Vasopressin, cortisol, and catecholamine concentrations in dogs with dilated cardiomyopathy

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Objective—To evaluate plasma concentrations and urinary excretion of vasopressin and cortisol and urinary excretion of catecholamines in dogs with dilated cardiomyopathy (DCM).

Animals—15 dogs with clinical signs of DCM, 15 dogs with preclinical DCM, and 15 control dogs.

Procedure—Physical examinations, thoracic radiography, ECG, and echocardiography were performed on all dogs. Blood and urine samples were collected.

Results—Plasma concentration of vasopressin and the urine cortisol-to-urine creatinine ratio were significantly increased in dogs with clinical signs of DCM and dogs with preclinical DCM, compared with control dogs. Plasma vasopressin concentration was significantly higher in dogs with clinical signs of DCM, compared with dogs with preclinical DCM. Urine vasopressin-to-urine creatinine ratio was significantly increased in dogs with clinical signs of DCM, compared with dogs with preclinical DCM and control dogs. Urine epinephrine-to-urine creatinine ratio and urine norepinephrine-to-urine creatinine ratio were significantly increased in dogs with clinical signs of DCM, compared with control dogs. Plasma concentration of cortisol and urine dopamine-to-urine creatinine ratio did not differ significantly among groups.

Conclusions and Clinical Relevance—According to this study, the neuroendocrine pattern is changed in dogs with preclinical DCM. These changes are even more pronounced in dogs with clinical signs of DCM. Analysis of concentrations of vasopressin, cortisol, and catecholamines may aid in identification of the clinical stages of DCM. These findings may also provide a basis for additional studies of the possible beneficial effects of vasopressin antagonists and β-adrenergic receptor antagonists in the treatment of dogs with congestive heart failure and DCM. (*Am J Vet Res* 2005;66:1709–1717)

Dilated cardiomyopathy (DCM) is one of the most commonly diagnosed heart diseases in dogs.[1,2] It has been reported in dogs of many medium and large breeds.[3] Dilated cardiomyopathy was first reported in dogs as congestive heart failure in conjunction with dilatation of the cardiac chambers and a lack of other clinically important cardiovascular disease.[a] Since the advent of echocardiography, myocardial hypokinesis measured as low values for fractional shortening and dilatation of the left atrium and left ventricle (ie, eccentric hypertrophy) without other detectable cardiac abnormalities have been regarded as diagnostic criteria for DCM.[2,4] Echocardiographic evidence of hypertrophy of the left ventricle attributable to volume overload and hypokinesis without clinical signs of congestive heart failure may, in animals lacking other cardiovascular disease, be classified as preclinical DCM.[5,b,c]

Cortisol and catecholamines are recognized as indicators of the degree of stress in healthy dogs and people, and the actions of these hormones have been extensively investigated.[6,7] Vasopressin has traditionally been regarded to be primarily involved in osmoregulation and regulation of the extracellular fluid volume through its renal effects and, at higher plasma concentrations, in mediating vasoconstriction in response to decreases in blood pressure. It has been suggested[8] that vasopressin may also be an indicator of stress in dogs, goats, and cattle.

Heart failure leads to activation of several endocrine compensatory mechanisms. As activity of the renin-angiotensin-aldosterone system and sympathetic nervous system increases, plasma concentrations of vasopressin increase.[9,10] The sympathetic nervous system provides inotropic and chronotropic support to the heart and causes systemic vasoconstriction and renin release. Vasopressin and the renin-angiotensin-aldosterone system induce strong vasoconstrictor responses and retention of sodium and water. These changes initially provide support for the failing heart and circulation by their peripheral vasoconstrictive and blood volume–expanding effects, but they are considered to be detrimental over long periods because they increase the workload of a failing heart.[9] Actions of the substances that promote vasocontriction and fluid retention are counteracted by substances that promote vasodilatation and fluid excretion, such as the natriuretic peptides. Therefore, equilibrium is maintained. Decompensated heart failure is characterized by an imbalance of this equilibrium.[11]

Catecholamines and many other vasoactive hormones have been extensively studied in animals with induced heart failure, including dogs.[12-14] However, experimental situations are not identical to naturally developing heart failure, and less is known about the hormonal balance in the latter. Timing of events for the vasoactive hormones is of particular interest because it may yield a better understanding of the pathophysiologic processes of

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heart failure and may suggest that clinical trials would be worthwhile. A study[15] of neurohormones in dogs with DCM revealed that plasma catecholamine concentrations are already increased and β-adrenergic receptors are downregulated during the preclinical phase in dogs with DCM. On the contrary, in a study[16] conducted by our laboratory group, we reported minor changes in the activity of the renin-angiotensin-aldosterone system and **atrial natriuretic peptide (ANP)** activity in dogs with preclinical DCM, whereas substantial changes were found in dogs with clinical signs of DCM. Accordingly, it has been suggested that DCM is characterized by an abnormally high adrenergic drive that may have consequences for progression of the disease. However, it is not known whether the abnormal adrenergic drive involves only the sympathetic nervous system or is a consequence of a more generalized state of stress. We hypothesized that vasopressin and cortisol concentrations would be increased in concert with increased adrenergic activity, indicating that changes may already be evident during the preclinical phase in dogs with DCM. The purpose of the study reported here was to investigate plasma concentrations and urinary excretion of vasopressin and cortisol and urinary excretion of catecholamines in dogs with naturally developing DCM and clinical signs of the disease and dogs with preclinical DCM.

Materials and Methods

Animals—Forty-five client-owned dogs representing 17 medium and large breeds were included in the study. Dogs were allocated to 1 of 3 groups on the basis of defined criteria. Group 1 consisted of 15 dogs with clinical signs of DCM (ie, fractional shortening < 25%)[17] as determined by use of M-mode echocardiography, a lack of echocardiographic lesions other than chamber dilatation (eccentric hypertrophy) detected during 2-dimensional echocardiography, and radiographic evidence of left-sided or biventricular cardiac enlargement in association with pulmonary edema or pleural effusion. Group 2 consisted of 15 dogs with DCM as determined on the basis of the aforementioned echocardiographic criteria used for dogs of group 1, but dogs of group 2 did not have clinical or radiographic evidence of congestive heart failure. Group 3 consisted of 15 clinically normal control dogs (ie, no abnormalities were detected during clinical, echocardiographic, or radiographic examinations). Control dogs were similar to dogs in the other 2 groups with respect to age, sex, and breed. None of the dogs received any treatments or were fed a sodium-restricted diet before entering the study. All examinations were conducted and samples (blood and urine) collected between 10 AM and 4 PM. Conduct of the study was approved by the Local Ethical Committee in Uppsala, Sweden, and consent was obtained from all owners for use of their dogs in the study.

Echocardiography—Two-dimensional and M-mode echocardiography were performed by use of a 5-MHz transducer[d] placed on the right precordium. Dogs were positioned in right lateral recumbency on a table that enabled dogs to be scanned from the dependent side of the thorax. Echocardiograms were recorded and analyzed in accordance with recommendations of the American Society of Echocardiography[18] and the Echocardiographic Committee of the Specialty of Cardiology, American College of Veterinary Internal Medicine.[19]

Echocardiographic measurements of left atrial and left ventricular dimensions were indexed in accordance with a modified method described elsewhere.[20,21] Cardiac dimensions and body weight were measured and recorded. To index cardiac dimensions, several variables were calculated by use of the following equations: LVEDD index = $LVEDD/BW^{0.32}$, where LVEDD is the left ventricular end-diastolic diameter and BW is body weight; LVESD index = $LVESD/BW^{0.41}$, where LVESD is the left ventricular end-systolic diameter; LA index = $LA/BW^{0.30}$, where LA is the left atrial diameter; and Ao index = $Ao/BW^{0.35}$, where Ao is the aortic root diameter. All echocardiographic recordings were reevaluated by a single cardiologist (JH) who was unaware of the group assignment for each dog.

Radiography—Thoracic radiography by use of 2 orthogonal views was performed in all dogs. All radiographs were evaluated for heart size, signs of pulmonary congestion and edema, and pleural effusion.[22] Radiographs were evaluated by a single radiologist (KH) who was unaware of the group assignment for each dog.

ECG—A standard 6-lead ECG was recorded for each dog. The ECGs were analyzed by use of standard ECG criteria for dogs.[23] All ECG recordings were analyzed by a single cardiologist (JH) who was unaware of the group assignment for each dog.

Collection of blood and urine samples—Blood samples were collected via direct venipuncture of a cephalic vein into chilled, silicon-coated evacuated tubes containing various additives. Samples were collected into tubes in the following order: a 10-mL, K3-EDTA tube[e] containing 0.5 mL of 10,000 kallikrein-inactivator U/mL of aprotinin[e] for cortisol and vasopressin analyses; a 5-mL tube containing aprotinin[f] for use in cortisol and vasopressin analysis; and a 5-mL lithium-heparin tube[g] for use in sodium, potassium, and osmolarity analysis. Thus, 20 mL of blood was collected from each dog. Blood samples were centrifuged (15,000 × *g* for 15 minutes) within 15 minutes after collection. Plasma was then harvested, placed in polyethylene tubes, and frozen initially at –20°C. The samples were then stored at –85°C until assayed.

Urine was collected from each dog by midstream catch during natural voiding and placed into plain silicon-coated evacuated tubes.[h] A small portion of the urine sample was removed for creatinine analysis, and the remainder was initially frozen at –20°C and then stored at –85°C until assayed.

All urinary and plasma samples were grouped into batches and analyzed in the same assay. All assays were conducted within 1 year after samples were frozen at –85°C. Long-term storage (> 1 year) at –85°C causes only minor degradation of cortisol[24] and vasopressin[25] in plasma samples and vasopressin,[26] cortisol,[24] and catecholamines[27] in urine samples.

Analysis of plasma and urine concentrations of vasopressin and cortisol—Plasma and urine concentrations of cortisol and vasopressin were measured by use of radioimmunoassays.[i,j] These assays were validated in our laboratory for use in canine plasma and urine samples on the basis of parallelism and recovery. Plasma and urine cortisol concentrations were measured without extraction. Before vasopressin was analyzed, the plasma was extracted with acetone[k] and petroleum benzene[l] with a recovery of 92%. Urine concentrations of vasopressin were measured on unextracted samples. The intra-assay **coefficient of variation (CV)** for the cortisol assay ranged between 3% and 14.5%, whereas the interassay CV for the cortisol assay was < 12.5%. Intra-assay CV for the vasopressin assay ranged between 6.5% and 25%,

whereas the interassay CV for the vasopressin assay was < 15%. The limit of detection was 6.5 nmol/L for the cortisol assay and 1 pmol/L for the vasopressin assay.

Analysis of urine concentrations of vasopressin, epinephrine, norepinephrine, and dopamine—Urine catecholamine concentrations were measured by use of ion-exchange liquid chromatography with electrochemical detection, as described elsewhere.[28] Purification of the urine samples was performed by use of C18-columns.[m] Catecholamines were then separated by use of cation-exchange, high-performance liquid chromatography columns.[n] Catecholamines were detected by use of an electrochemical detector.[o] Lower limit of detection for this method was 25 nmol/L for norepinephrine and 10 nmol/L for epinephrine. The intra-assay CV for norepinephrine, epinephrine, and dopamine ranged between 1.2% and 1.4%, 1.6% and 2.3%, and 1.4% and 1.6%, respectively. The interassay CV ranged between 5.3% and 5.5% for norepinephrine, 7.5% and 8.0% for epinephrine, and 5.5% and 6.5% for dopamine.

Other blood and urine biochemical analyses—Urine and plasma concentrations of creatinine were measured by use of commercially available kits,[p,q] as described elsewhere.[29] Sodium and potassium concentrations were measured in plasma and urine by use of ion-selective electrodes.[r] Osmolarity was determined in plasma and urine by measuring freezing point depression.[s]

Statistical analyses—All statistical calculations were performed by use of a computerized statistical program.[t] Differences among groups for categoric data were determined by use of the χ^2 test. The Fisher exact test was used when the sample size was < 5. A 1-way ANOVA was applied for multiple comparisons among groups for normally distributed data sets. When significant differences were detected among groups, a multiple comparison *t* test (ie, Tukey-Kramer test) was used to determine significant differences. Equal variance among groups was tested by use of the *F* test (ie, variance ratio test). Logarithmic transformation was conducted to correct for unequal variances among the 3 groups for serum concentrations of vasopressin and cortisol and the urine epinephrine-to-urine creatinine ratio. For urine norepinephrine-to-urine creatinine ratios, urine dopamine-to-urine creatinine ratios, and plasma sodium concentrations, the difference in distributions among the 3 groups could not be corrected by logarithmic transformation and nonparametric methods (eg, the Wilcoxon rank sum test) were applied. Linear regression analysis was used to compare continuous variables, and the Spearman correlation coefficient was used as a measure of association between 2 variables. The distribution of residuals in the regression analysis was tested for normality by use of the Shapiro-Wilk W test. Significance was defined as values of $P < 0.05$ (2-tailed test). Data were reported as the arithmetic mean ± SD.

Results

Animals—Significant differences were not detected among the 3 groups of dogs (ie, dogs with clinical signs of DCM, dogs with preclinical DCM, and control dogs) with regard to breed, age, sex, or body weight (**Table 1**). Twenty-six of 45 (58%) dogs were male (7 males and 8 females in the control group, 9 male and 6 female dogs with preclinical DCM, and 10 male and 5 female dogs with clinical signs of DCM). Dogs ranged from 2.4 to 11.9 years of age (mean ± SD, 7 ± 2.6 years). Body weight ranged from 12 to 70 kg (mean, 35.8 ± 14 kg).

Results of ECG—Heart rate was significantly increased in dogs with clinical signs of DCM, compared with the heart rate in dogs with preclinical DCM and control dogs (Table 1). Results of ECG included a normal sinus rhythm in 21 dogs (1 dog with clinical signs of DCM, 5 dogs with preclinical DCM, and 15 control dogs), sinus tachycardia in 11 dogs (10 with clinical signs of DCM and 1 with preclinical DCM), atrial fibrillation in 9 dogs (4 with clinical signs of DCM and 5 with preclinical DCM), ventricular premature depolarization in 3 dogs with preclinical DCM, and sinus bradycardia in 1 dog with preclinical DCM.

Plasma concentrations of vasopressin and cortisol—Plasma vasopressin concentration was significantly increased in dogs with clinical signs of DCM and dogs with preclinical DCM, compared with the concentration in control dogs (**Table 2**). Vasopressin concentration was significantly higher in dogs with clinical signs of DCM, compared with the concentration in dogs with preclinical DCM. Plasma cortisol concentrations did not differ significantly among the 3 groups of dogs.

Urine hormone concentrations—To compensate for differences in urine concentrations, hormonal values were expressed as a ratio, with the urine creatinine concentration as the denominator. Urine vasopressin-to-urine creatinine ratio was significantly increased in dogs with preclinical DCM and dogs with clinical signs of DCM, compared with the ratio for control dogs. Urine norepinephrine-to-urine creatinine ratio was sig-

Table 1—Mean ± SD age, body weight, heart rate, and indexed echocardiographic variables for 15 clinically normal dogs (control dogs), 15 dogs with dilated cardiomyopathy (DCM) without clinical signs of DCM (dogs with preclinical DCM), and 15 dogs with clinical signs of DCM.

Variable	Control dogs	Dogs with preclinical DCM	Dogs with clinical signs of DCM
Age (y)	6.5 ± 2.6	7.4 ± 2.5	7.2 ± 2.8
Body weight (kg)	33.6 ± 14.1	39.2 ± 14.9	34.8 ± 13.2
Heart rate (beats/min)	95 ± 16[a]	126 ± 48[a]	183 ± 49[b]
LVEDD index*	14.6 ± 1.1[a]	18.2 ± 2.2[b]	21.8 ± 2.4[c]
LVESD index*	7.3 ± 1.1[a]	11.1 ± 2.0[b]	13.2 ± 2.5[c]
FS (%)	32.0 ± 4.4[a]	16.1 ± 4.1[b]	13.0 ± 5.7[b]
LA index*	9.2 ± 0.9[a]	11.2 ± 2.0[a]	15.3 ± 3.2[b]
Ao index*	7.6 ± 0.8[a]	7.8 ± 1.1[a]	6.4 ± 0.9[b]
LA-to-Ao ratio	1.02 ± 0.08[a]	1.23 ± 0.31[a]	2.06 ± 0.71[b]

*Heart diameters were indexed as described elsewhere.[15]
[a–c]Within a row, values with different superscript letters differ significantly ($P < 0.05$).
LVEDD = Left ventricular end-diastolic diameter. LVESD = Left ventricular end-systolic diameter. FS = Fractional shortening. LA = Left atrium. Ao = Aortic root diameter.

Table 2—Mean ± SD values for various neurohumoral and biochemical variables in the plasma and urine of 15 clinically normal dogs (control dogs), 15 dogs with preclinical DCM, and 15 dogs with clinical signs of DCM.

Variable	Control dogs	Dogs with preclinical DCM	Dogs with clinical signs of DCM
Plasma			
Vasopressin (pmol/L)	0.79 ± 0.82[a]	1.43 ± 0.50[b]	3.47 ± 2.97[c]
Cortisol (nmol/L)	94.8 ± 81.4	102.0 ± 91.8	137.9 ± 110.8
Sodium (mmol/L)	146.4 ± 1.6	149.1 ± 4.8	148.0 ± 2.3
Potassium (mmol/L)	4.60 ± 0.59	4.30 ± 0.41	4.4 ± 0.45
Potassium-to-sodium ratio (mmol/mmol)	0.15 ± 0.37	0.25 ± 0.45	0.18 ± 0.37
Osmolarity (mOsm/L)	307 ± 3	306 ± 6	308 ± 6
Urine			
Urine vasopressin-to-urine creatinine ratio (pmol/mmol)	0.24 ± 0.36[a]	0.62 ± 0.57[a]	1.20 ± 0.30[b]
Specific gravity	1.03 ± 0.01	1.02 ± 0.01	1.02 ± 0.01
Creatinine (µmol/L)	17,523 ± 11,358	10,725 ± 7,011	9,262 ± 4,757
Urine cortisol-to-urine creatinine ratio (nmol/mmol)	0.38 ± 0.46[a]	0.93 ± 0.57[b]	1.32 ± 0.39[b]
Urine sodium-to-urine creatinine ratio (mmol/mmol)	6.3 ± 7.7	6.7 ± 6.3	7.4 ± 4.3
Urine potassium-to-urine creatinine ratio (mmol/µmol)	6.5 ± 3.8	7.8 ± 5.0	11.0 ± 6.6
Osmolarity (mOsm/L)	945 ± 606	709 ± 412	971 ± 478
Urine norepinephrine-to-urine creatinine ratio (nmol/mmol)	0.59 ± 0.25[a]	1.10 ± 0.47[b]	1.65 ± 0.20[b]
Urine epinephrine-to-urine creatinine ratio (nmol/mmol)	59.13 ± 76.53[a]	71.00 ± 78.00[a]	93.62 ± 56.81[b]
Urine dopamine-to-urine creatinine ratio (nmol/mmol)	0.97 ± 0.35	1.21 ± 0.26	1.21 ± 0.29

See Table 1 for remainder of key.

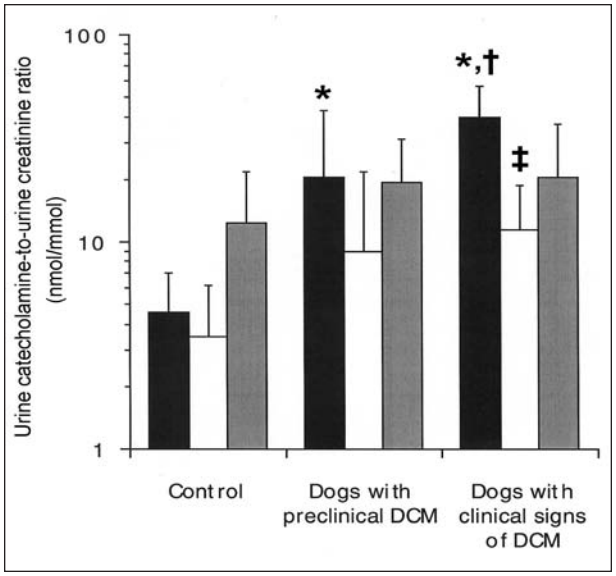

Figure 1—Mean ± SD values for urine norepinephrine-to-urine creatinine ratios (black bars), urine epinephrine-to-urine creatinine ratios (white bars), and urine dopamine-to-urine creatinine ratios (gray bars) for 15 control dogs, 15 dogs with preclinical (DCM), and 15 dogs with clinical signs of DCM. *Within the urine norepinephrine-to-urine creatinine ratios, value differs significantly ($P = 0.01$) from the value for the control dogs. †Within the urine norepinephrine-to-urine creatinine ratios, value differs significantly ($P = 0.01$) from the value for the dogs with preclinical DCM. ‡Within the urine dopamine-to-urine creatinine ratios, value differs significantly ($P = 0.01$) from the value for the control dogs.

nificantly increased in dogs with clinical signs of DCM, compared with the ratio for dogs with preclinical DCM and control dogs. Urine epinephrine-to-urine creatinine ratio was significantly increased in dogs with clinical signs of DCM, compared with the ratio for control dogs. Mean urine norepinephrine-to-urine creatinine ratio and urine epinephrine-to-urine creatinine ratio for the dogs with preclinical DCM were between the mean ratios for the control dogs and dogs with clinical signs of DCM; however, ratios for the dogs with preclinical DCM did not differ significantly from the ratios for the other 2 groups (**Figure 1**).

Urine cortisol-to-urine creatinine ratio was significantly increased in dogs with clinical signs of DCM and dogs with preclinical DCM, compared with the ratio for control dogs. However, although the ratio was higher in the dogs with clinical signs of DCM, the ratios did not differ significantly between dogs with clinical signs of DCM and dogs with preclinical DCM. Urine dopamine-to-urine creatinine ratios did not differ significantly among groups.

Other blood and urine biochemical analyses—Plasma concentrations of sodium and potassium, plasma osmolarity, and plasma sodium-to-plasma potassium ratio did not differ significantly among groups (Table 2). Similarly, urine osmolarity, urine potassium-to-urine creatinine ratio, and urine sodium-to-urine creatinine ratio did not differ significantly among groups.

Bivariate analysis—Plasma concentrations of vasopressin and cortisol; urine concentrations of vasopressin, cortisol, norepinephrine, and epinephrine; and urine hormone-to-urine creatinine ratios were significantly correlated (**Figure 2**; **Table 3**). The urine dopamine-to-urine creatinine ratio was significantly correlated with the urine norepinephrine-to-urine creatinine ratio.

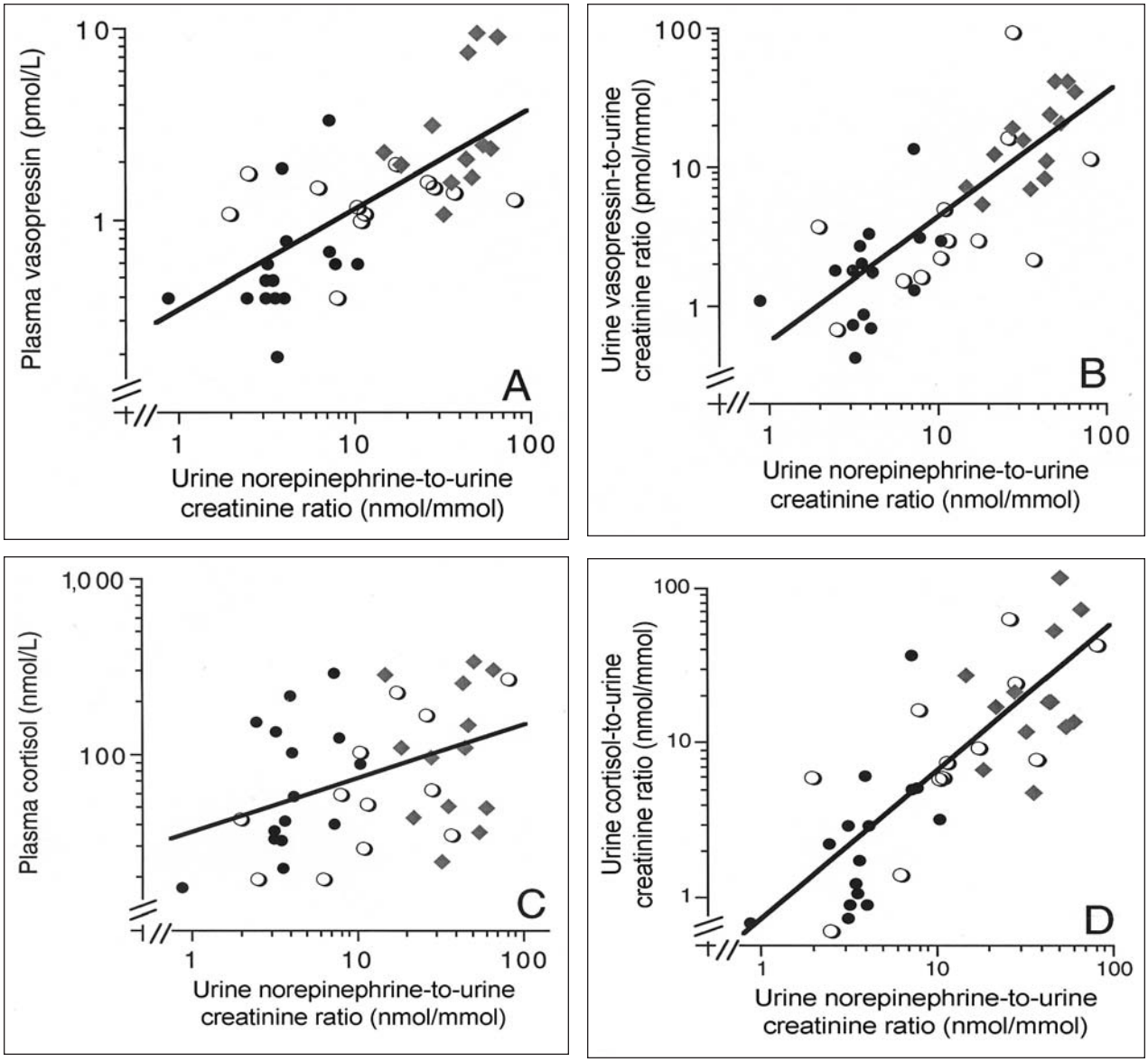

Figure 2—Plasma concentrations of vasopressin versus urine norepinephrine-to-urine creatinine ratios (A), urine vasopressin-to-urine creatinine ratios versus urine norepinephrine-to-urine creatinine ratios (B), plasma concentrations of cortisol versus urine norepinephrine-to-urine creatinine ratios (C), and urine cortisol-to-urine creatinine ratios versus urine norepinephrine-to-urine creatinine ratios in 15 clinically normal dogs (black circles), 15 dogs without clinical signs of DCM (white circles), and 15 dogs with clinical signs of DCM (black diamonds). The urine norepinephrine-to-urine creatinine ratio was significantly ($P < 0.05$) correlated with urine vasopressin-to-urine creatinine ratios, urine cortisol-to-urine creatinine ratios, plasma vasopressin concentrations, and plasma cortisol concentrations. The line represents the line of best fit for each set of data.

Table 3—Summary of the Spearman coefficient of correlation between values for various hormones.

	Variable						
Variable	**Log NE: Creat**	**Log Epi: Creat**	**Log dopamine: Creat**	**Log urine Vaso:urine Creat**	**Log urine cortisol: urine Creat**	**Log Vaso**	**Log cortisol**
Log NE:Creat	—	0.80*	0.48*	0.80*	0.80*	0.70*	0.40*
Log Epi:Creat	0.80*	—	0.31	0.69*	0.73*	0.49*	0.32*
Log dopamine:Creat	0.48*	0.31	—	0.16	0.24	0.21	0.12
Log urine Vaso:urine Creat	0.80*	0.69*	0.16	—	0.85*	0.71*	0.38*
Log urine cortisol:urine Creat	0.80*	0.73*	0.24	0.85*	—	0.72*	0.62*
Log Vaso	0.70*	0.49*	0.21	0.71*	0.72*	—	0.49*
Log cortisol	0.40*	0.32*	0.12	0.38*	0.62*	0.49*	—

*Values are significantly ($P < 0.05$) correlated.
NE = Norepinephrine. Epi = Epinephrine. Vaso = Vasopressin. Creat = Creatinine. — = Not applicable.

Discussion

Analysis of results of the study reported here revealed that vasopressin concentration increased in concert with other classical stress hormones in dogs with DCM. Indeed, with the exceptions of plasma cortisol concentration and the urine dopamine-to-urine creatinine ratio, the plasma concentrations of vasopressin and the urine vasopressin-to-urine creatinine ratio increased almost linearly with the urine cortisol-to-urine creatinine ratio and urine catecholamine-to-urine creatinine ratio.

It was reported > 30 years ago that vasopressin concentrations are increased in human patients with congestive heart failure.[30] Results of several subsequent studies[31-33] as well as results of the study reported here are in agreement with that finding. Concentrations of vasopressin are also increased in human patients with left ventricular dysfunction without clinical signs,[33] which is in accordance with findings of the study reported here. To the authors' knowledge, the study reported here is the first to characterize vasopressin concentrations in dogs with naturally developing DCM with and without clinical signs.

Vasopressin release is stimulated by increased osmolarity in the extracellular fluid compartment,[34] increased systemic activity of angiotensin II and relaxin,[34] and unloading of vascular and atrial baroreceptors (ie, decreased blood pressure).[35] The antidiuretic and fluid retentive actions of vasopressin are achieved at comparably low plasma concentrations, whereas a rapid decrease in blood pressure stimulates a surge in vasopressin release.[36] The latter is mediated primarily through vascular baroreceptors, whereas atrial baroreceptors appear to mediate reflex control of renin secretion.[36] The increased concentration of vasopressin in plasma and urine in the dogs of our study is less likely to be a part of osmoregulation because the osmolarity in plasma and urine was similar among the 3 groups of dogs.

Furthermore, it is less likely that the increased vasopressin concentrations found in the 2 groups of dogs with DCM were caused by atrial baroreceptors because these dogs had left atrial dilatation, which is a situation known to suppress renin and, possibly, vasopressin release, rather than to increase it. However, left atrial dilatation in the dogs with preclinical DCM may have prevented an increase in renin release reported in another study[16]; that increase was mediated through a combination of a decrease in signaling of atrial baroreceptors and a release of ANP. Therefore, the most likely cause for the increased concentrations of vasopressin in the study reported here is activation of the vascular baroreceptors in response to reduced cardiac output[36] and, possibly, stress.[8] However, our study was not designed to quantify the relative contribution of these 2 stimuli for vasopressin release. It is interesting that some of these compensatory mechanisms are recruited during the preclinical phases of the disease. Presumably, activation of these factors helps affected dogs to remain in the preclinical phase even when there is substantial systolic dysfunction of the left ventricle. Thus, increased concentrations of vasopressin more likely reflect a response, which is characterized by recruitment of several neuroendocrine factors, to a reduction in cardiac output.

Vasopressin causes vasoconstriction and exerts a positive inotropic effect on the myocardium by enhancing the activity of the inositol triphosphate pathway, thereby increasing intracellular calcium in a non–adenyl cyclase–dependent pathway. The effect on the myocardium may contribute to the maladaptive ventricular hypertrophy and remodeling because vasopressin can stimulate protein synthesis in myocytes.[37] A local vasopressin system has also been detected in isolated perfused rat hearts.[38] In the kidneys, vasopressin interacts with receptors in the renal-collecting tubules, which leads to retention of free water, volume expansion, and, potentially, hyponatremia, particularly when patients are treated with diuretics. The lack of differences in plasma sodium and potassium concentrations between the groups of dogs may have been attributable to the balance between the renin-angiotensin-aldosterone system and ANP activity (as reported in another study[16] conducted by our laboratory group) or to factors influencing the distribution of these ions in the various fluid compartments. Volume expansion may contribute to an increase in ventricular wall stress during diastole, which may induce the type of eccentric hypertrophy that characterizes the remodeling process in DCM.[39] Thus, there is a growing interest in the use of vasopressin antagonists for the treatment of patients with congestive heart failure.[39,40] A study[41] conducted on dogs with rapid pacing–induced congestive heart failure revealed a beneficial hemodynamic response with the use of vasopressin-receptor antagonists.

Plasma cortisol concentrations were not significantly different among groups of dogs in the study reported here, which is in accordance with 1 study[42] but contrary to another study[43] in humans. However, the urine cortisol-to-urine creatinine ratio was increased in dogs with DCM, compared with the ratio in control dogs. This discrepancy between plasma and urine concentrations may be explained by a small difference in plasma cortisol concentrations among the groups but a large amount of variation. Indeed, similar to the urine cortisol-to-urine creatinine ratio, plasma cortisol concentrations were correlated with the plasma and urine concentrations of all other hormones, except dopamine. The correlation coefficients were lower for the plasma values, compared with corresponding urinary values. Although cortisol (a steroid hormone) is not secreted in the same pulsatile manner as the catecholamines, concentrations of cortisol change over time more rapidly in plasma than they do in urine. Thus, the urine concentration of cortisol presumably reflects cortisol secretion over longer periods than do plasma values. Other suppressive factors of cortisol secretion, such as ANP and brain natriuretic peptide, which are increased in humans[10] and dogs[16,44] with congestive heart failure, can inhibit secretion of cortisol.[42] These factors are less likely to have caused the apparent discrepancy because a true suppression of cortisol secretion would have also influenced urinary excretion of cortisol.

Measuring hormone concentrations in plasma has the weakness that it only reflects the plasma concen-

trations at a specific moment in time. Many hormones, such as vasopressin and catecholamines in particular, are secreted in a pulsatile manner, which means that there may be substantial changes in plasma concentrations in a relatively limited time period.[45-47] Furthermore, there is diurnal variation that must be considered for all the hormones measured in the study reported here, although the greatest diurnal variation is usually between day and night.[46-49]

In addition to plasma concentrations of cortisol and vasopressin, we also measured urine concentrations of cortisol, vasopressin, and the catecholamines. Urine and blood samples were collected during the day at the same time the dogs underwent a clinical examination. To compensate for differences in urine concentrations, hormonal values were expressed as a ratio, with the urine creatinine concentration as the denominator.[50] With the exception of the urine dopamine-to-urine creatinine ratio, these urinary ratios were highly correlated with each other and with plasma concentrations of hormones, except for the plasma cortisol concentration. This finding was surprising because the urine samples were not collected during a specified time period and urine production was not measured. Obtaining such data would have required catheterization of the urinary balder and collection of urine during a specific time period,[51] which were not technically or ethically possible in all the patients included in the study (particularly dogs with untreated, decompensated congestive heart failure). Therefore, urinary hormonal values represented excretion over a variable time period, thus causing an increase in variation. However, analysis of results of the study reported here clearly revealed that despite the aforementioned weaknesses and sources of variation, the urine values of hormones were highly correlated with the plasma concentrations. Mean values for plasma concentrations, except cortisol, were significantly different among the 3 groups of dogs. These facts cannot be attributed to a high overall variation.

Ample evidence exists from reports[10,43,52-55] that there is activation of catecholamines in human patients with congestive heart failure, although reports[56] on activation of these hormones in dogs with naturally developing heart disease are scant. Initially described in 1929,[57] increases in heart rate, force of contraction, and blood pressure in response to sympathetic stimulation permit a rapid increase in physical performance during emergency situations. Stimulation of β-adrenergic receptors increases cardiac output by increasing the heart rate and myocardial contractile force. However, α-adrenergic stimulation may reduce cardiac output by increasing peripheral resistance. The various catecholamines should be considered separately. Epinephrine is secreted from the medulla of the adrenal glands and transported to distant target organs. Norepinephrine, a neurotransmitter found in sympathetic nerve endings, acts locally on effector cells. Dopamine serves as a precursor of norepinephrine and also acts as a neurotransmitter. We elected to measure urine concentrations of catecholamines in preference to plasma concentrations because the former may better reflect daily secretion, rather than isolated peaks of activity.

In the study reported here, urine epinephrine-to-urine creatinine and urine norepinephrine-to-urine creatinine ratios, but not urine dopamine-to-urine creatinine ratios, were significantly increased in dogs with clinical signs of DCM, compared with ratios in control dogs. In studies[52,58,59] of human patients treated because of chronic heart failure and dogs treated because of congestive heart failure attributable to DCM or chronic valvular disease,[56] plasma concentrations of norepinephrine were increased, whereas concentrations of epinephrine were not. The fact that epinephrine and norepinephrine concentrations were increased in the dogs in the study reported here may be explained by the fact that our dogs were in acute congestive heart failure before any treatments were initiated. This may indicate that the medulla of the adrenal glands and the sympathetic nerve endings are activated during acute congestive heart failure, whereas the sympathetic nerve endings are the major source of plasma catecholamines following chronic adrenergic stimulation attributable to congestive heart failure. Inefficient reuptake mechanisms,[60,61] increased turnover of norepinephrine at the sympathetic nerve endings, and a decrease in the density of adrenoreceptors[62] may also contribute to increased plasma norepinephrine concentrations in patients with heart failure.

Plasma concentrations of norepinephrine may serve as a guide for the prognosis in human patients with chronic congestive heart failure because concentrations are considerably higher in patients with substantially shorter survival times.[63] The use of β-adrenergic receptor blocking agents in human patients with congestive heart failure has been advocated by investigators.[64] In a report[65] on a large number of randomized, double-blind, placebo-controlled studies, long-term treatment of human patients with chronic heart failure with β-receptor blockade was associated with a substantial reduction in mortality rate. The β_1-receptor blockers can prevent and reverse many of the structural and functional changes that are evident during the progression of heart failure, and β_2- and α_1-receptor blockade seems to enhance the ability of β_1-blockers to prevent toxic effects of catecholamines. Additional studies may elucidate whether agents that block α_1-, β_1-, and β_2-receptors may be more effective than agents that act selectively on β_1-receptors.[53]

Dopamine is a direct agonist of α- and β-adrenergic receptors as well as dopamine receptors. Effects of exogenous dopamine on these receptors are a dose-dependent phenomenon because lower doses will primarily affect vascular beds,[66] whereas higher doses will increase heart rate and myocardial contractility.[67] Norepinephrine inhibits dopamine production by negative feedback on tyrosine hydroxylase, which is the rate-limiting step in the production of dopamine.[68] Therefore, because the concentration of norepinephrine was increased in the study reported here, it is not surprising that the dopamine concentration was not increased.

In the study reported here, we documented that concentrations of vasopressin, cortisol, and catecholamines were increased in dogs with DCM. Furthermore, these changes were more pronounced in

dogs with clinical signs of DCM, compared with results for dogs with preclinical DCM.

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f. Trasylol, Bayer Pharmaceuticals Corp, Bayer Leverkusen, Germany.
g. 5-mL lithium-heparin tubes, Becton-Dickinson, Meylex Cedex, France.
h. 10-mL plain tubes, Becton-Dickinson, Meylex Cedex, France.
i. Coat-A-Count cortisol RIA, Diagnostic Product Corp, Los Angeles, Calif.
j. Vasopressin RIA, catalogue No. RB 319, Eurodiagnostika AB, Malmö, Sweden.
k. Acetone, GR, Merck, Darmstadt, Germany.
l. Petroleum benzen, GR, boiling point 40° to 60°C, Merck, Darmstadt, Germany.
m. Isolute MFC18-columns, International Sorbent Technology Ltd, Glamorgan, UK.
n. Nucleosil 5 SA, 5 µm, 150 × 3.9 mm, HiChrom, Scantec Lab AB, Partille, Sweden.
o. ESA Coulochem 5100 A, Coricon AB, Knivsta, Sweden.
p. Unimate 5 urea/BUN, article No. 073685, Roche, Basel, Switzerland.
q. Unimate 7 crea, article No. 0736678, Roche, Basel, Switzerland.
r. Model E2A electrolyte analyzer, Beckman Instruments, Stockholm, Sweden.
s. Osmometer VAO 1, Herman Roebling Messtechnik, Berlin, Germany
t. JMP 3.2, SAS Institute Inc, Cary, NC.

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