentally induced diabetes mellitus and resultant proteinuria, treatment with lisinopril lowered glomerular capillary pressure, attenuated the development of glomerular hypertrophy, and reduced proteinuria significantly, when compared to untreated dogs.[244] In a 5/6-nephrectomy model of CKD, dogs treated with enalapril had, on average, a systolic BP that was 5-15 mmHg lower than their untreated counterparts. This difference was significant at 3 months, yet not at 6 months. Furthermore, treated dogs had lower urine protein to creatinine ratios (UP:C) and significantly lower renal tubular and glomerular lesion scores.[243] Benazepril significantly reduces azotemia and systemic blood pressure in a short-term study of experimental renal insufficiency in dogs induced by a 7/8 nephrectomy model of CKD.[247] In a double-blind, randomized clinical trial of dogs with biopsy-proven, membranous and membranoproliferative glomerulonephritis, treatment with enalapril significantly lowered blood pressure, UP:C and UP:C corrected for GFR, while these indices rose in the placebo group.[245] In another study of benazepril vs. placebo in dogs with CKD, benazepril therapy was associated with a significant increase in GFR over the 180-day study, something not seen in the placebo group.[246] In this study the authors hypothesize that the increased GFR could have been due to reduced glomerular capillary hypertension, decreased release of extracellular matrix and collagen from mesangial and tubular cells, and potentially decreased glomerular and interstitial fibrosis, though these factors were not directly evaluated.

The magnitude of proteinuria in dogs newly diagnosed with CKD appears to be related to prognosis[248], as a reduction in proteinuria may prolong survival. Dogs with a UPC > 1.0 are known to have a relative risk of uremic crisis or death 3 times greater than dogs with a UPC < 1.0. In a small study of X-linked, hereditary nephritis, dogs treated with high-dose enalapril (2mg/kg PO q12h) had a significantly slower progression of proteinuria and survived significantly longer than those untreated.[249] For these reasons, it is now well accepted, that ACEI, administered chronically to both human and veterinary patients with naturally occurring proteinuric renal disease, are beneficial.[245,247,250-255] Mechanisms for this improvement are postulated to be the antihypertensive effect, reduction of AngII-induced mesangial cell proliferation, and renal vasodilatory effects of ACEI, the latter resulting in a treatment-induced fall in renal filtration pressure and proteinuria.[250,252,253] Therefore, RAAS suppression with an ACEI or, less commonly, an ARB, is considered standard of care for dogs with glomerular disease and reduction in proteinuria is considered a surrogate therapeutic endpoint.[256] Neither dual RAAS blockade with ACEI and ARBs, nor the addition of a MRA to standard pharmacotherapy, have been studied in dogs with naturally occurring renal disease. Controlled studies are needed to evaluate the effect of these combinations. There is no consensus regarding RAAS suppressive therapy in dogs with non-proteinuric CKD.

The RAAS is activated in experimental and natural CKD in cats. [234,257] Benazepril has been evaluated in cats with CKD (proteinuric and non-proteinuric), and it is well tolerated and

significantly reduces proteinuria.[258] Although this study did not document a benefit of benazepril therapy on renal survival (endpoint of death or euthanasia due to renal disease or need for parenteral fluid therapy), renal survival times were inversely related to initial UP:C. Importantly, proteinuria has been shown to predict progression of azotemia[259] and is negatively associated with survival in cats with chronic renal disease[260] and systemic hypertension.[261] More recently the ARB, telmisartan, has been compared to benazepril in cats with chronic kidney disease and proteinuria.[262] Telmisartan was non-inferior to benazepril in preventing an increase in proteinuria over the 6-month treatment period and led to a significant reduction in proteinuria at all time points, whereas the reduction seen in the benazepril group did not reach significance. As with dogs, combination RAAS blockade has not been evaluated in cats. Finally, benazepril has been shown to lower blood urea nitrogen, serum creatinine concentration, and blood pressure in cats with polycystic kidney disease.[255] This is in stark contradistinction to the findings in human polycystic kidney disease, in which patients so afflicted did not benefit from ACEI.[253]

Safety of RAAS Suppressive Therapy

We have learned through clinical experience with ACEIs that their negative impact on kidney function is minimal, even in the face of severe heart failure. When azotemia is observed, ACEIs are usually being administered in conjunction with diuretics, sodium restriction, and sometimes vasodilators, often with resultant hypotension. Typically, diuretic cessation or reduction in dosage results in the improvement or resolution of azotemia.[263] In studies of enalapril in NYHA

phase III and IV heart disease (moderate to severe heart failure), due to MMVD and DCM, there was actually a lower incidence of azotemia in the enalapril-treated than the placebo-treated groups.[183,185,187] Additionally, in a placebo-controlled, double-blind evaluation of enalapril (average dosage of 0.45 mg/kg/day) in aged, small breed dogs with ACVIM Stage B2 (Figure 4) MMVD, there was no difference between groups in average serum creatinine concentration at any time point, number of dogs developing renal failure, or change in serum creatinine (evaluated every 3 months during the study duration; Supplemental Figure 2).[264]

A safety analysis of dogs involved in the study of Bernay and colleagues showed that those receiving spironolactone, in addition to standard therapy (including an ACEI), were not at higher risk for adverse events (such as death from renal disease and abnormalities in serum sodium, potassium, urea nitrogen, and creatinine), when compared with dogs receiving placebo and standard therapy.[265] In fact, mortality due to cardiac disease, kidney disease, or both was lower in the group treated with spironolactone. Importantly, dogs receiving spironolactone in addition to conventional therapy for heart failure (loop diuretic, ACEI, and pimobendan) did not have a greater incidence of hyperkalemia. In a study of Doberman pinschers with occult DCM, significant increases in serum potassium concentration (vs. individual baseline values) were seen in dogs receiving both spironolactone and an ACEI.[266] Although the authors deemed these changes to be clinically insignificant, the serum potassium concentration exceeded the reference interval in 50% of dogs and regular monitoring of serum electrolytes and renal values (as often

as every 3 months) was recommended for Doberman pinschers receiving spironolactone and ACEI. Overall, regular monitoring of serum electrolytes and renal values is prudent in all animals receiving vasodilators and diuretics, including spironolactone and ACEI. Finally, spironolactone in combination with an ACEI appears to be safe, when used to treat dogs with naturally occurring, asymptomatic MMVD, as well those with occult DCM, without preexisting azotemia.[266,267]

Conclusions

Sustained RAAS activation adversely affects the heart, vessels, and kidneys. The more we learn about this system, the broader and more complex its web becomes, and redundancies that bypass our therapeutic blockade or inadvertent suppression of beneficial components of this system may be part of what reduces the efficacy of our current RAAS suppressive therapies. The future of medical therapy for cardiovascular and kidney diseases will therefore likely not include more comprehensive blockade, yet rather will include a more adept modulation of this system. This will require a better understanding of how this entire system changes during disease and its treatment.

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Figure legends

Figure 1.

The RAAS scheme: factors that lead to the release of renin from the juxtaglomerular cells of the kidney and the 'target organs' of AngII, whose actions (both physiologic and pathologic) are primarily mediated by the AT_1R. The actions of AngII at the AT_2R are thought to counter those of the AT_1R. The AT_2R is likely of greater importance in the developing fetus, yet might be up-regulated in certain disease states in adults. Angiotensin II is also a major secretagogue for aldosterone, which acts via the MR to increase sodium retention in the kidney and also amplifies the pathologic effects of AngII in the heart, kidney, and vasculature. AngI, angiotensin I; AngII, angiotensin II, AT_1R, angiotensin type 1 receptor; AT_2R, angiotensin type 2 receptor; CO, cardiac output; MR, mineralocorticoid receptor; SNS, sympathetic nervous system.

Figure 2.

The renin-angiotensin-aldosterone system peptide cascade (RAAS Fingerprint®) is illustrated as a pedigree starting at Angiotensin 1. Each intersection represents a specific peptide fragment symbolized by colored spheres; Enzymes involved in the reactions are annotated on connecting lines. Size of spheres and numbers beside them represent absolute concentrations of angiotensins

(pg/ml, median values) in serum samples from 6 middle-aged, healthy, male beagles; the concentrations were analyzed by mass spectrometry. Angiotensin (1,7) and (1,5) are breakdown products of both angiotensin I and II. The novel peptides angiotensin (1,12) and angiotensin (1,25) may be directly derived from angiotensinogen and serve as precursors for angiotensin peptides such as AngII. AngI, angiotensin I; AngII Angiotensin II; AngIII, Angiotensin III; Ang IV, Angiotensin IV; Aldo, aldosterone; AP, aminopeptidase; AT_1R, Angiotensin Type-1 Receptor; NEP, neutral endopeptidase.

Figure 3.

Schematic representation of experiments carried out by Brilla and Weber[7,268-271] using rats with varying kidney function and experimental hypertension. Panel A. Normotensive control rat heart, aorta, renal arteries, kidneys, and normal angiotensin II and aldosterone production. B) unilateral renal ischemia (unilateral renal artery banding) model with infra-renal aortic banding C) aldosterone infusion with high-sodium diet, and D) infra-renal banding. Increased circulating angiotensin II and/or aldosterone occur in models B and C, which are characterized by interstitial and perivascular fibrosis of both the hypertrophied left and non-hypertrophied right ventricles. In model B the angiotensin converting enzyme inhibitor, captopril, prevented interstitial and perivascular fibrosis in rats with renal ischemia, yet did not prevent this remodeling in rats with aldosterone infusion and high sodium diets (model C). When models B and C were treated with spironolactone before and after the induction of hypertension. The rats developed left ventricular

hypertrophy as expected, yet had significantly less interstitial and perivascular myocardial fibrosis, when compared to untreated controls. ACEI, angiotensin converting enzyme inhibitor; Aldo, aldosterone; AngII, angiotensin II; LV, left ventricle, LVH, left ventricular hypertrophy, MRA mineralocorticoid receptor antagonist; RV, right ventricle.

Figure 4.

The American College of Veterinary Internal Medicine (ACVIM) Classification of Cardiac Disease. From at risk of heart failure to refractory heart failure.[173]

Figure 5.

Seven RAAS suppression clinical trials, 6 of which are of placebo-controlled, double blind, multicenter design, are redrawn from the original publications.[37,182-186,190-192,210] All studies were funded by pharmaceutical companies.

The first 4 Kaplan-Meier curves represent trials involving dogs in congestive heart failure (ACVIM, Stage C2; CHF), due to mitral valve disease (MMVD) or dilated cardiomyopathy (DCM). Only the 4th panel was comprised exclusively of dogs with MMVD. All studies in panels 5 through 7 were made up entirely of dogs with *asymptomatic* MMVD (ACVIM Stage B). Panels 1 and 2 depict prospective, double blind clinical trials, involving enalapril. While the third

graph contains data from a retrospective study of dogs treated with benazepril vs. those left untreated in ACVIM Stage B1.

Symptomatic (panels 1 through 4)

Panel 1. The LIVE trial was 1 of the first placebo-controlled, double-blind clinical trials in veterinary cardiology. Significant improvement in survival (death or removal from study) was documented in a population of dogs in CHF (ACVIM, Stage C2), due to MMVD or DCM, when treated with enalapril plus standard therapy, as compared to placebo plus standard therapy. Placebo plus standard therapy, dashed line. Enalapril plus standard therapy, solid line.

Panel 2. The BENCH Trial compared benazepril to placebo in dogs with CHF due to MMVD or DCM, and demonstrated significant benefit in survival (time to death or removal from the study for worsening clinical signs), and quality of life, in the dogs receiving benazepril. Placebo plus standard therapy, dashed line. Benazepril plus standard therapy, solid line.

Panel 3. The FIRST (placebo-controlled, double-blind) and EFFIC trials (open label) respectively compared imidapril (solid line) or ramipril (not shown) to an established ACEI, enalapril (dashed line), in treating dogs with MMVD or DCM, NYHA Stage 2-4. Data are shown only for the 12-month, placebo-controlled FIRST trial. Both studies demonstrated comparable survival and quality of life scores between imidapril and the control ACEI.

Panel 4. The CEVA Spironolactone Trial compared spironolactone plus standard therapy (including benazepril) to placebo, in a prospective, double-blind trial involving dogs in ISACH International Small Animal Health Council (ISACHC) classification, II and III, due to MMVD. In general, these dogs were in relatively mild heart failure. Similar to the RALES trial, significant survival benefit was realized by the dogs receiving spironolactone along with standard therapy. Placebo plus standard therapy, dashed line. Standard therapy plus spironolactone, solid line.

Asymptomatic (panels 5 through 7)

Panel 5. The SVEP trial involved an approximate 50:50 distribution of dogs with ACVIM Stage B1 and Stage B2, (asymptomatic), with treated dogs receiving enalapril, at a dosage of 0.37 mg/kg/day.[190] Of these 229 dogs (all Cavalier King Charles Spaniels), approximately 45% reached the defined endpoint, onset of CHF. The 2% benefit seen in the number of days dogs remained in the study, free of CHF, between placebo- and enalapril- treated dogs was not significant (log-rank test, P = 0.85). Placebo, dashed line. Enalapril, solid line

Panel 6. The VETPROOF compared enalapril to placebo, in a double-blind, placebo-controlled trial of dogs in ACVIM B2. The graph depicting the combined endpoint of survival (all-cause death and CHF-free survival), expressed as a Kaplan-Meier curve, for 124 dogs that met entry

requirements is presented. Median times to this combined endpoint in the treatment and placebo groups were 851 and 534 days (59% difference of 317 days [10.6 months] in heart failure and survival benefit), respectively (P = 0.05). The primary endpoint (time to onset of CHF) was prolonged by enalapril vs. placebo, but was not significant (P = 0.06). However, the numbers of dogs not in CHF were significantly different between groups on days 500 and 1000, as were the curves delineating all-cause mortality. Placebo, dashed line. Enalapril, solid line

Panel 7. Kaplan-Meier survival curves of dogs treated with benazepril (solid line) or untreated (Placebo, dashed line), after the initial diagnosis of ISACHC class Ia (ACVIM Stage B1) MMVD, with moderate-to-severe mitral regurgitation, demonstrated with echocardiography. While retrospective, this study demonstrates a delay in the onset of CHF in the treatment group. This raises the possibility that ACEI may be of benefit even before cardiac remodeling is evident and supports the use of ACEI in MMVD, prior to the onset of congestive heart failure.

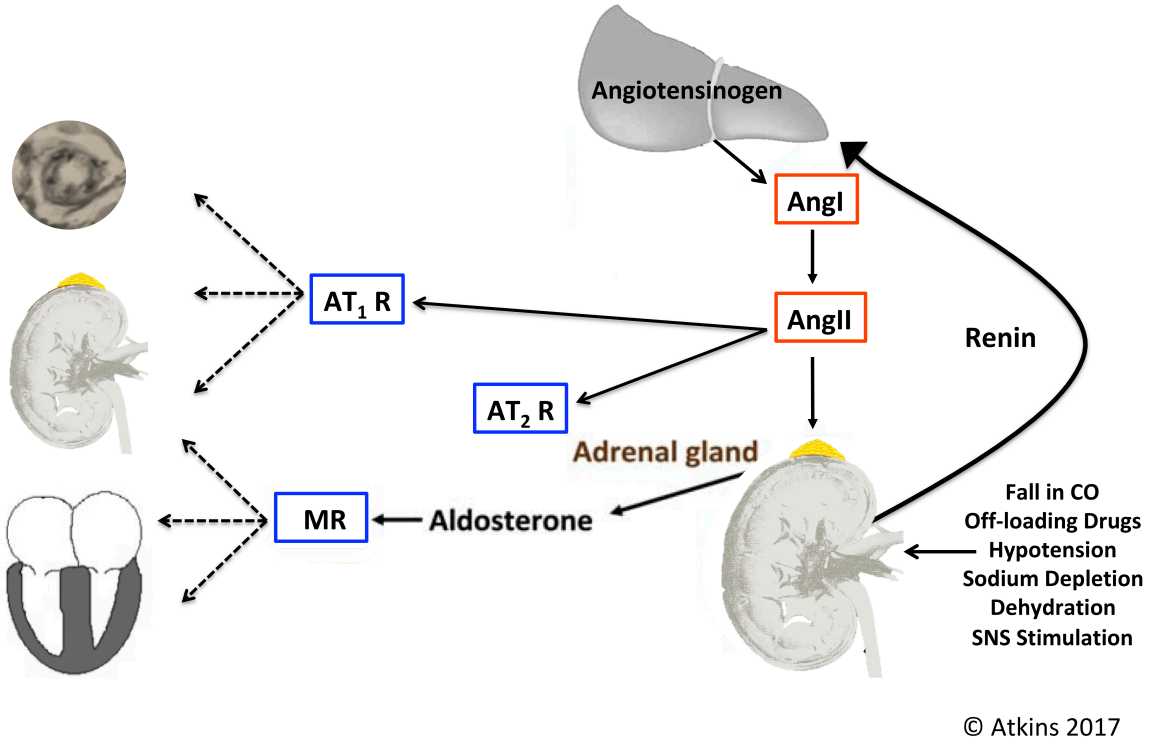

Figure 1.

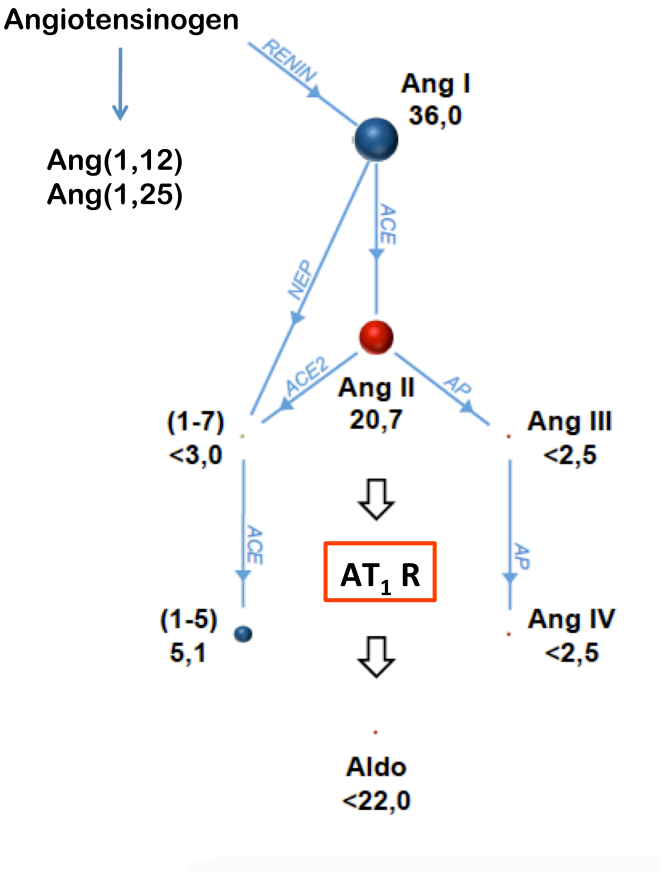

Figure 2.

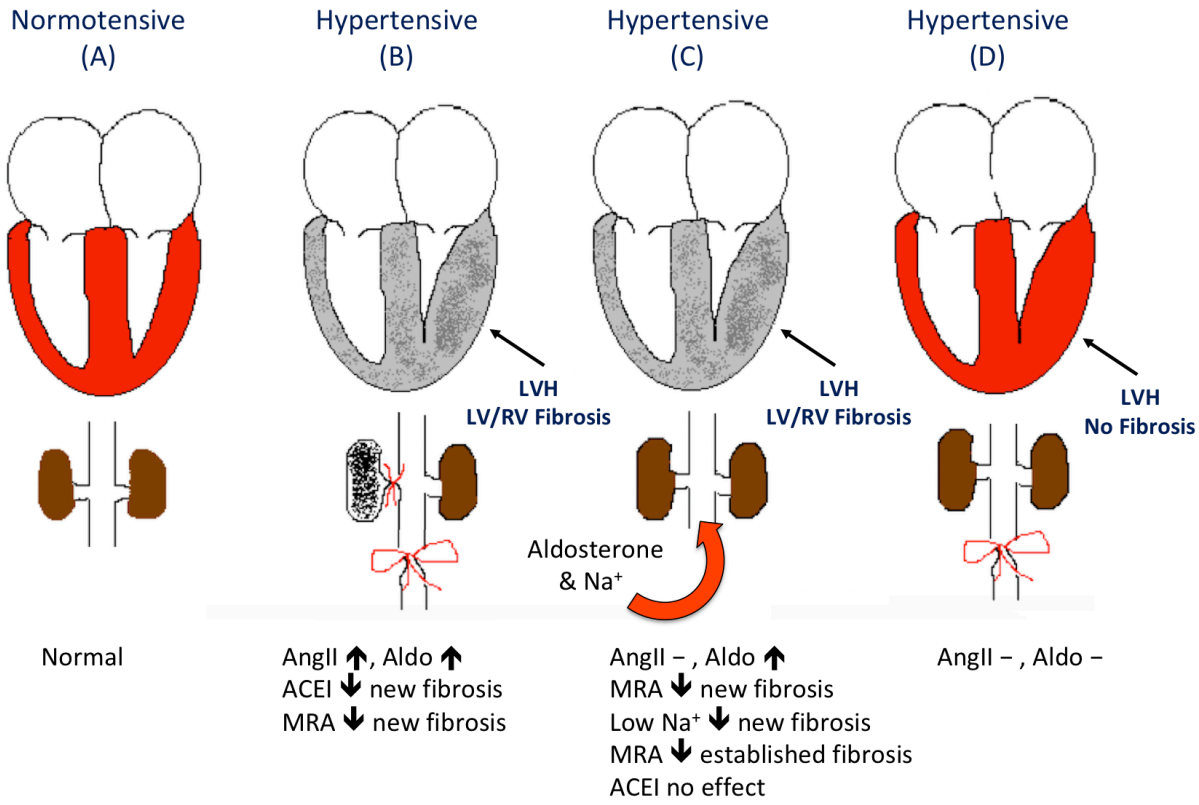

Figure 3.

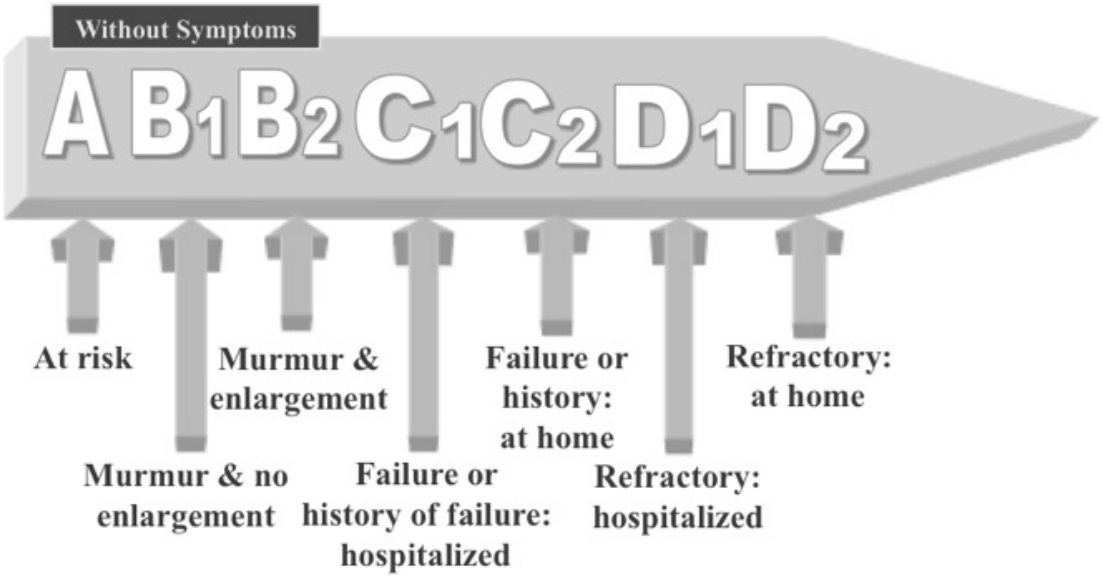

Figure 4.

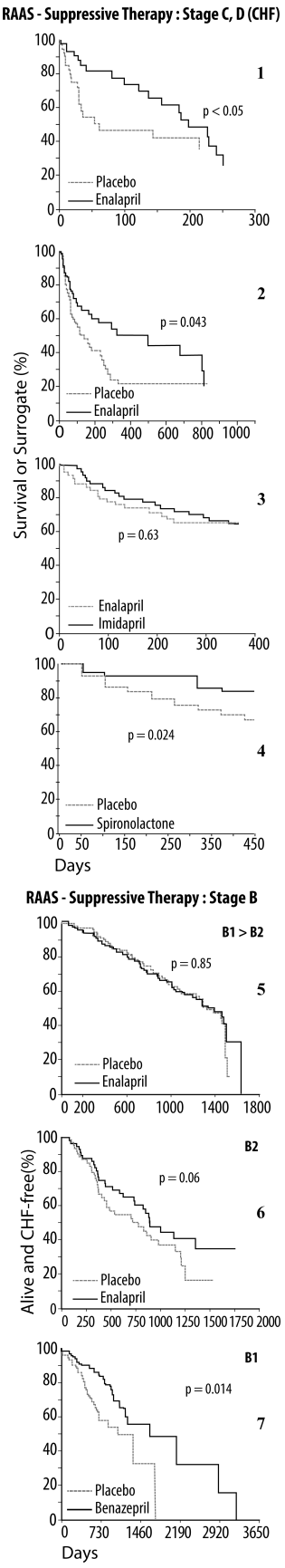

Figure 5.

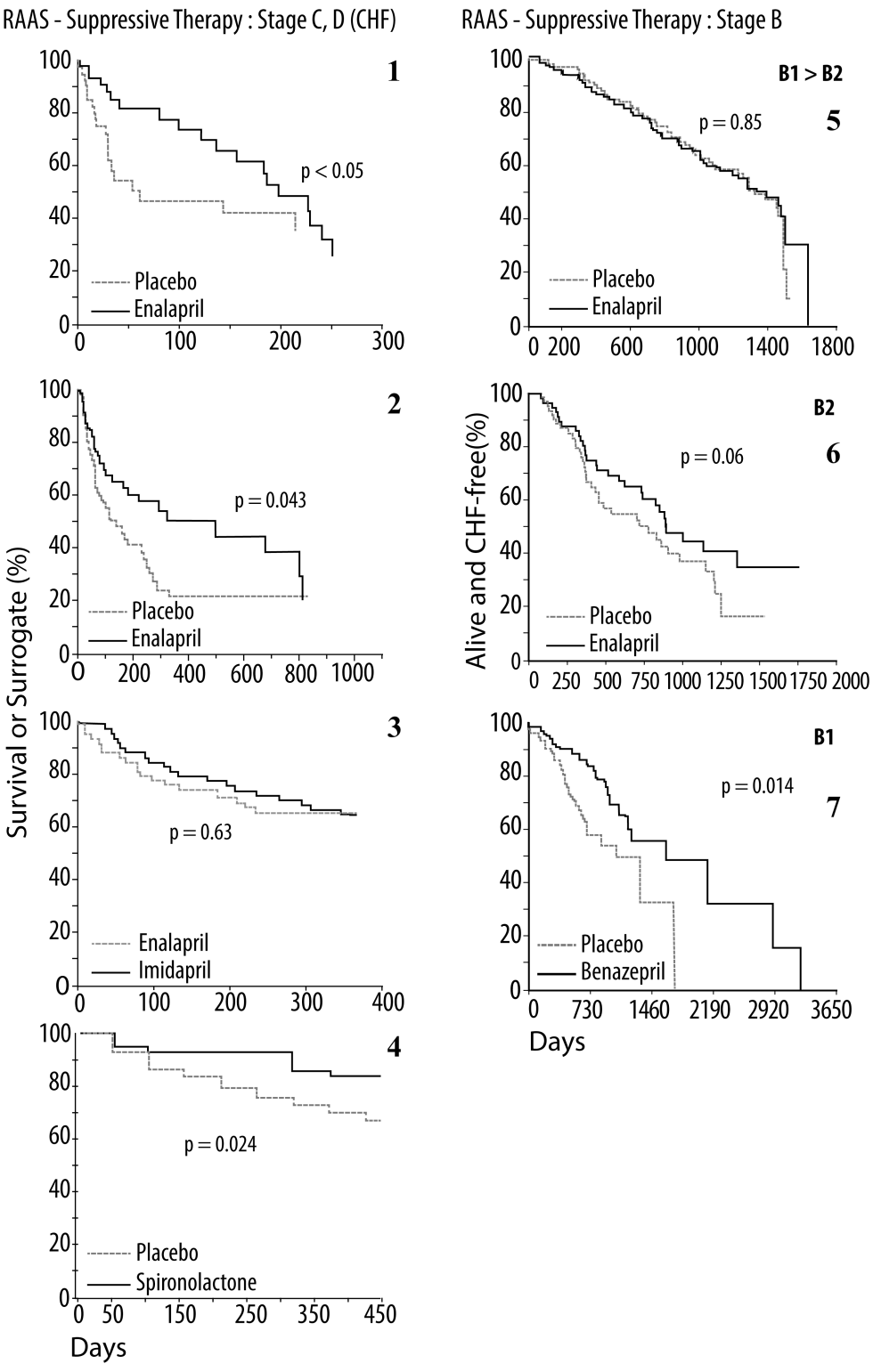

Figure 5. (Option 2)

Table 1: Aldosterone and Angiotensin II: Harmful Cardiovascular & Renal Effects

Adverse Effect	**Direct effects of Angiotensin II**	**Direct effects of Aldosterone**
Myocardial remodeling: Fibrosis, hypertrophy, necrosis, apoptosis	Yes	Yes
Vascular remodeling: Hypertrophy, fibrosis	Yes	Yes
Increase ROS	Yes	Yes
Pro-inflammatory (Cytokines, ROS)	Yes	Yes
Arrhythmogenic	Yes	Yes
Vascular endothelial dysfunction (ET-1, vasopressin, acetylcholine-mediated vasodilatory dysfunctin)	Yes	Yes
Systemic hypertension	Yes	Yes
Glomerular damage Glomerular dysfunction: proteinuria Increased intraglomerular pressure Tubulointerstitial injury	Yes Yes +++ (vasoconstriction) Yes	Yes Yes + (fluid retention & SNS) Yes
Baroreceptor dysfunction→HR increase Increased SNS tone Inotropy Direct HR increase Salt appetite Increased thirst	Maybe Yes Yes Yes Yes Yes	Yes Yes No No Yes No
Na^+ and H_2O retention, congestion K^+ wasting	Yes No	Yes Yes

ROS, reactive oxygen species, ET-1, endothelin 1; SNS, sympathetic nervous system; HR, heart rate.