Title
The renin-angiotensin-aldosterone system and its suppression.

Permalink
https://escholarship.org/uc/item/6qw0b5dw

Journal
Journal of veterinary internal medicine, 33(2)

ISSN
0891-6640

Authors
Ames, Marisa K
Atkins, Clarke E
Pitt, Bertram

Publication Date
2019-03-01

DOI
10.1111/jvim.15454

Peer reviewed
The renin-angiotensin-aldosterone system and its suppression

Marisa K. Ames1 © | Clarke E. Atkins2 | Bertram Pitt3

1Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado
2Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina
3Department of Medicine, University of Michigan School of Medicine, Ann Arbor, Michigan

Correspondence
Marisa K. Ames, Department of Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, 1678 Campus Deliver, Fort Collins, CO 80523. Email: marisa.ames@colostate.edu

1 | INTRODUCTION

Although renin-angiotensin-aldosterone system (RAAS) activation can be compensatory in the early stages of cardiovascular and renal disease, long-term activation is maladaptive. In patients with heart failure, relative increases in plasma renin activity and the blood aldosterone concentration are 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 angiotensin II (AngII) and aldosterone are outlined in Table 1.6-14 Suppression of RAAS is therefore a key strategy in the treatment 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. For example, Angiotensin II and aldosterone levels occasionally rise despite pharmacologic RAAS suppression, and the underlying mechanisms are still not well understood.15-32 Furthermore, some drugs and strategies used to treat cardiovascular disease, including furosemide, amlodipine, hydralazine and dietary sodium restriction, stimulate the RAAS (Supporting Information Figure S1).33-39 Continued research into this complex system is necessary to improve medical therapies for cardiovascular and renal disease.

Abbreviations: 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; ACE, angiotensin converting enzyme; ACEI, angiotensin converting enzyme inhibitor; ACVIM, American College of Veterinary Internal Medicine; ARB, angiotensin II type-1 receptor blocker; Angl, angiotensin I; AngII, angiotensin II type-1 receptor; AT1R, angiotensin II type-1 receptor; AT2R, angiotensin II type-2 receptor; CHF, congestive heart failure; CKCS, Cavalier King Charles Spaniel; 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; ROS, reactive oxygen species; UAldo: C, urine aldosterone to creatinine ratio; UPCR, urine protein to creatinine ratio.

[Correction added on 04 March 2019, after first online publication in the Abstract. “RAAS’s” was corrected to “RAAS”]

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. Journal of Veterinary Internal Medicine published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

DOI: 10.1111/jvim.15454
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. In the circulation, renin metabolizes angiotensinogen, liberating angiotensin I (AngI). Angiotensin converting enzyme (ACE), which is released from endothelial cells, converts AngI to AngII. Angiotensin II acts at 2 receptors, the angiotensin type-1 and type-2 receptors (AT1R and AT2R). Angiotensin II's actions at the AT1R 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 disease change associated with chronic RAAS activation summarized in Table 1. Actions of AT2R stimulation are counter regulatory to those of the AT1R, where type-2 receptor stimulation leads to anti-inflammatory, anti-fibrotic, and vasodilatory effects. The AT2R is the dominant receptor type in the fetus and plays a key role in development, but is less relevant in the normal adult. The AT2R, however, might be upregulated 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 adipocytes.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 activation of the small fraction of cell membrane localized MR and MR interaction

---

**TABLE 1  Aldosterone and angiotensin II: harmful cardiovascular and 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 dysfunction) | Yes                             | Yes                           |
| Systemic hypertension                      | Yes                             | Yes                           |
| Glomerular damage                          | Yes                             | Yes                           |
| Glomerular dysfunction: proteinuria        | Yes                             | Yes                           |
| Increased intraglomerular pressure         | +++ (vasoconstriction)           | + (fluid retention & SNS)     |
| Tubulointerstitial injury                  | Yes                             | Yes                           |
| Baroreceptor dysfunction to HR increase    | Maybe                           | Yes                           |
| Increased SNS tone                         | Yes                             | Yes                           |
| Inotropy                                   | Yes                             | No                            |
| Direct HR increase                         | Yes                             | No                            |
| Salt appetite                              | Yes                             | No                            |
| Increased thirst                           | Yes                             | No                            |
| Na⁺ and H₂O retention, congestion         | Yes                             | Yes                           |
| K⁺ wasting                                 | No                              | Yes                           |

Abbreviations: ET-1, endothelin 1; HR, heart rate; SNS, sympathetic nervous system activation; ROS, reactive oxygen species.
with other receptors such as the G-protein coupled estrogen receptor, AT1R, and epidermal growth factor receptor. 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.

Angiotensin peptides and aldosterone are also produced in tissues such as brain, blood vessels, kidneys, and the heart. Locally produced RAAS hormones play important roles in normal cardiovascular function and electrolyte-fluid homeostasis, yet also mediate abnormal remodeling in the tissues. In-vitro work on cultured vascular smooth muscle cells and cardiomyocytes has shown that aldosterone upregulates components of the RAAS, including ACE activity and AngII-stimulated signal transduction, leading to increased local (tissue) activity of the RAAS. Tissue RAAS components are also modulated by mechanical stretch of the myocardium and vessels, adipoocyte secretions, presence of reactive oxygen species (ROS), and inflammation. Although the amount of aldosterone produced in the tissues is normally <1% of the amount produced by the adrenal glands, locally produced aldosterone likely plays a role in abnormal remodeling (hypertrophy and fibrosis), and tissue RAAS management will likely be part of future pharmacotherapeutic strategy.

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. 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.

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). Chymase, a serine protease, 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. 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. Chymase activation results in pro-fibrotic, anti-fibrotic, or pro-inflammatory phenotypes, with the exact phenotype or combination of phenotypes depending upon the tissue and the nature and timing of the stress. Chymase is an important player in AngII formation (and a pro-fibrotic phenotype) in cardiac disease. Chymase activation is hypothesized to exacerbate cell death and mitochondrial injury after cardiac ischemia/reperfusion. Additionally, mast cell activation and increased release of mediators such as chymase have been implicated in the pathophysiology of cardiometabolic disease, such as diabetes mellitus and obesity. The phenotype of chymase activation differs in canine models of hemodynamic overload and might contribute to both extracellular matrix degradation and fibrosis, contributing to or counter-balancing ventricular dilatation.

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 ACE2 processing of AngII, whereas in the circulation and kidney, Ang(1,7) arises from processing of angiotensin I by endopeptidases, such as nephrilysin.54,85 Angiotensin(1,7) and its metabolite Ala⁺-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 the 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 downregulation. For example, AngI can also be metabolized by ACE2 to form to Ang(1,9), which appears to bind at the AT₂R.88 Also, AngII is metabolized by aminopeptidase A to form angiotensin III, which like its parent peptide is capable of binding both the AT₁R and AT₂R.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 AT₁R 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.90,42,54,55

2.1 | 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 fluctuation in RAAS hormones in dogs92,96 is similar to that in 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 shows that, in dogs fed once daily (a.m.) with normal sodium meal, the 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

2.2 | 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. Renin-angiotensin-aldosterone system 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. This allows the kidney to "escape" from the sodium retaining effects of excess AngII (and increased aldosterone production). 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.

2.3 | 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 UAldoC. More recently, liquid chromatography-mass spectrometry has been used to create a comprehensive renin-angiotensin system Fingerprint (Attoquant Diagnostics GmbH, Vienna, Austria) from both blood and tissue samples (Figure 2). This 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 treatment might suggest very effective suppression, yet AngII and aldosterone do "break through" this treatment in some patients. Additional information regarding aldosterone metabolism and the assessment of RAAS activity in dogs and cats can be found in the Supporting Information of this review.

Genetic profiling of specific populations and individuals will also likely play a role in the development of future RAAS modulation strategies. Renin-angiotensin-aldosterone system 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 treatment. A more individualized and comprehensive approach to future RAAS modulation should include an RAAS profile, RAAS genotype, metabolic profile, and diet survey.

3 | AngII AND ALDOSTERONE EXCESS

3.1 | AngII 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). This is based on direct (hormone infusion, transgenic animal models) and indirect (RAAS suppression) evidence (Figure 3 and Supporting Information Table S1). These studies link the fibrosis that attends the abnormal 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. Ultimately, these changes lead to increased collagen gene expression and synthesis, as well as decreased fibrolysis. 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.

Renin-angiotensin-aldosterone system activation also contributes to the immune cell infiltration that contributes to the inflammation and fibrosis that attends renal ischemia, myocardial infarction, and systemic hypertension (HTN). 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. Antagonism or deletion of the macrophage MR is protective against cardiovascular remodeling, even in situations where aldosterone levels are normal. Oxidative stress also appears to amplify inflammation and directly activate the MR or allow glucocorticoids to activate the MR. 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. The mechanisms by which neurohormonal activation decreases baroreceptor sensitivity are not fully understood. Decreased baroreceptor-heart rate sensitivity is a key feature of the heart failure syndrome and is an independent predictor of poor prognosis in people with heart failure.

The deleterious effects of excess aldosterone are augmented when the relationship between sodium and aldosterone is abnormal. In the aforementioned animal studies (Table 1, Supporting Information), 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 among aldosterone, sodium status, metabolic state, inflammation, and oxidative stress can be found in the Supporting Information.

4 | SUPPRESSION OF RAAS

Angiotensin converting enzyme 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 Supporting Information Tables S2 and S3. 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. The plasma concentration-time profile has initial and protracted elimination phases,
because of clearance of free drug and release of drug from tissue-binding sites, respectively. Using circulating plasma ACE activity as a surrogate for efficacy, dosages have been determined for several ACEI in animals (Supporting Information Tables S2 and S3). 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 ACEIs. Variable efficacy among 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, differences in lipophilicity should be less important, except in the case of certain centrally acting ACEIs. The most common adverse effects of ACEI in humans include cough or angioedema, or both, because of 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 AT1 receptor and were developed because of the cough and angioedema adverse effects seen with ACE-inhibition in humans. Theoretical benefits of ARBs include blockade of the actions of AngII at the AT1R, regardless of the pathway of its formation (via ACE or non-ACE pathways, such as chymase). Also, increased circulating AngII, resulting from AT1R blockade, might stimulate the AT2R, thought to be counter regulatory to the maladaptive actions mediated by the AT1R. Commonly used ARBs are summarized in Supporting Information Table S4. 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 (CKD), and proteinuria suggests that this may be the case (see below). A recent meta-analysis of studies of dual (ACEI and ARB) treatment in patients with heart failure concluded that until further clinical trials are performed, dual treatment should not be routinely administered. Controlled clinical trials in dogs and cats evaluating combination of ACEI and ARB treatment have not been performed.
Spironolactone and eplerenone are synthetic steroidal MRA. Mineralocorticoid receptor 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 8 mg/kg/d.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 2 mg/kg PO once daily restored 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 (DRIs) such as aliskiren theoretically prevent the initiation of the renin-angiotensin cascade. First-generation DRIs have not proven 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 15 mg/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 20 mg/kg PO q12h) in a small study of dogs with the American College of Veterinary Internal Medicine (ACVIM) stage B2 myxomatous mitral valve disease (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) (Figure 2).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.

4.1 | Cardiac disease

Studies in humans have shown the benefits of ACEI in the treatment of systolic heart failure, associated with moderate to severe179 and mild to moderate clinical signs.180,181 Veterinary clinicians have had experience with enalapril, captopril, benazepril, lisinopril, imidapril, alacepril, and ramipril (Supporting Information Tables S2 and S3). 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 treatment 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 congestive heart failure (CHF) caused by 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).188 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 caused by MMVD or DCM subsequently showed an improvement in survival time and delay in the time to worsening of heart failure. 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. In a US FDA pivotal trial (double-blind and active-control), the outcome of dogs in CHF caused by MMVD and DCM was compared between those treated with enalapril plus standard therapy versus those receiving standard treatment plus the inodilator, pimobendan (Vetmedin). 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 caused by 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.

Although the use of ACEI in canine CHF is well accepted, the data for ACEI are less robust for dogs with cardiac disease, before 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 versus placebo) and 2 retrospective studies (benazepril versus no treatment). The MMVD studies are summarized in Figure 5. The first of these looked at dogs with MMVD where approximately half were

**FIGURE 5** Legend on next page.
ACVIM Stage B1 and half were Stage B2. Enalapril was dosed at 0.37 mg/kg/d.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/d. The result was a significant (P < .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 retrospective study,192 the CKCS did not benefit from RAAS suppression, when compared to other breeds. This raises the question of pharmacogenomic differences among 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 treatment. 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 treatment and the natural history of cardiac disease in this breed.

In dogs with experimentally created mitral regurgitation, captopril treatment 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 dilatation and extracellular matrix loss in other studies of experimentally created mitral regurgitation in dogs.196,197 These acute to subacute models add valuable insight to the pathophysiology of left ventricular volume.

**FIGURE 5** Seven renin-angiotensin-aldosterone system (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 (the American College of Veterinary Internal Medicine [ACVIM], Stage C2; congestive heart failure [CHF]), caused by myxomatous mitral valve disease (MMVD) or dilated cardiomyopathy (DCM). Only the 4th panel was composed 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. Although the third graph contains data from a retrospective study of dogs treated with benazepril versus 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), caused by MMVD or DCM, when treated with enalapril plus standard treatment, as compared to placebo plus standard treatment. Placebo plus standard treatment, dashed line. Enalapril plus standard treatment, solid line.

Panel 2. The BENCH Trial compared benazepril to placebo in dogs with CHF caused by 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 treatment, dashed line. Benazepril plus standard treatment, 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 angiotensin converting enzyme inhibitor (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 treatment (including benazepril) to placebo, in a prospective double-blind trial involving dogs in ISACH International Small Animal Health Council (ISACHC) classification, II and III, caused by 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 treatment. Placebo plus standard treatment, dashed line. Standard treatment 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/d.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 = .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 = .85). 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 II (ACVIM Stage B1) MMVD, with moderate-to-severe mitral regurgitation, demonstrated with echocardiography. Although 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, before the onset of congestive heart failure.
overload because of mitral regurgitation, yet further study is needed in patients with naturally occurring MMVD. Overall, the effect of ACE-inhibition in preclinical MMVD might be variable among dogs. Although improvement might result from reduced afterload, decreased size of the regurgitant orifice, and an anti-fibrotic 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 cardiomyopathy. This study showed that the inodilator pimobendan significantly prolonged the preclinical period in this cohort of dogs.196 Compared to the veterinary enalapril trial to prove reduction in onset of heart failure (VETPROOF) trial, the EPIC trial had more strictly defined cardiomyopathy 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 studies197–199 have shown a clear benefit from the inclusion of broader RAAS suppressive therapy in the treatment of HFrEF with the addition of an MRA to standard treatment, including an ACEI or ARB. The ground-breaking randomized aldactone evaluation study (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, an MRA (spironolactone or eplerenone) was added to optimal heart failure treatment, 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 treatment were 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 contributory. 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 those of controls.35,202 In dogs with heart failure caused by MMVD, 1 study found that, on average, dogs treated with enalapril had an initial decline in plasma aldosterone concentrations, when first reevaluated 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.25 Additionally, studies in dogs using pharmacologic RAAS activation and experimentally induced heart failure have found rising aldosterone levels despite ACEI treatment.38,203–207 Regardless of its frequency, chronic neurohormonal activation has consequences and has been linked to abnormal remodeling in dogs with naturally occurring heart disease.208 Overall, these data add support for the adjunctive use of an MRA in dogs with heart disease. Although low-dosage spironolactone (0.5 mg/kg PO q24h) did not significantly impact survival in dogs with advanced CHF caused by 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 caused by heart disease with the addition of spironolactone (2 mg/kg PO q24h) to “standard therapy” (ACEI, furosemide ± digoxin).209,210

The role of spironolactone in the treatment of heart failure, caused by ventricular diastolic dysfunction with preserved ejection fraction (HFpEF) is an area of ongoing 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 treatment 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 ongoing (https://clinicaltrials.gov/ct2/show/NCT02901184?term=SPIRITFTrank=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 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.
caused by cardiomyopathy, the majority being afflicted with HCM. 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 and heart failure are warranted.

4.2 | 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 HTN. Whereas in people, 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 CKD is often treated with RAAS suppression (ACEI or ARB). Hyperreninemia is a common endocrine disease in the dog and is often complicated by HTN, especially in dogs with untreated adenocortical tumors. The mechanisms involved in HTN in dogs with hyperreninemia are multifactorial and the general approach to treatment is an ACEI alone or in combination with amlodipine. Dogs with adenocortical tumors have significantly higher plasma aldosterone concentrations, when compared to normal dogs and dogs with pituitary-dependent hyperadrenocorticism, and may benefit from the addition of an MRA. The role of hyperaldosteronism in dogs with hypertension, secondary to pituitary dependent hyperadrenocorticism, is unclear as HTN often persists despite medical treatment and control of the hyperadrenocorticism. In these cases, the addition of an MRA should be considered.

In cats, HTN is usually secondary to renal disease, endocrine disease, or both. 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. In the non-azotemic hypertensive cats in this study, the average plasma aldosterone concentration was also elevated, but independent of plasma renin activity, which was actually low (suppressed). In this “low-renin” group, the cats’ aldosterone concentrations were not high enough to support primary hyperaldosteronism. Rather, their neurohumoral 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. Despite this, the authors are unaware of studies using MRA treatment 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 AngII-induced blood pressure response, whereas losartan did not. 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 AngII-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 either idiopathic or secondary to CKD or hyperthyroidism. This study excluded cats with pretreatment blood pressures >200 mm Hg 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. Amlodipine, a commonly used antihypertensive agent in both dogs and cats, is known to cause aldosterone levels to rise in normal dogs, yet this does not appear to be the case in cats with naturally occurring HTN. In summary, the role of RAAS in feline hypertension appears to be complex and, as yet, not adequately defined.

Addition of an MRA is increasingly employed in humans with resistant hypertension, already receiving an ACEI, ARB, or both. 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 HTN (persistent despite triple treatment with an ACE, ARB, and calcium channel blocker). From this study, it appears that spironolactone is the best additional drug for such resistant HTN. Mineralocorticoid receptor antagonist add-on treatment in dogs and cats with HTN has not yet been evaluated.

4.3 | 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. Clinical trials involving RAAS suppression in people with proteinuric kidney disease are summarized in Supporting Information Table S5. In people, the combination of ACEI and ARB is usually more effective at reducing proteinuria in various forms of kidney disease than either treatment alone. The reduction in proteinuria does not, however, always translate to improved renal outcomes (need for dialysis, increasing creatinine, and fall in estimated glomerular filtration rate [GFR]) and is sometimes associated with increased incidence of hyperkalemia and acute kidney injury when compared to monotherapy. The addition of an MRA to an ACE or ARB (alone or together) has also been shown to augment proteinuria reduction in people with either HTN, diabetic nephropathy, or CKD with and without HTN. The addition of an MRA to an ACE or ARB, however, increases the risk of hyperkalemia in patients with CKD. 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 an MRA to standard pharmacotherapy has not been studied in dogs and cats with naturally occurring renal disease.
The benefit of RAAS suppressive treatment in canine proteinuric CKD, including idiopathic glomerulonephritis, has been demonstrated in experimental models and small clinical studies. 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. In a 5/6-nephrectomy model of CKD, dogs treated with enalapril had, on average, a systolic BP that was 5-15 mm Hg 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. Benazepril significantly reduced 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. 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, whereas these indices rose in the placebo group. In another study of benazepril versus placebo in dogs with CKD, benazepril treatment was associated with a significant increase in GFR over the 180-day study, something not seen in the placebo group. In that study, the authors hypothesize that the increased GFR could have been because of reduced glomerular capillary hypertension, decreased release of extracellular matrix and collagen from mesangial and tubular cells, and decreased glomerular and interstitial fibrosis, although these factors were not directly evaluated.

The magnitude of proteinuria in dogs newly diagnosed with CKD appears to be related to prognosis, as a reduction in proteinuria may prolong survival. Dogs with an UP:C > 1.0 are known to have a relative risk of uremic crisis or death 3 times greater than dogs with an UP:C < 1.0. In a small study of X-linked, hereditary nephritis, dogs treated with high-dose enalapril (2 mg/kg PO q12h) had a significantly slower progression of proteinuria and survived significantly longer than those untreated. 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. 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. 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. Neither dual RAAS blockade with ACEI and ARB nor the addition of an MRA to standard pharmacotherapy has 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 treatment in dogs with non-proteinuric CKD.

The RAAS is activated in experimental and natural CKD in cats. Benazepril has been evaluated in cats with CKD (proteinuric and non-proteinuric), and it is well tolerated and significantly reduces proteinuria. Although this study did not document a benefit of benazepril treatment on renal survival (endpoint of death or euthanasia caused by 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 and is negatively associated with survival in cats with chronic renal disease and with HTN. More recently, the ARB, telmisartan, has been compared to benazepril in cats with CKD and proteinuria. 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. This is in stark contrast to the findings in human polycystic kidney disease, in which patients so afflicted did not benefit from ACE inhibition.

### 4.4 Safety of RAAS suppressive treatment

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. In studies of enalapril in NYHA phase III and IV heart disease (moderate to severe heart failure), caused by MMVD and DCM, there was actually a lower incidence of azotemia in the enalapril-treated than the placebo-treated groups. Additionally, in a placebo-controlled double-blind evaluation of enalapril (average dosage of 0.45 mg/kg/d) 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; Supporting Information Figure S2).

A safety analysis of dogs involved in the study of Bernay and colleagues showed that those receiving spironolactone, in addition to standard treatment (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 treatment. In fact, mortality caused by cardiac disease, kidney disease, or both was lower in the group treated with spironolactone. Importantly, dogs receiving spironolactone in addition to conventional treatment 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 (versus individual baseline values) were seen in dogs receiving both spironolactone and an ACEI. 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
recommneded 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

5 | 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 therapeutc blockade or inadvertent suppression of beneficial components of this system may reduce the efficacy of our current RAAS suppressive treatments. The future of medical treatment for cardiovascular and kidney diseases will have to be a more adept modulation of this system. This will require a better understanding of how this entire system changes during disease and its treatment.

CONFLICT OF INTEREST DECLARATION

Dr Pitt is a consultant for Bayer, Astra Zeneca, Sanofi, Sarfex, scPharmaceuticals, Relypsa/Vifor, Stealth Peptides, Cyphornerx (stock options); Dr Atkins is a consultant for Ceva Sante Animale, Vetoquinol, and Boehringer Ingelheim; Dr Ames is a consultant for Ceva Sante Animale, and Elanco.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Marisa K. Ames https://orcid.org/0000-0002-3865-6123

REFERENCES

1. Swedberg K, Eneroth P, Kjekshus J, Wilhelmsen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. CONSENSUS Trial Study Group. Circulation. 1990;82(5):1730-1736.

2. Anker SD, Chua TP, Ponikowski P, et al. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation. 1997;96(2):526-534.

3. Broqvist M, Dahlstrom U, Karlberg BE, et al. Neuroendocrine response in acute heart failure and the influence of treatment. Eur Heart J. 1989; 10(12):1075-1083.

4. Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN. Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. Am J Cardiol. 1982; 49:1659-1666.

5. Francis GS, Benedict C, Johnstone DE, et al. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. Circulation. 1990;82:1724-1729.

6. Weber KT. Aldosterone in congestive heart failure. N Engl J Med. 2001;345(23):1689-1697.

7. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. Circulation. 1991;83(6):1849-1865.

8. Wang W, McClain JM, Zucker IH. Aldosterone reduces baroreceptor discharge in the dog. Hypertens. 1992;19:270-277.

9. Brilla CG, Rupp H, Funck R, Malsch B. The renin-angiotensin-aldosterone system and myocardial collagen matrix remodeling in congestive heart failure. Eur Heart J. 1995;16(Suppl O):107-109.

10. Martinez FA. Aldosterone inhibition and cardiovascular protection: more important than it once appeared. Cardiovasc Drugs Ther. 2010; 24(4):345-350.

11. Schiffrin EL. Effects of aldosterone on the vasculature. Hypertension. 2006;47(3):312-318.

12. Waanders F, de Vries LV, van Goor H, et al. Aldosterone, from (patho)physiology to treatment in cardiovascular and renal damage. Curr Vasc Pharmacol. 2011;9(5):594-605.

13. Gilbert KC, Brown NJ. Aldosterone and inflammation. Curr Op Endocrinol Diabetes Obes. 2010;17(3):199-204.

14. Young M, Fullerton M, Dilley R, Funder JW. Mineralocorticoids, hypertension, and cardiac fibrosis. J Clin Invest. 1994;93:2578-2583.

15. Staessen J, Lijnen P, Fagard R, et al. Rise in plasma concentration of aldosterone during long-term angiotensin II suppression. J Endocrinol. 1981;91(3):457-465.

16. Girend N, Pang PS, Swedberg K, et al. Serum aldosterone is associated with mortality and re-hospitalization in patients with reduced ejection fraction hospitalized for acute heart failure: analysis from the EVEREST trial. Eur J Heart Fail. 2014;16(11):1228-1235.

17. Lijnen P, Fagard R, Staessen J, Amery A. Effect of chronic diuretic treatment on the plasma renin-angiotensin-aldosterone system in essential hypertension. Br J Clin Pharmacol. 1981;12(3):387-392.

18. Bomback AS, Klemmer PJ. The incidence and implications of aldosterone breakthrough. Nat Clin Pract Nephrol. 2007;3(9):486-492.

19. Sato A, Fukuda S. Effect of aldosterone breakthrough on albuminuria during treatment with a direct renin inhibitor and combined effect with a mineralocorticoid receptor antagonist. Hypertens Res. 2013; 36(10):879-884.

20. Sato A, Saruta T. Aldosterone escape during angiotensin-converting enzyme inhibitor therapy in essential hypertensive patients with left ventricular hypertrophy. J Intern Med. 2001;29:13-21.

21. Sato A, Hayashi K, Naruse M, Saruta T. Effectiveness of aldosterone blockade in patients with diabetic nephropathy. Hypertension. 2003; 41(1):64-68.

22. Schjoedt KJ, Andersen S, Rossing P, Tarnow L, Parving HH. Aldosterone escape during blockade of the renin-angiotensin-aldosterone system in diabetic nephropathy is associated with enhanced decline in glomerular filtration rate. Diabetologia. 2004;47(11):1936-1939.

23. Tang WHW, Vagelos RH, Yee Y-G, et al. Neurohormonal and clinical responses to high- versus low-dose enalapril therapy in chronic heart failure. J Am Coll Cardiol. 2002;39:70-78.
24. Cicoira M, Zanolla L, Rossi A, et al. Failure of aldosterone suppression despite angiotensin-converting enzyme (ACE) inhibitor administration in chronic heart failure is associated with ACE DD genotype. J Am Coll Cardiol. 2001;37:1808-1812.

25. Bomback AS, Rekhtman Y, Klemmer PJ, Canetta PA, Radhakrishnan J, Appel GB. Aldosterone breakthrough during aliskiren, valsartan, and combination (aliskiren + valsartan) therapy. J Am Soc Hypertens. 2012;6(5):338-345.

26. Yoneda T, Takeda Y, Usukura M, et al. Aldosterone breakthrough during angiotensin II receptor blockade in hypertensive patients with diabetes mellitus. Am J Hypertens. 2007;20(12):1329-1333.

27. MacFadyen RJ, Lee AFC, Morton JJ, et al. How often are angiotensin II and aldosterone concentrations raised during chronic ACE inhibitor treatment in cardiac failure? Heart. 1999;82(1):57-61.

28. Lee A, MacFadyen RJ. Neurohormonal reactivation in heart failure patients on chronic ACE inhibitor therapy: a longitudinal study. Eur J Heart Fail. 1999;1(4):401-406.

29. Horita Y, Taura K, Taguchi T, et al. Aldosterone breakthrough during angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in proteinuric patients with immunoglobulin A nephropathy. Nephrol Ther. 2006;11(5):462-466.

30. Pitt B. "Escape" of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: implications for therapy. Cardiovasc Drugs Ther. 1995;9(1):145-149.

31. van de Wal RMA, Plokker HWM, Lok DJA, et al. Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. Int J Cardiol. 2006;106(3):367-372.

32. Roig E, Perez-Villa F, Morales M, et al. Clinical implications of increased plasma angiotensin II despite ACE inhibitor therapy in patients with congestive heart failure. Eur Heart J. 2000;21(3):55-57.

33. Aronson D, Burger AJ. Neurohormonal prediction of mortality following admission for decompensated heart failure. Am J Cardiol. 2003;91(2):245-248.

34. Bayliss J, Norell M, Canepa-Anson R, Sutton G, Poole-Wilson P. Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics. Br Heart J. 1987;57(17):11-22.

35. Knowlen GG, Kittleson MD, Nachreiner RF, Eyster GE. Comparison of plasma aldosterone concentration among clinical status groups. J Am Vet Med Assoc. 1983;183(9):991-996.

36. Lovern CS, Swecker WS, Lee JC, Moon ML. Additive effects of a sodium chloride restricted diet and furosemide administration in healthy dogs. Am J Vet Res. 2001;62(11):1793-1796.

37. Haggstrom J, Hansson K, Karlberg BE, et al. Effects of long-term treatment with enalapril or hydralazine on the renin-angiotensin system and fluid balance in dogs with naturally acquired mitral valve regurgitation. Am J Vet Res. 1996;57(11):1645-1652.

38. Atkins CE, Rausch WP, Gardner SY, et al. The effect of amloidine and the combination of amloidine and enalapril on the renin-angiotensin-aldosterone system in dogs. J Vet Pharmacol Ther. 2007;30(5):394-400.

39. Lantis AC, Atkins CE, DeFrancesco TC, et al. Effects of furosemide and the combination of furosemide and the labeled dosage of pimo-bendan on the circulating renin-angiotensin-aldosterone system in clinically normal dogs. Am J Vet Res. 2011;72(12):1646-1651.

40. De Mello WC, Frohlich ED. Clinical perspectives and fundamental aspects of local cardiovascular and renal Renin-Angiotensin systems. Front Endocrinol (Lausanne). 2014;5:16.

41. De Mello WC. Chemical communication between heart cells is disrupted by intracellular renin and angiotensin ii: implications for heart development and disease. Front Endocrinol (Lausanne). 2015;6:1-6.

42. Bader M, ACE2, angiotensin-(1–7), and Mas: the other side of the coin. Pflugers Arch. 2013;465(1):79-85.

43. Seelinger E, Wrons T, Ladwig M, et al. The "body fluid pressure control system" relies on the renin-angiotensin-aldosterone system: balance studies in freely moving dogs. Clin Exp Pharmacol Physiol. 2005;32(6):394-399.

44. Hall JE, Guyton AC. Role of the kidneys in long-term control of arterial pressure and in hypertension: the integrated system for arterial pressure regulation. Guyton and Hall Textbook of Medical Physiology. Philadelphia, PA: Elsevier Health Sciences; 2010:213-228.

45. Beuschlein F. Regulation of aldosterone secretion: from physiology to disease. J Endocrinol. 2013;216(6):R85-R93.

46. Funder JW, Pearce PT, Smith R, Smith AL. Mineralocorticoid receptor: target tissue specificity is enzyme, not receptor, mediated. Science. 1988;242(4878):583-585.

47. Funder JW, Pearce PT, Smith R, Campbell J. Vascular type I aldosterone binding sites are physiological mineralocorticoid receptors. Endocrinology. 1989;129(4):2224-2226.

48. Jaisser F, Farman N. Emerging roles of the mineralocorticoid receptor in pathology: toward new paradigms in clinical pharmacology. Pharmacol Rev. 2015;68(1):49-75.

49. Marzolla V, Armani A, Zennaro M-C, et al. The role of the mineralocorticoid receptor in adipocyte biology and fat metabolism. Mol Cell Endocrinol. 2012;350(2):281-288.

50. Marzolla V, Armani A, Feraco A, et al. Mineralocorticoid receptor in adipocytes and macrophages: a promising target to fight metabolic syndrome. Steroids. 2014;91:46-53.

51. Gomez-Sanchez E, Gomez-Sanchez CE. The multifaceted mineralocorticoid receptor. Compr Physiol. 2014;4(3):965-994.

52. Gros R, Ding Q, Liu B, Chorazycewski J, Feldman RD. Aldosterone mediates its rapid effects in vascular endothelial cells through GPER activation. Am J Physiol. 2013;304(6):C532-C540.

53. Fuller PJ, Yang J, Young MJ. 30 years of the mineralocorticoid receptor: coregulators as mediators of mineralocorticoid receptor signaling diversity. J Endocrinol. 2017;234(1):T23-T34.

54. Bader M. Tissue renin-angiotensin-aldosterone systems: targets for pharmacological therapy. Annu Rev Pharmacol Toxicol. 2010;50(1):439-465.

55. Bader M, Ganten D. Update on tissue renin-angiotensin systems. J Mol Med. 2008;86(6):615-621.

56. Takeda Y, Miyamori I, Yoneda T, et al. Production of aldosterone in isolated rat blood vessels. Hypertension. 1995;25(2):170-173.

57. Dzau VJ, Bernstein K, Celermajer D, et al. The relevance of tissue angiotensin-converting enzyme: manifestations in mechanistic and endpoint data. Am J Cardiol. 2001;88(9A):1L-20L.

58. Harada K, Izawa H, Nishizawa T, et al. Beneficial effects of torasemide on systolic wall stress and sympathetic nervous activity in asymptomatic or mildly symptomatic patients with heart failure: comparison with azosemide. J Cardiovasc Pharmacol. 2009;53(6):468-473.

59. Ullian ME, Schelling JR, Linas SL. Aldosterone enhances angiotensin II receptor binding and inositol phosphate responses. Hypertension. 1992;20(1):67-73.

60. Ullian ME, Hutchison FN, Hazen-Martin DJ, Morinelli TA. Angiotensin II-aldosterone interactions on protein synthesis in vascular smooth muscle cells. Am J Physiol. 1993;264:C1525-C1531.

61. Ullian ME, Fine JJJ. Mechanisms of enhanced angiotensin. J Cell Physiol. 1994;161(2):201-208.

62. Harada E, Yoshimura M, Yasue H, et al. Aldosterone induces cardiac hypertrophy in pathology: toward new paradigms in clinical pharmacology. Hypertension. 2005;46(5):483-489.
84. Yamane T, Fujii Y, Orito K, Osamura K, Kanai T, Wakao Y. Comparison of the effects of candesartan cilexetil and enalapril maleate on right ventricular myocardial remodeling in dogs with experimentally induced pulmonary stenosis. Am J Physiol Heart Circ Physiol. 2008;295(1):H152-H160.

85. Chappell MC. Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute? Am J Physiol Heart Circ Physiol. 2016;310(2):H137-H152.

86. Chappell MC. Emerging evidence for a functional angiotensin-converting enzyme 2-angiotensin-(1-7)-MAS receptor axis: more than regulation of blood pressure? Hypertension. 2007;50(4):596-599.

87. Metzger R, Bader M, Ludwig T, Berberich C, Bunnenmann B, Ganten D. Expression of the mouse and rat mas proto-oncogene in the brain and peripheral tissues. FEBS Lett. 1995;357(1):27-32.

88. Flores-Muñoz M, Smith NJ, Haggerty C, Milligan G, Nicklin SA. Angiotensin1-9 antagonises pro-hypertrophic signalling in cardiomyocytes via the angiotensin type 2 receptor. J Physiol. 2011;589(4):939-951.

89. Park BM, Gao S, Cha SA, Park BH, Kim SH. Cardioprotective effects of angiotensin III against ischemic injury via the AT2 receptor and KATP channels. Physiol Rep. 2013;1(6):e00151.

90. Park BM, Oh Y-B, Gao S, Cha SA, Kang KP, Kim SH. Angiotensin III stimulates high stretch-induced ANP secretion via angiotensin type 2 receptors. Peptides. 2013;42:131-137.

91. Park BM, Cha SA, Han BR, Kim SH. Angiotensin IV stimulates high atrial stretch-induced ANP secretion via insulin regulated aminopeptidase. Peptides. 2015;63:30-37.

92. Gordon CR, Lavie P. Day-night variations in urine excretions and hormones in dogs: role of autonomic innervation. Physiol Behav. 1985;35(2):175-181.

93. Kawasaki T, Cugini P, Uezono K, et al. Circadian variations of total renin and active renin. Horm Metab Res. 2008;40(12):636-639.

94. Cugini P, Scavo D, Halberg F, et al. Ageing and circadian rhythm of plasma renin and aldosterone. Maturitas. 1981;3(2):173-182.

95. Pelliccia P, Pugliesi T, Zanetti E, et al. Circadian variations in plasma renin activity and aldosterone concentration in dogs. Circ Res. 2005;97(2):152-156.

96. Reinhardt HW, Seelig E, Lohmann K, et al. Changes of blood pressure, sodium excretion and sodium balance due to variations of the renin-angiotensin-aldosterone system. Am J Physiol. 1997;273(3):H961-H970.

97. Itoh K, Morita S, Ikeda Y, et al. Circadian variations of plasma renin and aldosterone levels in dogs. J Physiol. 2003;553(2):545-552.

98. Metzger R, Bader M, Ludwig T, Berberich C, Bunnenmann B, Ganten D. Expression of the mouse and rat mas proto-oncogene in the brain and peripheral tissues. FEBS Lett. 1995;357(1):27-32.

99. Flores-Muñoz M, Smith NJ, Haggerty C, Milligan G, Nicklin SA. Angiotensin1-9 antagonises pro-hypertrophic signalling in cardiomyocytes via the angiotensin type 2 receptor. J Physiol. 2011;589(4):939-951.

100. Park BM, Gao S, Cha SA, Park BH, Kim SH. Cardioprotective effects of angiotensin III against ischemic injury via the AT2 receptor and KATP channels. Physiol Rep. 2013;1(6):e00151.

101. Park BM, Oh Y-B, Gao S, Cha SA, Kang KP, Kim SH. Angiotensin III stimulates high stretch-induced ANP secretion via angiotensin type 2 receptors. Peptides. 2013;42:131-137.

102. Park BM, Cha SA, Han BR, Kim SH. Angiotensin IV stimulates high atrial stretch-induced ANP secretion via insulin regulated aminopeptidase. Peptides. 2015;63:30-37.

103. Gordon CR, Lavie P. Day-night variations in urine excretions and hormones in dogs: role of autonomic innervation. Physiol Behav. 1985;35(2):175-181.
110. Xue C, Siragy HM. Local renal aldosterone system and its regulation by salt, diabetes, and angiotensin II type 1 receptor. Hypertension. 2005;46(3):584-590.

111. Bolignano D, Palmer SC, Navaneethan SD, Strippoli GFM. Aldosterone nongenomic action: Clinical implications. J Am Soc Nephrol. 2011;22(2):252-261.

112. Bolignano D, Palmer SC, Navaneethan SD, Strippoli GFM. Aldosterone nongenomic action: Clinical implications. J Am Soc Nephrol. 2011;22(2):252-261.

113. Bolignano D, Palmer SC, Navaneethan SD, Strippoli GFM. Aldosterone nongenomic action: Clinical implications. J Am Soc Nephrol. 2011;22(2):252-261.

114. van den Berg TNA, Rongen GA, Fröhlich GM, Deinum J, Hausenloy DJ, van den Berg TNA, Rongen GA, Fröhlich GM, Deinum J, Hausenloy DJ. Aldosterone signaling in cardiac hypertrophy: Impact on myocardial fibrosis. Hypertension. 2012;60(3):464-472.

115. Villarreal FJ, Kim NN, Ungab GD, Printz MP, Dillmann WH. Identification of functional angiotensin II receptors on rat cardiac fibroblasts. Circulation. 1993;88(6):2849-2861.

116. Lassègue B, Sorensen D, Szécs K, et al. Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res. 2001;89(9):888-894.

117. Ni W, Zhan Y, He H, Maynard E, Balschi JA, Oettgen P. Ets-1 is a transcriptional repressor of angiotensin II-induced cardiac hypertrophy and remodeling in mice. J Clin Invest. 2005;115(1):48-57.

118. Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Pavo N, Wurm R, Goliasch G, et al. Renin-angiotensin system finger-prints of heart failure with reduced ejection fraction. J Am Coll Cardiol. 2016;68(25):2912-2914.

119. Villarreal FJ, Dillmann WH. Cardiac hypertrophy-induced changes in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res. 2001;89(9):888-894.

120. Brilla CG, Zhou G, Rupp H, Maisch B, Weber KT. Role of angiotensin II and prostaglandin E2 in regulating cardiac fibroblast collagen turnover. Am J Cardio. 1995;76(13):8D-13D.

121. Villarreal FJ, Dietmann WR. Cardiac hypertrophy-induced changes in mRNA levels for TGF-beta1, fibronectin, and collagen. Am J Physiol. 1992;262(Pt 2):H1861-H1866.

122. Azizani F, Benard L, Schlossarek S, Merval R. Aldosterone inhibits antifibrotic factors in mouse hypertensive heart. Hypertension. 2012;59:1179-1187.

123. Schreier B, Rabe S, Schneider B, Ruhs S. aldosterone/NaCl-induced renal and cardiac. Hypertension. 2011;34(5):623-629.

124. Tsymbouleva N, Zhang L, Chen S, et al. Aldosterone, through novel signaling proteins, is a fundamental molecular bridge between the genetic defect and the cardiac phenotype of hypertrophic cardiomyopathy. Circulation. 2004;109(10):1284-1291.

125. Lariviere R, Thibault G, Schiffrin EL. Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. Hypertension. 1993;21(3):294-300.

126. Xavier FE, Aras-López R, Arroyo-Villa I, et al. Aldosterone induces endothelial dysfunction in resistance arteries from normotensive and hypertensive rats by increasing thromboxane A2 and prostacyclin. Br J Pharmacol. 2008;154(6):1225-1235.

127. Fujita M, Minamino T, Asanuma H, et al. Aldosterone nongenomically worsens ischemia via protein kinase C-dependent pathways in hypoperfused canine hearts. Hypertension. 2005;46(1):113-117.

128. Iglarz M, Touyz RM, Viel EC, et al. Involvement of oxidative stress in the profibrotic action of aldosterone: interaction with the renin-angiotensin system. Am J Hypertens. 2004;17:597-603.

129. Ikeda U, Kanbe T, Nakayama I, Kawahara Y, Yokoyama M, Shimada K. Aldosterone inhibits nitric oxide synthesis in rat vascular smooth muscle cells by interleukin-1 beta. Eur J Pharmacol. 1995;290(2):49-73.

130. Dewald O, Zymek P, Winkelmann K, et al. CCL2/monocyte chemoattractant protein-1 regulates inflammatory responses critical to healing myocardial infarcts. Circ Res. 2005;96(8):881-889.

131. Persy V, Verhulst A, Ysebaert DK, de Greef KE, de Broe ME. Reduced postischemic macrophage infiltration and interstitial fibrosis in osteopontin knockout mice. Kidney Int. 2003;63(2):543-553.

132. Rickard AJ, Morgan J, Tesch G, Funder JW, Fuller PJ, Young MJ. Deletion of mineralocorticoid receptors from macrophages protects against deoxycorticosterone/salt-induced cardiac fibrosis and increased blood pressure. Hypertension. 2009;54(3):537-543.

133. Bienvenu LA, Morgan J, Rickard AJ, et al. Macrophage mineralocorticoid receptor signaling plays a key role in aldosterone-independent cardiac fibrosis. Endocrinology. 2012;153(7):3416-3425.

134. Rafatian N, Westcott KV, White RA, Leenen FHH. Cardiac macrophages and apoptosis after myocardial infarction: effects of central MR blockade. Am J Physiol Reg Cardiol Physiol. 2014;307(7):R879-R887.

135. Usher MG, Duan SZ, Ivaschenko CY, et al. Myeloid mineralocorticoid receptor controls macrophage polarization and cardiovascular hypertrophy and remodeling in mice. J Clin Invest. 2010;120(9):3350-3364.

136. Nagase M, Matsui H, Shibata S, Gotoda T, Fujita T. Salt-induced nephropathy in obese spontaneously hypertensive rats via paradoxical activation of the mineralocorticoid receptor: role of oxidative stress. Hypertension. 2007;50(5):877-883.

137. Wang H, Shimosawa T, Matsui H, et al. Paradoxical mineralocorticoid receptor activation and left ventricular diastolic dysfunction under high oxidative stress conditions. J Hypertens. 2008;26(7):1453-1462.

138. Monahan KD, Leuenberger UA, Ray CA. Aldosterone impairs baroreflex sensitivity in healthy adults. Am J Physiol Heart Circ Physiol. 2006;292(1):H190-H197.

139. Wang W. Chronic administration of aldosterone depresses baroreceptor reflex function in the dog. Hypertension. 1994;24(5):571-575.

140. Tucker IH, Pliquet RU. Novel mechanisms of sympatho-excitation in chronic heart failure. Heart Fail Monit. 2002;3(1):2-7.

141. Eckberg DL, Drabinsky M. Defective cardiac parasympathetic control of heart rate in chronic heart failure. Circulation. 2008;118(7):1877-1883.

142. MacFadyen RJ, Meredith PA, Elliott HL. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. An overview. Circulation. 1994;90(1):84-91.

143. Toutain PL, Lefebvre HP, King JN. Benazeprilat disposition and metabolite formation in normotensive and hypertensive rats by increasing thromboxane A2 and prostacyclin. J Pharmacol Exp Ther. 2000;292(3):1087-1093.

144. MacFaddyen RJ, Meredith PA, Elliott HL, Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. An overview. Clin Pharmacokinet. 1993;25(4):274-282.

145. Bernstein KE, Martin BM, Edwards AS, Bernstein EA. Mouse angiotensin-converting enzyme is a protein composed of two homologous domains. J Biol Chem. 1989;264(20):11945-11951.

146. Rousseau A, Michaud A, Chauvet MT, Lenfant M, Corvol P. The angiotensin-converting enzyme is a protein composed of two homologous domains. J Biol Chem. 1989;264(20):11945-11951.

147. Tan J, Wang J, Leenen F. Inhibition of brain angiotensin-converting enzyme by peripheral administration of trandolapril versus lisinopril in Wistar rats. Am J Hypertens. 2005;18(2):158-164.
185. The COVE Study Group. Controlled clinical evaluation of enalapril in dogs with heart failure: results of the Cooperative Veterinary Enala-
pril Study Group. J Vet Intern Med. 1995;9:243-252.

186. Besche B, Chetboul V, Lachaud Lefay M-P, Grandemange E. Clinical evaluation of imidapril in congestive heart failure in dogs: results of the Efficacy study. J Small Anim Pract. 2007;48(5):265-270.

187. The IMPROVE Study Group. Acute and short-term hemodynamic, echocardiographic, and clinical effects of enalapril maleate in dogs with naturally acquired heart failure: results of the Invasive Multi-
center PROspective Veterinary Evaluation of Enalapril Study. J Vet Intern Med. 1995;9(4):234-242.

188. FDA New Drug Application. Freedom of Information Summary, VETMEDIN – Pimobendan chewable tablets for dogs. 2007 Apr 30:1-36.

189. Häggström J, Boswood A, O’Grady M, et al. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: the QUEST Study. J Vet Intern Med. 2008;22(5):1124-1135.

190. Kwart C, Häggström J, Pedersen HD, et al. Efficacy of enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation. J Vet Intern Med. 2002;16(1):80-88.

191. Atkins CE, Keene BW, Brown WA, et al. Results of the veterinary enalapril trial to prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency. J Am Vet Med Assoc. 2007;231(7):1061-1069.

192. Pouchelon JL, Jamet N, Gouni V, et al. Effect of benazepril on survival and cardiac events in dogs with asymptomatic mitral valve disease: a retrospective study of 141 cases. J Vet Intern Med. 2008;22(4):905-914.

193. O’Grady MR, O’Sullivan ML, Minors SL, Horne R. Efficacy of benazepril hydrochloride to delay the progression of occult dilated cardiomyopathy in doberman pinschers. J Vet Int Med. 2009;23(5):977-983.

194. Meurs KM, Stern JA, Atkins CE, et al. Angiotensin-converting enzyme activity and inhibition in dogs with cardiac disease and an angiotensin-converting enzyme polymorphism. J Renin Angiotens Aldosterone Syst. 2017;18(4):1-4.

195. Blackford LW, Golden AL, Bright JM, et al. Captopril provides sustained hemodynamic benefits in dogs with experimentally induced mitral regurgitation. Vet Surg. 1990;19(2):237-242.

196. Bonnemann A, Haggstrom J, Gordon SG, et al. Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiac-megaly: The EPIC Study—a randomized clinical trial. J Vet Intern Med. 2016;30(6):1765-1779.

197. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. N Engl J Med. 1999;341(10):709-717.

198. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldoste-
rone blocker, in patients with left ventricular dysfunction after myo-
cardial infarction. 2003;348(14):1309-1321.

199. Zannad F, McMurray J, Krum H, et al. Eplerenone in patients with systolic heart failure and mild symptoms. N Engl J Med. 2011;364(1):11-21.

200. Butler J, Ezekowitz JA, Collins SP, et al. Update on aldosterone antagonists use in heart failure with reduced left ventricular ejection fraction. Heart Failure Society of America Guidelines Committee. J Card Fail. 2012;18(4):265-281.

201. Rousseau MF, Gurné O, Duprez D, et al. Beneficial neurohormonal profile of spironolactone in severe congestive heart failure: results from the RALES neurohormonal substudy. J Am Coll Cardiol. 2002; 40(9):1596-1601.

202. Pedersen HD, Olsen LH, Arnorsdottir H. Breed differences in the plasma renin activity and plasma aldosterone concentration of dogs. Zentralbl Veterinarmed A. 1995;42(7):435-441.

203. Lantis AC, Ames MK, Atkins CE, et al. Aldosterone breakthrough with benazepril in furosemide-activated renin-angiotensin-aldosterone system in normal dogs. J Vet Pharmacol Ther. 2014;38(1):65-73.

204. Lantis AC, Ames MK, Werre S, Atkins CE. The effect of enalapril on furosemide-activated renin-angiotensin-aldosterone system in healthy dogs. J Vet Pharmacol Ther. 2015;38(5):513-517.

205. Ames MK, Atkins CE, Lee S, Lantis AC, zumBrunnen JR. Effects of high doses of enalapril and benazepril on the pharmacologically acti-
vated renin-angiotensin-aldosterone system in clinically normal dogs. Am J Vet Res. 2015;76(12):1041-1050.

206. Suzuki G. Effects of long-term monotherapy with eplerenone, a novel aldosterone blocker, on progression of left ventricular dys-
function and remodeling in dogs with heart failure. Circulation. 2002;106(23):2967-2972.

207. Cataliotti A. Differential actions of vasopeptidase inhibition versus angiotensin-converting enzyme inhibition on diuretic therapy in exper-
imental congestive heart failure. Circulation. 2002;105(5):639-644.

208. Lee J, Mizuno M, Mizuno T, Harada K, Uechi M. Pathologic mani-
festations on surgical biopsy and their correlation with clinical indices in dogs with degenerative mitral valve disease. J Vet Intern Med. 2015;29(5):1313-1321.

209. Schuller S, van Israël N, Vanbelle S, et al. Lack of efficacy of low-
dose spironolactone as adjunct treatment to conventional conges-
tive heart failure treatment in dogs. J Vet Pharmacol Ther. 2010;34 (4):322-331.

210. Bernay F, Bland JM, Haggstrom J, et al. Efficacy of spironolactone on survival in dogs with naturally occurring mitral regurgitation caused by myxomatous mitral valve disease. J Vet Intern Med. 2010; 24(2):331-341.

211. Edelmann F, Wachter R, Schmidt AG, et al. Effect of spironolactone on diastolic function and exercise capacity in patients with heart fail-
ure with preserved ejection fraction: the Aldo-DHF randomized con-
trolled trial. JAMA. 2013;309(8):781-791.

212. Mottram PM, Haluska B, Leano R, Cowley D, Stowasser M, Marwick TH. Effect of aldosterone antagonism on myocardial dys-
fuction in hypertensive patients with diastolic heart failure. Circula-
tion. 2004;110(5):558-565.

213. Pitt B, Pfeffer MA, Assmann SF, et al. Spironolactone for heart fail-
ure with preserved ejection fraction. N Engl J Med. 2014;370(15): 1383-1392.

214. de Denus S, O-Meara E. Spironolactone metabolites in TOPCAT - new insights into regional variation. N Engl J Med. 2017;376(17):1-3.

215. Bristow MR, Silva Enciso J, Gersh BJ, et al. Detection and manage-
ment of geographic disparities in the TOPCAT trial. JACC Basic Transl Sci. 2016;1(3):180-189.

216. Lewis EF, Kim H-Y, Claggott B, et al. Impact of spironolactone on longitudinal changes in health-related quality of life in the treatment of preserved cardiac function heart failure with an aldosterone antagonist trial. Circ Heart Fail. 2016;9(3):e001937.

217. Kosmala W, Rojek A, Prewlocka-Kosmala M, Wright L, Mysiak A, Marwick TH. Effect of aldosterone antagonism on exercise toler-
ance in heart failure with preserved ejection fraction. J Am Coll Car-
diol. 2016;68(17):1823-1834.

218. MacDonald KA, Kittleson MD, Larson RF, et al. The effect of ramipril on left ventricular mass, myocardial fibrosis, diastolic function, and plasma neurohormones in canine coon cats with familial hypertro-
phic cardiomyopathy without heart failure. J Vet Intern Med. 2006; 20(5):1093-1105.

219. MacDonald KA, Kittleson MD, Kass PH. Effect of spironolactone on diastolic function and left ventricular mass in canine coon cats with familial hypertrophic cardiomyopathy. J Vet Intern Med. 2008;22(2): 335-341.

220. James R, Guillot E, Garelli-Paar C, Huxley J, Grassi V, Cobb M. The SEISICAT study: a pilot study assessing efficacy and safety of
spironolactone in cats with congestive heart failure secondary to cardiomyopathy. J Vet Cardiol. 2018;20(1):1-12.

221. Krum H, Nolly H, Workman D, et al. Efficacy of eplerenone added to renin-angiotensin blockade in hypertensive patients. Hypertension. 2002;40(2):117-123.

222. Kawada N, Isaka Y, Kitamura H, Rakugi H, Moriyama T. A pilot study of the effects of eplerenone add-on therapy in patients taking renin-angiotensin system blockers. J Renin Angiotensin Aldosterone Syst. 2015;16(2):360-365.

223. Jansen PM, Danser AHJ, Imholz BP, van den Meiracker AH. Aldosterone-receptor antagonism in hypertension. J Hypertens. 2009;27(4):680-691.

224. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med. 2007;21:542-558.

225. Ortega TM, Feldman EC, Nelson RW, Willits N, Cowgill LD. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. J Am Vet Med Assoc. 1996;209(10):1724-1729.

226. Javadi S, Kooistra HS, Mol JA, Boer P, Boer WH, Rijkenberk A. Plasma aldosterone concentrations and plasma renin activity in healthy dogs and dogs with hyperadrenocorticism. Vet Rec. 2003;153(17):521-525.

227. Goy-Thollot I, Péchereau D, Kérouz S, et al. Investigation of the role of aldosterone in hyperadrenocorticism associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. J Small Anim Pract. 2002;43(11):489-492.

228. Wengen M, Sieber-Ruckstuhl NS, Müller C, Reusch CE. Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism. Am J Vet Res. 2004;65(9):1245-1250.

229. Jepson RE, Syme HM, Elliott J. Plasma renin activity and aldosterone concentrations in hypertensive cats with and without azotemia and in response to treatment with amlopidine besylate. J Vet Intern Med. 2013;28(1):144-153.

230. Flack JM, Oparil S, Pratt JH, et al. Efficacy and tolerability of eplerenone and losartan in hypertensive black and white patients. J Am Coll Cardiol. 2003;41(7):1148-1155.

231. Jenkins TL, Coleman AE, Schmidt WW, Brown SA. Attenuation of the pressor response to exogenous angiotensin by angiotensin receptor blockers and benazepril hydrochloride in clinically normal cats. Am J Vet Res. 2015;76(9):807-813.

232. Glaus TM, Elliott J, Herberich E, et al. Effects of enalapril versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. Lancet. 2015;386:2059-2068.

233. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. 2006;116(2):288-296.

234. Walls J. Relationship between proteinuria and progressive renal disease. Am J Kidney Dis. 2001;37(1):513-516.

235. Williams TL, Elliott J, Syme HM. Renin-Angiotensin-Aldosterone System Activity in Hyperthyroid Cats with and without Concurrent Hypertension. J Vet Intern Med. 2013;27(3):522-529.

236. Jensen J, Henik RA, Brownfield M, Armstrong J. Plasma renin activity and angiotensin I and aldosterone concentrations in cats with hypertension associated with chronic renal disease. Am J Vet Res. 1997;58(5):535-540.

237. Williams B, MacDonald TM, Morant S, et al. Spironolactone versus placebo, bisoprolol, and doxazosin to determine the optimal treatment for drug-resistant hypertension (PATHWAY-2): a randomised, double-blind, crossover trial. Lancet. 2015;386:2059-2068.

238. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. J Clin Invest. 2006;116(2):288-296.

239. Walls J. Relationship between proteinuria and progressive renal disease. Am J Kidney Dis. 2001;37(1):513-516.

240. Cheng J, Zhang X, Tian J, Li Q, Chen J. Combination therapy an ACE inhibitor and an angiotensin receptor blocker for IgA nephropathy: a meta-analysis. Int J Clin Pract. 2012;66(10):917-923.

241. Ando K, Ohitsu H, Uchida S, et al. Anti-albuminuric effect of the aldosterone blocker eplerenone in non-diabetic hypertensive patients with albuminuria: a double-blind, randomised, placebo-controlled trial. Lancet Diabetes Endocrinol. 2014;2(12):944-953.

242. Esteghamati A, Noshad S, Jarrah S, Mousavizadeh M, Khoei SH, Nakjavan M. Long-term effects of addition of mineralocorticoid receptor antagonist to angiotensin II receptor blocker in patients with diabetic nephropathy: a randomized clinical trial. Nephrol Dial Transplant. 2013;28(11):2823-2833.

243. Brown SA, Finco DR, Brown CA, et al. Evaluation of the effects of inhibition of angiotensin converting enzyme with enalapril in dogs with induced chronic renal insufficiency. Am J Vet Res. 2003;64(3):321-327.

244. Brown SA, Walton CL, Crawford P, Bakris GL. Long-term effects of antihypertensive regimens on renal hemodynamics and proteinuria. Kidney Int. 1993;43(6):1210-1218.

245. Grauer GF, Greco DS, Getzy DM, et al. Effects of enalapril versus placebo as a treatment for canine idiopathic glomerulonephritis. J Vet Intern Med. 2000;14(5):526-533.

246. Tenhundfeld J, Weftaedi P, Nolte J. A randomized controlled clinical trial of the use of benazepril and heparin for the treatment of chronic kidney disease in dogs. J Am Vet Med Assoc. 2009;234(8):1031-1037.

247. Mishima M, Watanabe T. Development of hypertension and effects of benazepril hydrochloride in a canine remnant kidney model of chronic renal failure. J Vet Med Sci. 2008;70(5):455-460.

248. Jacob F, Polzin DJ, Osborne CA, et al. Evaluation of the association between initial proteinuria and morbidity rate or death in dogs with naturally occurring chronic renal failure. J Am Vet Med Assoc. 2005;226(3):393-400.

249. Grodecki K, Gains MJ, Baumal R, et al. Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting enzyme (ACE) inhibitor. J Comp Pathol. 1997;117(3):209-225.

250. Abraham PA, Opsahl JA, Halstenson CE, Keane WF, et al. Efficacy and renal effects of enalapril therapy for hypertensive patients with chronic renal insufficiency. Arch Intern Med. 1988;148(11):2358-2362.

251. Brown SA, Brown CA, Jacobs G. Hemodynamic effects of angiotensin converting enzyme inhibition (Benazepril) in cats with chronic renal insufficiency. J Vet Intern Med. 1999;17:716.

252. Praga M, Hernandez E, Montoyo C, et al. Long-term beneficial effects of angiotensin-converting enzyme inhibition in patients with nephrotic proteinuria. Am J Kidney Dis. 1992;20(3):240-248.

253. Maschio G, Aliberti D, Janin G, et al. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. N Engl J Med. 1996;334(15):939-945.

254. Hou FF, Zhang X, Zhang GH, et al. Efficacy and safety of benazepril for advanced chronic renal insufficiency. N Engl J Med. 2006;354(2):131-140.

255. Miller RH, Lehmkuhl LB, Smek DD, DiBartola S, Radin J. Effect of enalapril on blood pressure, renal function, and the renin-angiotensin-aldosterone system in cats with autosomal dominant polycystic kidney disease. Am J Vet Res. 1999;60(12):1516-1525.

256. IRIS Canine GN Study Group Standard Therapy Subgroup, Brown S, Elliott J, Franc ey T, Polzin D, Vaden S. Consensus recommendations for standard therapy for glomerular disease in dogs. J Vet Intern Med. 2013;27(s1):S27-S43.

257. Steele J, Henik R, Stepien R. Effects of angiotensin-converting enzyme inhibition on plasma aldosterone concentration, plasma renin activity, and blood pressure in spontaneously hypertensive cats with chronic renal disease. Vet Ther. 2002;3(2):157-166.
258. King JN, Tasker S, Gunn-Moore DA, Strehlau G, BENRIC (benazepril in renal insufficiency in cats) Study Group. Prognostic factors in cats with chronic kidney disease. *J Vet Intern Med*. 2007;21(5):906-916.

259. Chakrabarti S, Syme HM, Elliott J. Clinicopathological variables predicting progression of azotemia in cats with chronic kidney disease. *J Vet Intern Med*. 2012;26(2):275-281.

260. Syme HM, Markwell PJ, Pfeiffer D, Elliott J. Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria. *J Vet Intern Med*. 2006;20(3):528-535.

261. Jepson RE, Elliott J, Brodbelt D, Syme HM. Effect of control of systolic blood pressure on survival in cats with systemic hypertension. *J Vet Intern Med*. 2007;21(3):402-409.

262. Sent U, Gössl R, Elliott J, Syme HM, Zimmering T. Comparison of efficacy of long-term oral treatment with telmisartan and benazepril in cats with chronic kidney disease. *J Vet Intern Med*. 2015;29(6):1479-1487.

263. Wynckel A, Ebikili B, Melin JP, et al. Long-term follow-up of acute renal failure caused by angiotensin converting enzyme inhibitors. *Am J Hypertens*. 1998;11(9):1080-1086.

264. Atkins CE, Brown WA, Coats JR, et al. Effects of long-term administration of enalapril on clinical indicators of renal function in dogs with compensated mitral regurgitation. *J Am Vet Med Assoc*. 2002;221(5):654-658.

265. Lefebvre HP, Olivier E, Atkins CE, et al. Safety of spironolactone in dogs with chronic heart failure because of degenerative valvular disease: a population-based, longitudinal study. *J Vet Intern Med*. 2013;27(5):1083-1091.

266. Thomason JD, Rapoport G, Fallaw T, Calvert CA. The influence of enalapril and spironolactone on electrolyte concentrations in Doberman pinschers with dilated cardiomyopathy. *Vet J*. 2014;202(3):1-5.

267. Thomason JD, Rockwell JE, Fallaw TK, Calvert CA. Influence of combined angiotensin-converting enzyme inhibitors and spironolactone on serum K⁺, Mg²⁺, and Na⁺ concentrations in small dogs with degenerative mitral valve disease. *J Vet Cardiol*. 2007;9(2):103-108.

268. Brilla CG, Weber KT. Mineralocorticoid excess, dietary sodium. *J Lab Clin Med*. 1992;120(6):893-901.

269. Brilla CG, Pick R, Tan LB, Janicki JS, Weber KT. Remodeling of the rat right and left ventricles in experimental hypertension. *Circ Res*. 1990;67(6):1355-1364.

270. Brilla CG, Matsubara LS, Weber KT. Antifibrotic effects of spironolactone in preventing myocardial fibrosis in systemic arterial hypertension. *Am J Cardiol*. 1992;71(3):A12-A16.

271. Jalil JE, Janicki JS, Pick R, Weber KT. Coronary vascular remodeling and myocardial fibrosis in the rat with renovascular hypertension. Response to captopril. *Am J Hypertens*. 1991;4(1 Pt 1):51-55.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

---

**How to cite this article:** Ames MK, Atkins CE, Pitt B. The renin-angiotensin-aldosterone system and its suppression. *J Vet Intern Med*. 2019;33:363–382. https://doi.org/10.1111/jvim.15454