Plasma Renin Activity and Aldosterone Concentrations in Hypertensive Cats with and without Azotemia and in Response to Treatment with Amlodipine Besylate

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Background: Role of renin-angiotensin aldosterone system (RAAS) in feline systemic hypertension is poorly understood.

Objectives: Examine plasma renin activity (PRA) and plasma aldosterone concentrations (PAC) in normotensive and hypertensive cats with variable renal function and in response to antihypertensive therapy.

Animals: One hundred and ninety-six cats >9 years from first opinion practice.

Methods: PRA, PAC, and aldosterone-to-renin ratio (ARR) were evaluated in cats recruited prospectively and grouped according to systolic blood pressure (SBP) and renal function (nonazotemic normotensive [Non-Azo-NT], nonazotemic hypertensive [Non-Azo-HT], azotemic normotensive [Azo-NT], azotemic hypertensive [Azo-HT]). Changes in PRA and PAC were evaluated with antihypertensive therapy (amlodipine besylate).

Results: Plasma renin activity (ng/mL/h; $P = .0013$), PAC (pg/mL; $P < .001$), and ARR ($P = 0.0062$) differed significantly among groups. PRA (ng/mL/h) was significantly lower in hypertensive (Non-Azo-HT; n = 25, median 0.22 [25th percentile 0.09, 75th percentile 0.39], Azo-HT; n = 44, 0.33 [0.15, 0.48]) compared with Non-Azo-NT cats (n = 57, 0.52 [0.28, 1.02]). Azo-HT cats had significantly higher PAC (n = 22, 149.8 [103.1, 228.7]) than normotensive cats (Non-Azo-NT; n = 26, 45.4 [19.6, 65.0], Azo-NT; n = 18, 84.1 [38.6, 137.8]). ARR was significantly higher in Azo-HT (n = 20, 503.8 [298.8, 1511]) than Azo-NT cats (n = 16, 97.8 [77.0, 496.4]). Significant increase in PRA was documented with antihypertensive therapy (pretreatment [n = 20] 0.32 [0.15–0.46], posttreatment 0.54 [0.28, 1.51]), but PAC did not change.

Conclusions and Clinical Importance: Hypertensive cats demonstrate significantly increased PAC with decreased PRA. PRA significantly increases with antihypertensive therapy. Additional work is required to determine the role of plasma aldosterone concentration in the pathogenesis of hypertension and whether this relates to autonomous production or activation of RAAS without demonstrable increase in PRA.

Key words: Blood pressure; Cats; Chronic kidney disease.

Systemic hypertension in cats has been widely described in the veterinary literature, but its pathogenesis remains poorly understood.[1] The kidneys are integral to sodium balance and fluid volume regulation. Altered renal function and pressure natriuresis play potential roles in the development of systemic hypertension. Stimulation of the renin-angiotensin-aldosterone system (RAAS) is often considered as a link among renal disease, sodium retention, volume expansion, peripheral vascular resistance, and systemic hypertension. In recent years, the importance of tissue-specific RAAS, which operates independently from systemic RAAS, has also been realized.[2]

To date, only a limited number of studies have examined components of the RAAS in feline CKD and systemic hypertension.[3,4] In such studies, results

Abbreviations:

ACEi	angiotensin-converting enzyme inhibitor
Ang-1	angiotensin 1
ARR	aldosterone-to-renin ratio
BP	blood pressure
CKD	chronic kidney disease
CV	coefficient of variance
GFR	glomerular filtration rate
IRIS	International Renal Interest Society
PAC	plasma aldosterone concentration
PRA	plasma renin activity
RIA	radioimmunoassay
SBP	systolic blood pressure
UP:C	urine protein-to-creatinine ratio
USG	urine specific gravity

have been contradictory but have suggested increased plasma aldosterone concentrations (PAC; pg/mL) but variable plasma renin activity (PRA; ng/mL/h). However, these studies have been limited by small group sizes, prior introduction of low sodium renal diets, and lack of appropriate age-matched control groups.

Previous epidemiologic studies have documented that cats with systemic hypertension have lower plasma potassium concentration than normotensive cats with comparable renal function.[1,5] Relative or absolute hyperaldosteronism could be a possible cause. Hypertensive cats with naturally occurring CKD and experimentally induced renal insufficiency respond poorly to treatment with angiotensin-converting

enzyme inhibitors (ACEi).[4,6–8] The study by Steele and colleagues evaluated the effect of ACEi (benazepril or enalapril) therapy in cats with systemic hypertension and CKD. They documented no change in systolic blood pressure (SBP) and in concordance with this finding, no significant change in PRA or PAC potentially argues against a role for RAAS in the genesis of systemic hypertension in the cat.[9] However, alternative explanations for inadequate antihypertensive response to ACEi therapy in this study could be inadequate dosage or poor bioavailability of the drugs studied.

Approximately 20% of cats diagnosed with systemic hypertension are nonazotemic and do not have an identifiable cause for their hypertension.[10] These cats demonstrate variable urine-concentrating ability, and therefore mild CKD that has not resulted in azotemia could be contributing to the development of systemic hypertension.[11] PRA and PAC have not been explored previously in this group of cats. Amlodipine besylate, a dihydropyridine calcium channel antagonist, has been established as the first-line antihypertensive agent for cats.[10,12] The effect of this medication on PRA and PAC in hypertensive cats has not previously been investigated.

The aims of this study were to perform a cross-sectional analysis of PRA, PAC after solvent extraction from feline plasma and resuspension in human matrix, and aldosterone-to-renin ratios (ARR) in cats with variable renal function and in the pathogenesis of systemic hypertension. An additional aim was to evaluate the effect of the first-line antihypertensive agent, amlodipine besylate, on PAC and PRA. Based on the previously identified finding of significantly lower plasma potassium concentrations in cats with systemic hypertension, it was hypothesized that nonazotemic and azotemic cats with systemic hypertension would have significantly increased PAC that was driven by increased PRA.

Materials and Methods

Case Selection and Sample Collection

Cats (>9 years) were recruited to the study between 1999 and 2011 from 2 first opinion practices in central London (People's Dispensary for Sick Animals, Bow and Beaumont Sainsbury Animal Hospital, Camden). Cats included in this study have been included in previous studies published by the authors, but PRA and PAC data for these cats have not been reported previously. At recruitment, a full history was obtained and physical examination performed. SBP was assessed using the Doppler technique[a] and indirect fundic examinations performed as previous described.[11]

The collection and storage of blood samples was performed with owner consent and the protocols adhered to within this study had been approved by the Ethics and Welfare Committee at the Royal Veterinary College. Blood samples were obtained by jugular venipuncture and collected into lithium heparin and EDTA. Samples were held on ice (4°C) for a maximum of 6 hours before centrifugation[b] and separation. Heparinized and EDTA plasma samples were subsequently aliquoted and frozen at −80°C until required for assessment of PRA and PAC, respectively. Plasma biochemistry (heparin plasma) was performed at

an external commercial laboratory.[c] Total serum thyroxine concentration was assessed in all cats in which the history (eg, polyphagia, weight loss), physical examination findings (eg, tachycardia, arrhythmia, poor body condition, palpable goiter), or serum biochemical findings (increased alanine transferase or alkaline phosphatase activities) raised concern for hyperthyroidism. Any cat diagnosed with hyperthyroidism (total T4 > 55 nmol/L) or with a previous diagnosis of hyperthyroidism was excluded from the study. In all cases in which the urinary bladder was palpable, a urine sample was collected by cystocentesis. Urine sample handling, urinalysis, storage, and assessment of urine protein-to-creatinine measurements (UP:C) were performed as previously described.[11] Urine culture was performed when there was microscopic evidence of bacteriuria or pyuria or where clinical signs (stranguria) were compatible with the presence of a urinary tract infection.

Cats entered in the cross-sectional analysis were classified according to their plasma biochemical profile and SBP. The International Renal Interest Society (IRIS) provides a staging system, based on plasma creatinine concentration, for the classification of cats diagnosed with CKD, where IRIS stage 1 (plasma creatinine concentration <1.6 mg/dL) and early IRIS stage 2 (plasma creatinine concentration 1.6–2.8 mg/dL) reflect cats with nonazotemic CKD. In this study, cats were diagnosed with azotemic IRIS stage 2 CKD or greater on the basis of history (eg, polyuria, polydipsia), physical examination findings (renal palpation), the presence of azotemia (plasma creatinine concentration >2 mg/dL [177 μmol/L]), which reflected the upper limit of the laboratory reference interval and, where possible, inappropriate urine-concentrating ability (USG <1.035). Cats diagnosed with azotemic CKD were subsequently enrolled into a renal care program allowing long-term monitoring of their CKD. This ensured that persistence of azotemia was documented in all cats diagnosed with IRIS stage 2 or greater CKD, on at least 2 occasions, a minimum of 2 weeks apart, and that where urine samples were not available for assessment of USG on the initial visit, measurements were available on subsequent visits.

Systemic hypertension was diagnosed on the basis of SBP >170 mmHg on at least 2 occasions 7–14 days apart or on a single occasion if in association with clinical manifestations of systemic hypertension, most often hypertensive choroidopathy or retinopathy. All cats diagnosed with systemic hypertension were treated with amlodipine besylate[d] (0.625 mg/cat PO q24 h). Owners of cats were offered a re-examination appointment 7–14 days after initiating antihypertensive therapy to document efficacy of treatment, with a target SBP <160 mmHg. In those cats in which response to treatment was inadequate, the dose was increased to 1.25 mg/cat PO q24 h. Cats diagnosed with CKD could therefore be classified according to their BP status: azotemic normotensive (Azo-NT) and azotemic hypertensive (Azo-HT).

Cats were considered nonazotemic normotensive (Non-Azo-NT) if they were deemed healthy by their owners and the clinician responsible for the case, no abnormalities were identified on physical examination, they were documented to be nonazotemic on plasma biochemical profile, and SBP <160 mmHg was recorded. These cats did not have a diagnosis of CKD, although no requirement was made for USG. An additional group of cats was nonazotemic on the basis of a plasma biochemical profile, but these cats were documented to be persistently hypertensive warranting antihypertensive medication in each case. No requirement was made for USG. The presence of hypertension in these cats suggests impaired renal response to hypertension and therefore these cats were considered to have IRIS stage I and early-stage 2 CKD. As such, this group of cats is referred to as nonazotemic hypertensive (Non-Azo-HT) rather than idiopathic hypertensive.

Clinicopathologic variables, PRA, PAC, and ARR were compared among groups based on SBP and renal function (Non-Azo-NT, Non-Azo-HT, Azo-NT, Azo-HT). The ARR was calculated by dividing the PAC by PRA. To provide groups of normotensive and hypertensive cats that were matched according to renal status, cats with IRIS stage 4 CKD (plasma creatinine concentration >5 mg/dL) were excluded from the Azo-NT group.[1] Storage time from sample date to date of PRA and PAC assay was calculated and compared among groups.

An additional cross-sectional analysis was performed to evaluate the effect of renal function on PRA and PAC. Cats were classified according to their plasma creatinine concentration: Group 1 (plasma creatinine concentration <2.0 mg/dL), Group 2 (plasma creatinine concentration 2.0–2.8 mg/dL), and Group 3 (plasma creatinine concentration ≥2.9 mg/dL). Group 2 reflects cats classified with azotemic IRIS stage 2 CKD and Group 3 cats classified as IRIS stage 3 or 4 CKD. However, Group 1 potentially contains a combination of cats both with and without a diagnosis of nonazotemic CKD. All 3 groups contain both normotensive and hypertensive cats.

Exclusion criteria for cats entered into both cross-sectional analyses included concurrent medical conditions other than CKD, systemic hypertension, or both, recent IV fluid therapy, recent administration of medications other than antibiotic therapy or routine endo- or ectoparasiticides, potassium supplementation, use of a low protein and low phosphorous (renal) diet, or antihypertensive medications at the time of sampling. Every attempt was made to exclude cats with evidence of prerenal azotemia based on both clinical and biochemical information including USG whenever available.

In a smaller number of cats diagnosed with systemic hypertension (azotemic or nonazotemic), PRA and PAC were evaluated once target BP (<160 mmHg) had been achieved. In all cases, posttreatment samples were obtained within 3 months of starting antihypertensive therapy.

Measurement of Plasma Renin Activity

PRA was measured in EDTA plasma using a commercially available kit.[e] The assay involves an initial incubation phase to generate angiotensin 1 (Ang-1) followed by quantitation of Ang-1 by radioimmunoassay (RIA). The assay was performed in accordance with the manufacturer's instructions except that reaction volumes were halved in the Ang-1 generation phase to halve the volume of EDTA plasma required. Given this methodological variation, inter- and intra-assay variability was assessed. EDTA samples from multiple cats were pooled to provide plasma with low and high PRA. Dilutional parallelism was assessed for the RIA component of this assay.

Preliminary assessment of cryoactivation of renin was assessed by comparing PRA of feline plasma after standard handling (held on ice; 4°C) or maintaining samples at room temperature (21°C) until analysis (6 hours). PRA was also compared when measured immediately or after 40 days frozen at −80°C. PRA from normal healthy cats >9 years was used to generate a reference interval. Whenever PRA was undetectable, samples were assigned the arbitrary value of 0.05 ng/mL/h, which was determined to be the lower limit of quantitation of the assay.

Measurement of Solvent-Extracted Aldosterone Concentration

Aldosterone was measured using a commercially available RIA[f] performed in accordance with the manufacturer's recommendations and which has previously been validated for use with heparinized feline plasma samples.[4,13,14] Before RIA, aldosterone was extracted from feline plasma and resuspended in human matrix.[15]

For the extraction process, 10 mL dicloromethane[g] was added to 0.5 ml EDTA plasma and 5000 disintegrations per minute [^{3}H]-aldosterone (50 pg)[h] and shaken vigorously for 20 minutes. The aqueous phase was discarded and the extract was evaporated to dryness at 40°C under a constant stream of room air. Then, the residue was dissolved in 0.5 mL human matrix (zero human standard from kit). To evaluate recovery from the extraction process, disintegrations per minute were compared when 50 μL of extracted sample or 50 μL of [^{3}H]-aldosterone[k] was added to 4.5 mL of scintillation fluid[i] and counted in a scintillation counter.[j] An adjustment was made to the RIA measurement of aldosterone to correct for both the efficiency of the extraction process and the [^{3}H]-aldosterone[k] added to the samples.

A reference interval was generated from normal healthy geriatric cats using this technique. Intra- and interassay variability was assessed using pooled heparinized feline plasma to give low and high PAC. Dilutional parallelism was assessed after extraction of aldosterone.

Statistical Analysis

Computerized statistical software[k,l] was used for all statistical analyses. Probabilities <.05 were considered significant. Throughout the study, data are presented as median [25th, 75th percentile]. Inter- and intra-assay variability were assessed by calculation of coefficient of variance (CV). Upper limit of reference intervals for PRA, extracted PAC, and ARR were calculated from the group of normal geriatric cats as mean + 2SD. The influence of sample handling on PRA was assessed with a Wilcoxon signed rank test.

Data for the cross-sectional analyses were assessed for normality using the Kolmogorov-Smirnov test. For data approaching normality, significant differences among groups were assessed using a one-way ANOVA with Bonferroni's multiple comparison posthoc analysis. For data that were not normally distributed, the nonparametric Kruskall-Wallis test with Dunn's multiple comparison posthoc test was used. PRA, extracted PAC, and ARR were not considered normally distributed because of some samples being below the limit of detection (PRA) and the presence of outliers. A Spearman's rank correlation was used to assess for any relationship between plasma potassium concentration and extracted aldosterone concentration either within the group of cats as a whole or more specifically within those nonazotemic cats that were diagnosed with systemic hypertension.

For paired data pre- and postamlodipine besylate therapy, a Kolmogorov-Smirnov test was used to establish whether the differences between pre- and posttreatment analyte values were normally distributed. If differences were normally distributed, the data were analysed using a paired student t-test. If differences were not normally distributed, the Wilcoxon signed rank test was used.

Results

One hundred and ninety-six cats were included in the study, of which 105 (53.6%) were female neutered, 85 (43.4%) male neutered, 3 (1.5%) were intact males, and 3 (1.5%) intact females. The median age of cats recruited to the study was 14.0 [25th percentile 11.6, 75th percentile 16.0] years. The majority of cats were domestic shorthair (n = 154, 78.5%) or longhair

(n = 15, 7.7%), with other breeds represented including Persian (n = 13), British Shorthair (n = 4), Burmese (n = 4), Siamese (n = 2), and 1 each of Birman, Chinchilla, Devon Rex and Bengal. One cat, which was nonazotemic and hypertensive, was documented to have hyperaldosteronism attributable to persistent hypokalemia, the presence of an adrenal mass on ultrasound examination, and marked hyperaldosteronism (617 pg/mL). This cat was excluded from further data analysis. Of the remaining cats, 39.5% (n = 77) were hypertensive with 76.6% of hypertensive cats demonstrating evidence of hypertensive retinopathy, choroidopathy, or both.

Measurement of Plasma Renin Activity

The mean measured PRA for pooled feline plasma were 0.50 ng/mL/h and 7.50 ng/mL/h. Intra-assay coefficients of variation (n = 6) for the low and high samples were 16.8% and 12.6%, respectively. Inter-assay CVs (n = 5) for the low and high samples were 22.5% and 20.9%, respectively. Dilutional parallelism was demonstrated. There was no significant difference ($P = 0.875$) in PRA whether samples (n = 4) were held on ice (4°C; 0.75 ng/mL/h [0.62, 0.96]) or at room temperature (21°C; 0.68 ng/mL/h [0.59, 1.00]) or whether samples (n = 4) were analyzed immediately ($P = 0.625$; 0.47 ng/mL/h [0.29, 2.15]) or frozen at −80°C for 40 days (0.48 ng/mL/h [0.29, 1.97]). The

upper limit of a reference interval generated from normal cats >9 years (mean + 2SD) was 1.52 ng/mL/h.

Measurement of Solvent-Extracted Aldosterone Concentration

The upper limit of reference interval defined as mean + 2SD for PAC after solvent extraction was 106.5 pg/mL. The mean PAC concentrations for the pooled samples used to assess assay variability were 104 and 564 pg/ml. Intra-assay CVs (n = 6) for the samples with low and high aldosterone concentration were 8.6 and 9.6%, respectively. Interassay CVs for samples with low and high aldosterone concentration were 10.3% (n = 5) and 20.1% (n = 6), respectively. Dilutional parallelism was demonstrated for the aldosterone RIA after extraction.

Cross-Sectional Analysis of RAAS in Cats with Variable Renal Function and Blood Pressure

Clinicopathologic data for cats grouped according to their renal and SBP status are presented in Table 1. The median storage time from sample collection until analysis of PAC and PRA was 351 days (148, 706). No significant difference in storage time was found when cats were grouped according to SBP and renal status ($P = .785$). PAC differed significantly between groups ($P < .001$, Fig 1A). Cats in the Azo-HT group demonstrated a significantly higher PAC (pg/mL,

Table 1. Clinicopathologic parameters for cats included in the cross-sectional study grouped according to blood pressure and renal status.

Parameter	n	Nonazotemic Normotensive	n	Nonazotemic Hypertensive	n	Azotemic Normotensive	n	Azotemic Hypertensive	P
		68		28		41		46	
	n	Median [25th, 75th percentile]	n	Median [25th, 75th percentile]	n	Median [25th, 75th percentile]	n	Median [25th, 75th percentile]	P
Age (years)	64	12.0[a] [11.0, 14.0]	27	15.3[b] [13.8, 16.0]	40	14.5[b] [11.4, 17.1]	42	15.2[b] [13.8, 17.0]	<.001
Weight (kg)	66	4.13 [3.44, 4.8]	24	3.78 [3.11, 4.55]	39	3.84 [3.10, 4.60]	39	4.00 [3.10, 4.80]	.300
Body condition (/9)	9	6.0 [4.0, 6.5]	5	4.0 [3.5, 4.5]	17	4.0 [4.0, 5.0]	9	5.0 [2.5, 5.0]	–
Body condition (/5)	18	3.5 [2.5, 4.0]	6	2.5 [1.5, 3.5]	4	2.0 [1.5, 2.5]	11	2.0 [1.5, 2.5]	–
Creatinine (mg/dL)	68	1.5[a] [1.3, 1.67]	28	1.6[a] [1.4, 1.8]	41	2.5[b] [2.2, 2.9]	46	2.2[b] [2.2, 2.9]	<.001
Urea (mmol/L)	68	9.7[a] [8.2, 10.7]	28	12.2[b] [11.2, 14.8]	41	17.9[c] [14.8, 20.6]	46	16.9[c] [13.9, 22.7]	<.001
Sodium (mEq/L)	68	151.6[a] [150.0, 153.0]	28	152.6[ab] [150.2, 154.0]	41	153.3[b] [151.5, 155.0]	46	153.0[ab] [151.0, 156.0]	.006
Potassium (mEq/L)	68	3.96 [3.60, 4.22]	28	3.82 [3.49, 4.09]	41	4.08 [3.80, 4.30]	46	3.89 [3.38, 4.21]	.162
Phosphate (mg/dL)	68	3.81[a] [3.34, 4.26]	28	4.30[ab] [3.58, 3.36]	41	4.37[b] [3.47, 5.76]	46	4.30[b] [3.73, 6.26]	.007
USG	62	1.050[a] [1.040, 1.065]	22	1.030[b] [1.022, 1.042]	35	1.020[c] [1.017, 1.024]	37	1.018[c] [1.014, 1.025]	<.001
UP:C	56	0.14[a] [0.11, 0.17]	14	0.24[bc] [0.19, 0.35]	26	0.15[ab] [0.10, 0.26]	33	0.37[c] [0.21, 0.84]	<.001
SBP (mmHg)	68	133.2[a] [114.3, 145.9]	28	200.1[b] [187.5, 211.0]	41	133.6[a] [120.8, 145.4]	46	189.1[b] [177.2, 216.0]	<.001

n, number of cats; USG, urine specific gravity; UP:C, urine protein-to-creatinine ratio; SBP, systolic blood pressure; P, significance; superscript letters identify groups, which differed significantly.
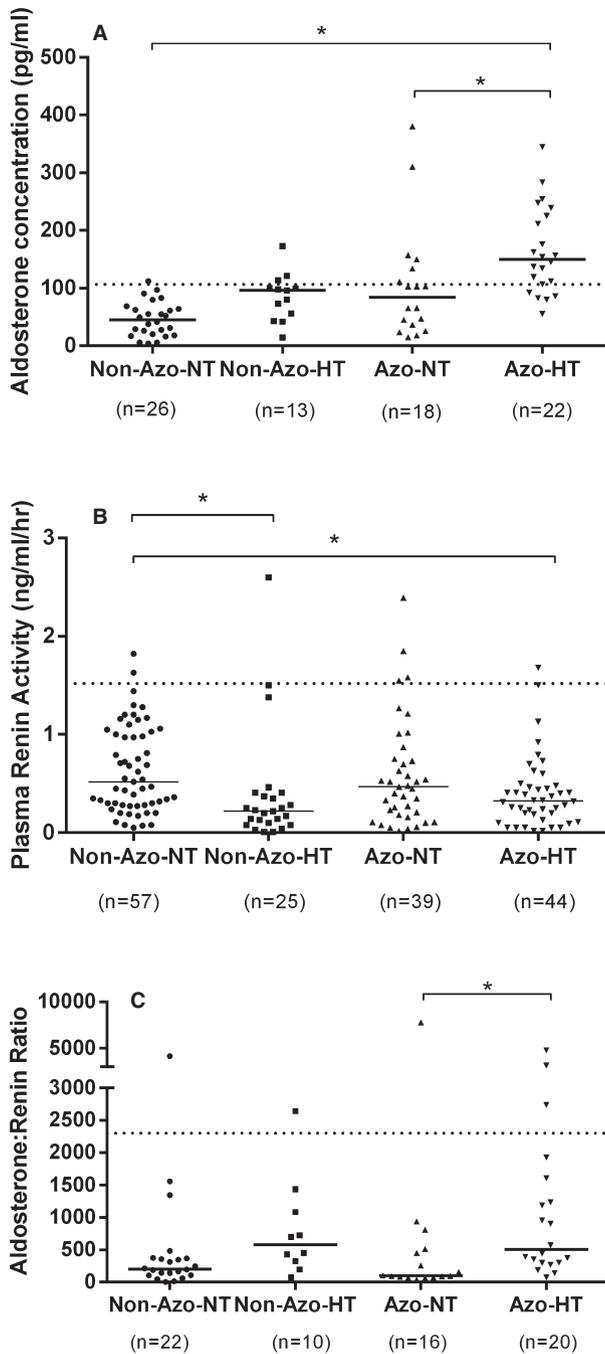
Fig 1. Comparison of extracted aldosterone concentration, plasma renin activity, and aldosterone-to-renin ratios in cats grouped according to renal and blood pressure status. Comparison of (A) extracted aldosterone concentration (PAC), (B) plasma renin activity (PRA), (C) aldosterone-to-renin ratio (ARR). Horizontal bars represent the median PAC, PRA, or ARR for each group. The dashed line represents the upper limit of the reference interval generated from the group of normal geriatric cats for each parameter. Non-Azo-NT; nonazotemic normotensive, Non-Azo-HT; nonazotemic cats with systemic hypertension, Azo-NT; azotemic cats normotensive, Azo-HT; azotemic cats with systemic hypertension. *indicates statistical significance ($P < .05$) was attained on posthoc analysis.

n = 22, 149.8 pg/mL [103.1, 228.7]) compared with normotensive cats with comparable renal function (Azo-NT n = 18, 84.1 pg/mL [33.6, 137.8]) and the Non-Azo-NT group (n = 26, 45.4 pg/mL [19.6, 65.0]). There was no significant difference in PAC between the Non-Azo-HT group (n = 13, 96.1 pg/mL [49.4, 106.8]) and the Non-Azo-NT group. PRA differed significantly between cats grouped according to SBP status ($P = .0013$, Fig 1B). Hypertensive cats in both the Non-Azo-HT (n = 25, 0.22 ng/mL/h [0.09, 0.39]) and Azo-HT group (n = 44, 0.33 ng/mL/h [0.15, 0.48]) demonstrated significantly lower PRA compared with Non-Azo-NT cats (n = 57, 0.52 ng/mL/h [0.28, 1.02]). There was no significant difference in PRA between Azo-HT cats and normotensive cats matched for renal function (Azo-NT; n = 39, 0.47 ng/mL/h [0.18, 0.75]). ARR also differed significantly between groups ($P = .0062$, Fig 1C). On posthoc analysis of Azo-HT cats (n = 20, 503.8 [298.8, 1511]) demonstrated significantly higher ARR compared with their normotensive counterparts (Azo-NT; n = 16, 97.8 [77.0, 496.4]), but there was no statistically significant difference between Non-Azo-NT (n = 22, 200.7 [106.0, 368.5]) and Non-Azo-HT cats (n = 10, 573.7 [290.9, 1170]). No significant correlation could be identified between plasma potassium concentration and extracted PAC either in the population of cats as a whole ($r = -0.096$, $P = .371$) or in those nonazotemic cats with systemic hypertension ($r = -0.22$, $P = .453$).

When cats were classified according to their renal status regardless of SBP, there were no significant differences in extracted PAC ($P = .917$; Fig 2A), PRA ($P = .903$; Fig 2B) or ARR ($P = .772$; Fig 2A) among groups. Data were also analyzed (data not shown) including only normotensive cats, but there were no significant differences among groups. Clinicopathologic data for cats grouped according to plasma creatinine concentration are presented in Table 2.

Twenty-three hypertensive cats were included in the comparison of PRA and PAC pre- and posttreatment with amlodipine besylate. Fourteen (60.9%) were azotemic before treatment. Clinicopathologic data for these cats at the pre- and posttime points are summarized in Table 3. As expected, a significant decrease in SBP was documented with a median decrease of 50.4 mmHg [33.3, 67.6]. There was no change in PAC (Fig 3A; $P = .345$, (n = 23) pre 122.8 pg/mL [92.3, 162.4], post 108.4 [57.1, 176.5]), but a significant increase in PRA (Fig 3B; $P < .001$ (n = 20), pre 0.32 ng/mL/h [0.15, 0.46], post 0.54 [0.28, 1.51]), and hence a significant decline in ARR ($P = .001$ (n = 20), 440.6 [229.0, 927.1], post 148.6 [70.2, 398.1]) was documented with antihypertensive therapy.

Discussion

Results of this study show that, in nonazotemic hypertensive cats, PRA is suppressed. PAC was significantly increased in azotemic hypertensive cats compared with normotensive cats matched for renal function. Based on these findings, it is speculated that
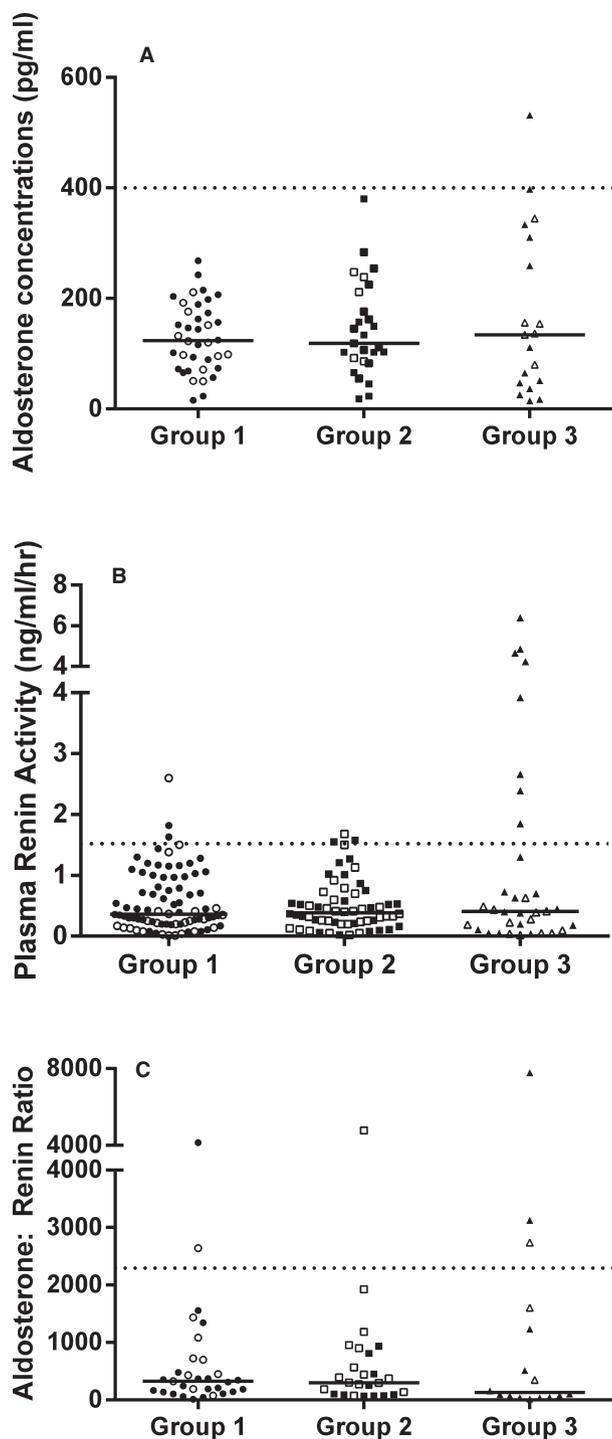
Fig 2. Comparison of extracted aldosterone concentration, plasma renin activity, and aldosterone-to-renin ratios in cats grouped according to renal status. Comparison of (A) extracted aldosterone (PAC; $P = .917$; Group 1 n = 39, Group 2 n = 28, Group 3 n = 20), (B) plasma renin activity (PRA; $P = .903$; Group 1 n = 82, Group 2 n = 60, Group 3 n = 33), or (C) aldosterone-to-renin ratio (ARR; $P = .772$; Group 1 n = 32, Group 2 n = 25, Group 3 n = 16) between groups. Horizontal bars represent the median PAC, PRA, or ARR. The dashed line represents the upper limit of the reference interval generated from the group of nonazotemic geriatric cats for each parameter. Open symbols represent hypertensive cats within each group.

cats may have a low renin/high PAC hypertension. However, heterogeneity is present such that, despite substantially larger numbers of cats, when compared to previous studies, uncertainty still exists as to whether this is the case for all cats.[4] Decrease in PRA may be the result of a combination of decreased renal mass, appropriate response to increased blood pressure or could be the consequence of age. Previous studies in cats have documented that PRA decreases with age, and cats in the nonazotemic normotensive group were significantly younger than those in other groups.[16] Concern is often raised regarding the diagnosis of hypertension in cats without evidence of azotemia and the potential for white-coat hypertension. Suppression of PRA in these cats suggests that systemic hypertension is likely to be a genuine finding.

PRA is an important parameter for classification of systemic hypertension in human medicine.[17] Hypertensive patients with low renin/high PAC can be classified as having low-renin essential hypertension or primary aldosteronism (PA).[17] Another group of hypertensive human patients that demonstrates suppressed renin activity includes people of African-American descent. Such patients are deemed to have 'salt-sensitive' hypertension because SBP fluctuates with variable sodium intake.[18]

Previous epidemiologic studies have shown that cats with systemic hypertension demonstrate significantly decreased plasma potassium concentrations in comparison with their normotensive counterparts.[1] This observation may reflect a relative increase in PAC. In this study, there was no significant difference in potassium concentration between hypertensive and normotensive cats with azotemia, but there was a small but significant difference between nonazotemic hypertensive and normotensive cats. However, no association could be identified between plasma potassium concentration and PAC. Failure to demonstrate an association between systemic hypertension and hypokalemia in this study may reflect sample sizes. Approximately 25% of cats had plasma potassium concentrations below the laboratory reference interval (3.5–5.5 mEq/L) and this was true regardless of group. However, the results obtained were not unexpected for cats with CKD, and no cats had overt clinical signs of hypokalemic polymyopathy.[19]

Several case series have described cats with hyperaldosteronism secondary to adrenal neoplasia.[20–23] Such cats usually demonstrate PAC substantially higher than cats in this study, and yet not all of them will have systemic hypertension. It therefore seems unlikely that increased PAC is the only contributor to the development of hypertension. In this study, increased PAC did not appear to be driven by an increase in systemic PRA and therefore could be primary and adrenal-dependent. A recent study suggests that bilateral adrenocortical hyperplasia is an almost ubiquitous finding in cats >9 years (97%), but there was no significant difference in histopathologic grading between hypertensive (n = 37) and normotensive cats (n = 30), supporting that adrenocortical hyperplasia is unlikely to be a primary factor in feline hypertension.[m] A number of

Table 2. Clinicopathologic parameters for cats grouped according to renal status.

Parameter		Group 1		Group 2		Group 3	
	n	96		62		37	
	n	Median [25th, 75th percentile]	n	Median [25th, 75th percentile]	n	Median [25th, 75th percentile]	P
Age (years)	91	13.0[a] [11.0, 15.0]	58	15.0[b] [12.8, 17.0]	34	14.2[ab] [11.8, 17.0]	.001
Weight (kg)	90	4.0[a] [3.33, 4.70]	56	4.04[a] [3.41, 4.82]	32	3.24[b] [3.00, 3.96]	<.001
Creatinine (mg/dL)	96	1.5[a] [1.3, 1.7]	62	2.4[b] [2.2, 2.7]	37	3.7[c] [3.0, 5.27]	<.001
Urea (mmol/L)	96	10.2[a] [8.7, 12.1]	62	16.4[b] [13.1, 19.0]	37	26.2[c] [20.7, 46.2]	<.001
Sodium (mEq/L)	96	152.0[a] [150.0, 153.6]	62	153.0[b] [151.0, 154.5]	37	154.0[b] [151.0, 156.7]	.003
Potassium (mEq/L)	96	3.90 [3.60, 4.20]	62	4.02 [3.69, 4.30]	37	3.96 [3.68, 4.28]	.438
Phosphate (mg/dL)	96	3.93[a] [3.41, 4.57]	62	4.18[a] [3.49, 5.25]	37	6.71[b] [4.54, 10.45]	<.001
USG	84	1.044[a] [1.033, 1.060]	50	1.020[b] [1.018, 1.026]	30	1.016[c] [1.014, 1.018]	<.001
UP:C	70	0.16[a] [0.12, 0.20]	38	0.23[b] [0.13, 0.41]	31	0.45[c] [0.15, 0.89]	<.001
SBP (mmHg)	96	142.2[a] [127.1, 184.0]	62	173.2[b] [132.8, 207.2]	37	155.6[ab] [134.0, 184.2]	.03

Group 1: nonazotemic cats with plasma creatinine <2.0 mg/dL; Group 2: azotemic cats with plasma creatinine 2–2.8 mg/dL; Group 3: azotemic cats with plasma creatinine ≥2.9 mg/dL; n, number of cats; USG, urine specific gravity; UP/C, urine protein-to-creatinine ratio; SBP, systolic blood pressure; P, significance; superscript letters identify groups, which differed significantly.

Table 3. Clinicopathologic parameters for cats before and after treatment with amlodipine besylate.

Parameter	n	Pretreatment Median [25th, 75th percentile]	n	Posttreatment Median [25th, 75th percentile]	P
SBP (mmHg)	23	193.6 [184.0, 213.6]	23	148.0 [139.2, 156.4]	<.001
Creatinine (mg/dL)	23	2.2 [1.6, 2.5]	23	1.8 [1.6, 2.4]	.110
Urea (mmol/L)	23	13.6 [11.7, 20.3]	23	13.7 [11.6, 18.0]	.486
Sodium (mEq/L)	23	153.0 [151.0, 156.0]	23	152.0 [149.0, 153.0]	.025
Potassium (mEq/L)	23	3.68 [3.46, 4.27]	23	3.57 [3.40, 3.87]	.251
Phosphorus (mg/dL)	23	4.1 [3.4, 4.6]	23	4.1 [3.7, 5.1]	.441
UP:C	19	0.29 [0.21, 0.43]	19	0.23 [0.15, 0.44]	.454
USG	19	1.026 [1.020, 1.033]	19	1.027 [1.018, 1.036]	.313

n, number of cats; USG, urine specific gravity; UP/C, urine protein-to-creatinine ratio; SBP, systolic blood pressure; P, significance value comparing pre- and posttreatment.

other factors have been demonstrated to modulate aldosterone production apart from angiotensin II including extracellular potassium concentration, adrenocorticotropic hormone, adrenomedullin, atrial natriuretic peptide, dopamine, endogenous digitalis-like factors (eg, ouabain) and endothelin-1, but the effect these play in regulating aldosterone production in the cat has not been investigated.[24–29]

An alternative hypothesis is that cats may demonstrate low-renin hypertension similar to the situation identified in hypertensive black human patients. The pathophysiologic mechanism underlying low-renin activity in hypertensive black patients is uncertain, but most likely reflects differences in renal handling of sodium among ethnic groups and a tendency for renal retention of sodium.[18] Studies suggest that hypertensive black patients have significantly lower PRA than their white counterparts and demonstrate a disproportionately increased PAC.[30–32] Such patients have a greater propensity to target organ damage and demonstrate a better response to antihypertensive agents such as calcium channel blockers and diuretics than those targeting the RAAS.[33,34] However, the role of

activation of the RAAS in salt-sensitive hypertension in black human patients is currently debated.[35] Similar to hypertensive black patients, cats demonstrate a limited antihypertensive response to ACEi therapy, but respond well to calcium channel blockade.[7,12] However, studies that have investigated the effect of sodium intake on SBP and renal function in cats have not reported an association.[36–39] Additional work is warranted to evaluate salt sensitivity in naturally occurring hypertension in cats and the role of angiotensinogen in cats with variable BP and renal function.

In the cross-sectional analysis, when cats were grouped according to renal function, although there was no significant difference in PRA or PAC among groups, the variance in PRA increased with severity of azotemia. Reviewing the data from individual cats, those cats with the highest PRA were also more markedly azotemic. In these cats, it can be hypothesized that increased PRA is an appropriate response to dehydration and decrease renal perfusion, even if this was not detected clinically.

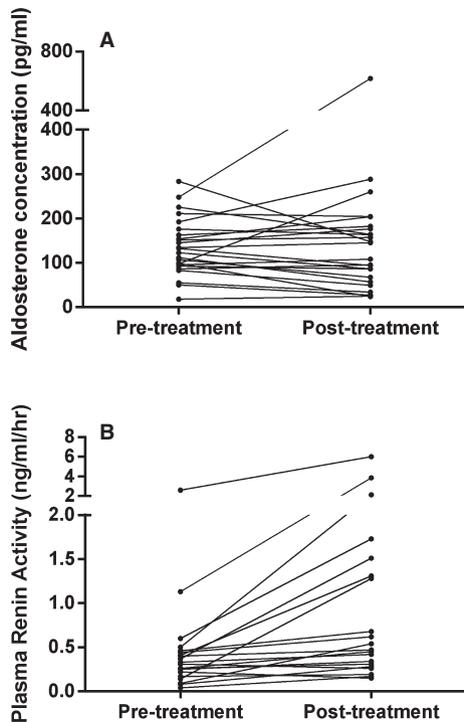Cats treated with the dihydropyridine calcium channel blocker, amlodipine besylate, showed a significant

Fig 3. (A) Line chart demonstrating change in plasma aldosterone concentration before and after treatment with amlodipine besylate. No significant change in plasma aldosterone concentration was demonstrated with amlodipine besylate therapy ($P = .584$; n = 24). Median time between measurements 35 days (25th percentile 14, 75th percentile 56). (B) Line chart demonstrating change in plasma renin activity before and after treatment with amlodipine besylate. A significant increase ($P < .001$; n = 20) in plasma renin activity was demonstrated with amlodipine besylate therapy. Median time between measurements 28 days (25th percentile 14, 75th percentile 56).

increase in PRA but no change in PAC. Previous studies in human patients receiving nifedipine and amlodipine have demonstrated a significant increase in PRA most likely because of decreased BP and sympathetic stimulation, but effects on PAC have been conflicting.[40–43] The response of PRA and hence ARR to amlodipine treatment in cats appears appropriate and comparable to that in humans.

There are a number of limitations to this study. The PRA assay involves an initial incubation phase to generate Ang-1 and is dependent on angiotensinogen as a substrate and enzymatic kinetics. Variable concentrations of angiotensinogen in individual samples could restrict generation of Ang-1 despite low, normal, or increased PRA. Previous studies have documented cryoactivation of prorenin to renin when human plasma is held at temperatures <6°C.[44,45] Interspecies differences in cryoactivation of prorenin have been documented.[45–47] Although sample numbers were low (n = 4), failure to identify a difference in PRA between samples stored at 4 and 21°C supports the hypothesis that cryoactivation of prorenin in the cat is minimal or that there is lack of a substantial quantity of prorenin present in feline plasma to be activated.[48] The inter- and intra-assay

CVs for the PRA assay were higher than ideal in this study and it is possible that using a smaller volume of plasma than advocated by the manufacturer contributed to these results. However, this was a necessity given the residual volume of sample available from feline clinical cases.

Plasma aldosterone concentrations have previously been measured by either direct RIA or with solvent extraction before RIA.[4,13,16,49] In human medicine, polar metabolites of aldosterone are known to interfere with PAC quantitation and increase with decreased renal function.[15,50] No studies have evaluated the influence of polar metabolites on PAC in cats. A recent study by Syme and colleagues demonstrated that concentrations of aldosterone excreted in feline urine were substantially lower than reported in human urine and that, unlike dogs and humans, cats do not excrete glucuronidated polar metabolites of aldosterone in their urine.[51] As such, it is possible that accumulation of polar metabolites of aldosterone is of lesser concern in the cat, but the definitive pathway of aldosterone metabolism and route of excretion in the cat remain to be determined.

Substantially more PRA measurements were available than PAC measurements. In fact, missing PAC data were available for many of the cats in this study but had been performed using direct assessment of PAC. Given the different methodology and reference intervals, it was not considered appropriate to combine these data. There was no significant difference in storage times among cats grouped according to SBP and renal status. Previous validation work from studies in humans demonstrates stability of PRA in plasma samples frozen for up to 15 months and steroid hormones for between 1 and 10 years[52,53] Although it cannot be precluded that prolonged storage has impacted the PRA and PAC measurements, the distribution of this effect among groups should remain comparable.

Cats in the Non-Azo-HT group had significantly lower USG and higher UP:C than those in the Non-Azo-NT group. Whether this reflects the presence of nonazotemic kidney disease or pressure diuresis remains uncertain. Assessment of glomerular filtration rate (GFR) could be useful to assist in clarifying this, but still would not exclude the possibility of hyperfiltration occurring in a decreased number of nephrons. Changes in sodium intake can influence volume status and BP, although evidence to support this in cats is limited.[38,39] In this study, the majority of cats were eating commercially produced diets, although the brands varied widely. Therefore, sodium intake may have varied, but consumption of renal diets (which are often low in sodium) was an exclusion criterion.

At the visit when PRA and PAC were assessed, total thyroxine concentrations were available for 47.4% (93/196) of cats and confirmed euthyroid status. A recent study by Williams and colleagues demonstrated that PRA and PAC significantly decreased with treatment of hyperthyroidism in normotensive

cats, but that PAC did not change significantly in those cats that became hypertensive with treatment.[54] Occult hyperthyroidism in the current cohort of cats could therefore have impacted the PRA and PAC results. Whenever possible, urine samples were obtained from cats. However, because of financial constraints, it was not routine for urine culture to be performed unless an active sediment, bacteriuria, or both were evident on urinalysis. It is possible that renal scarring because of recurrent episodes of chronic pyelonephritis influenced the kidney's ability to respond to the RAAS. Within the limits of this study protocol, occult urinary tract infections or episodes of pyelonephritis may not have been identified. Finally, cats did not undergo routine diagnostic imaging and it is therefore possible that adrenal mass lesions may have gone undetected. The study by Keele and colleagues examining adrenal gland histopathology from cats >9 years reported a prevalence of only 3% (2/67) for adrenal adenomas.[m] Given that this study was performed using a tissue archive from the authors' group, it can be anticipated that the prevalence would be similar.

In conclusion, on a population basis, azotemic hypertensive cats showed significantly increased PAC and ARR independent of PRA. Nonazotemic hypertensive cats showed significantly suppressed PRA. However, there was substantial overlap among all groups of cats such that, despite larger numbers of cats in this study, it remains difficult to fully establish the role of the RAAS that is playing in the genesis of hypertension in cats. Additional work is required to establish whether suppressed PRA and relative increase in PAC reflects autonomous aldosterone production or if the cat represents a potential model for low-renin hypertension identified in hypertensive black humans.

Footnotes

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