Validation of a commercially available enzyme immunoassay for measurement of plasma antidiuretic hormone concentration in healthy dogs and assessment of plasma antidiuretic hormone concentration in dogs with congestive heart failure

Katherine F. Scollan, DVM; Barret J. Bulmer, DVM, MS; D. David Sisson, DVM

Objective—To validate the use of a human enzyme immunoassay (EIA) kit for measurement of plasma antidiuretic hormone (ADH) concentration in dogs and evaluate plasma ADH concentrations in dogs with congestive heart failure (CHF) attributable to acquired cardiac disease, compared with findings in healthy dogs.

Animals—6 healthy dogs and 12 dogs with CHF as a result of chronic degenerative valve disease or dilated cardiomyopathy.

Procedures—Plasma samples from the 6 healthy dogs were pooled and used to validate the EIA kit for measurement of plasma ADH concentration in dogs by assessing intra-assay precision, dilutional linearity, and spiking recovery. Following validation, plasma ADH concentrations were measured in the 6 healthy dogs and in the 12 dogs with CHF for comparison.

Results—The EIA kit measured ADH concentrations in canine plasma samples with acceptable intra-assay precision, dilutional linearity, and spiking recovery. The intra-assay coefficient of variation was 11%. By use of this assay, the median plasma concentration of ADH in dogs with CHF was 6.15 pg/mL (SD, 3.2 pg/mL; range, 4.18 to 15.47 pg/mL), which was significantly higher than the median concentration in healthy dogs (3.67 pg/mL [SD, 0.93 pg/mL; range, 3.49 to 5.45 pg/mL]).

Conclusions and Clinical Relevance—Plasma ADH concentrations in dogs can be measured with the tested EIA kit. Plasma ADH concentrations were higher in dogs with CHF induced by acquired cardiac disease than in healthy dogs. This observation provides a basis for future studies evaluating circulating ADH concentrations in dogs with developing heart failure. (Am J Vet Res 2013;74:1206–1211)

Received December 12, 2012. Accepted April 18, 2013.

From the Department of Clinical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331. Dr. Bulmer’s present address is Tufts Veterinary Emergency Treatment and Specialties, 525 South St, Walpole, MA 02081.

Supported by gifts from Dr. Dana Buoscio to the Cardiology Research Fund, College of Veterinary Medicine, Oregon State University.


Address correspondence to Dr. Scollan (kate.scollan@oregonstate.edu).

Chronic degenerative valve disease and DCM are the 2 most common acquired cardiac diseases of dogs. Both are progressive disorders that eventually result in overt CHF, causing considerable morbidity and death in affected dogs. Antidiuretic hormone, also referred to as arginine vasopressin or vasopressin, is a nonapeptide hormone synthesized within the hypothalamus; it contributes to the regulation of plasma osmolality and blood pressure. In addition to other well-characterized neurohormonal alterations in dogs with heart failure, excessive circulating concentrations of ADH may have a role in the relentless progression of cardiac decompensation over time. Such changes are well characterized in humans with CHF. Two important biological actions of ADH of particular relevance in patients with heart disease include vasoconstriction and expansion of plasma volume via the retention of solute-free water. In this context, ADH excess promotes the development...
of hyponatremia, particularly when high doses of loop diuretics are used to relieve clinical signs of congestion.

Circulating concentrations of ADH in dogs with heart failure have not been studied extensively, in part because of the difficulty of measuring ADH plasma concentrations. In the past, ADH concentration has been measured mainly by radioimmunoassay, which is expensive to perform and has the disadvantages inherent to the preparation and handling of radioactive reagents. For humans, the development of an EIA kit has allowed faster and less hazardous measurement of plasma ADH concentration, albeit with limits of detection that are modestly less sensitive than those of the radioimmunoassay. Given the homologous structure of human and canine ADH, we hypothesized that a human EIA kit assay would be less sensitive than those of the radioimmunoassay. Assuming this objective could be met, the intent was to evaluate plasma ADH concentrations in dogs with CHF attributable to acquired cardiac disease and compare those data with findings in healthy dogs. We hypothesized that plasma ADH concentrations would be higher in dogs with CHF induced by acquired cardiac disease than in healthy dogs.

Materials and Methods

Animals—Six healthy dogs without evidence of cardiac disease owned by students and employees at the veterinary teaching hospital at Oregon State University were used in the study. Plasma samples obtained from these dogs were used to validate the use of a human EIA kit for measurement of plasma ADH concentration in dogs and were analyzed for comparison with data from dogs with CHF. Dogs in this group were adults (>4 years old) and determined to be free of cardiac disease on the basis of results of physical and echocardiographic examinations. Six breeds were represented (Greater Swiss Mountain Dog, Australian Shepherd, Labrador Retriever, Standard Poodle, Labrador Retriever mix, and Boxer mix). There were 3 spayed females and 3 neutered males.

Twelve client-owned dogs with CHF as a result of CDVD (7 dogs) or DCM (5 dogs) were also used in the study. Among the dogs with CDVD, there were 7 breeds (Cairn Terrier, Miniature Schnauzer, Rhodesian Ridgeback, Maltese, Chihuahua, Pekingese, and a medium-sized mixed-breed dog). Among the dogs with DCM, there were 4 breeds (Doberman Pinscher [n = 2], Wirehair Pointer [1], Great Dane [1], and a large mixed-breed dog). In the CHF group, there were 6 spayed females, 1 sexually intact female, 3 neutered males, and 2 sexually intact male dogs. Dogs were included in the CHF group if they had radiographic evidence of pulmonary edema (determined at a previous hospital visit) but were not currently in heart failure (n = 5; 3 dogs with CDVD and 2 dogs with DCM) or if they had active heart failure at the time of blood sample collection (7; 4 dogs with CDVD and 3 dogs with DCM). Dogs were excluded if radiographic evidence of pulmonary edema was not conclusive. At the time of blood sample collection, 2 dogs with CHF had not received any treatment, whereas 10 dogs with CHF had been treated with furosemide (10/10 dogs), enalapril (10/10 dogs), digoxin (2/10 dogs), and pimobendan (7/10 dogs). The Oregon State University Institutional Animal Care and Use Committee approved all procedures used in the study, and written consent was obtained from all owners of the study dogs.

Collection of blood samples—Twelve milliliters of blood was collected via direct venipuncture from each dog, and 3-mL aliquots of each sample were placed into 1 of 4 identical chilled tubes containing EDTA and a combination of protease inhibitors. The protease inhibitor concentrations per mL of blood containing EDTA were as follows: 3-phenylalanine-arginine-chloromethketone, 10 µg/mL; benzamidine, 700 µg/mL; aprotinin, 20 µg/mL; leupeptin hemisulfate, 100 µg/mL; and DL-thiorphan, 50 µg/mL. The tubes were placed on ice immediately and centrifuged at 4°C (1,600 X g for 15 minutes) within 10 minutes after collection. Plasma samples were then transferred to polyethylene tubes and stored at −80°C until time of analysis.

Validation of EIA kit for use with canine plasma samples—Validation of the commercially available human ADH EIA kit was performed with plasma samples obtained from the 6 healthy dogs. Sensitivity of the kit provided by the manufacturer was 3.39 pg/mL. Plasma samples were collected and stored as described, and then one 3-mL aliquot of dog plasma was thawed prior to validation assays. Once thawed, the plasma samples were combined into a pooled plasma sample. The pooled canine plasma sample and ADH standard supplied with the EIA kit were used to produce a standard curve. Concentrations of ADH in pooled canine plasma were 1,000, 400, 160, 64, 25.6, 10.24, 4.1, and 1.64 pg/mL. A kit standard containing a known amount of ADH was included with each assay plate. Accuracy of the EIA kit was determined by measuring the spiked and unspiked plasma samples that were not spiked with ADH. The kit was used in accordance with the manufacturer’s instructions. The results were recorded in an Excel spreadsheet (Microsoft Excel 2007, Redmond, WA). The intra-assay variability was determined by performing the assay in triplicate for each spiked or unspiked sample. Dilutional linearity was assessed by measuring ADH concentrations in the plasma sample spiked with 10.24 pg of ADH/mL before and after serial dilutions (1:2, 1:4, and 1:8). The measured ADH concentrations were compared to predicted concentrations. Accuracy was assessed by spike and recovery, comparing measured ADH concentrations to expected values in samples.
spiked at low (4.1 pg/mL), medium (10.24 pg/mL), and high (64.0 pg/mL) concentrations of ADH.

Echocardiography—Transthoracic 2-D, M-mode, color flow, and spectral Doppler echocardiographic evaluations were performed with a commercial echocardiographic unit and 7-, 5-, or 3-MHz phased array probes. Each of the 18 dogs was placed in right and then left lateral recumbency to obtain standard right and left parasternal views. Left atrial-to-aortic root ratio, LVIDd, LVIDs, and fractional shortening were measured from M-mode right parasternal short axis images. Measurements of LVIDd and LVIDs were indexed to body weight (BW) by use of formulas described by Cornell et al as follows:

\[
\text{LVIDd index} = \frac{\text{LVIDd}}{\text{BW}^{0.33}} \\
\text{LVIDs index} = \frac{\text{LVIDs}}{\text{BW}^{0.33}}
\]

All echocardiographic measurements were made in triplicate and averaged.

In the dogs with CHF, CDVD was diagnosed when echocardiography revealed characteristic valvular lesions (thickened mitral valve leaflets) and enlargement of the left atrium and ventricle in association with a large mitral regurgitant jet on 2-D Doppler color flow Doppler images. For the other dogs with CHF, DCM was diagnosed when echocardiography revealed increased left ventricular systolic and diastolic dimensions (compared with expected values based on body weight) with a reduction in fractional shortening (%) and moderate to severe left atrial enlargement in the absence of other identifiable cardiac disorders.

Radiography—For each dog with suspected CHF, 2-view orthogonal thoracic radiographs obtained either during a previous hospital visit or during the visit at which the blood sample was collected were reviewed. Radiographic views were evaluated by a board-certified cardiologist (KFS) and radiologist for evidence of pulmonary edema and assessment of heart size by use of the vertebral heart scale method. Cardiogenic pulmonary edema was determined to be present when the pulmonary veins were distended, the left atrium was enlarged, and an interstitial or alveolar pulmonary pattern was observed.

Analysis of plasma ADH concentrations—Blood samples from the 12 dogs with CHF that met the inclusion and exclusion criteria were collected, and plasma was obtained and stored as described. The unused plasma aliquots obtained from the healthy dogs were also analyzed. At the time of analysis, plasma samples were thawed and all samples were assayed in duplicate following the protocol recommended by the EIA kit manufacturer.

Statistical analysis—Data were assessed for normality by the D’Agostino and Pearson omnibus normality test. Data that were not normally distributed were reported as median with SD or range. Median age, body weight, La: Ao ratio, LVIDd index, LVIDs index, fractional shortening, and plasma ADH concentration for healthy dogs and dogs with CHF were compared by use of nonparametric Mann-Whitney U tests. Dilutional linearity was evaluated by ordinary linear regression analysis in which the measured concentrations of ADH in plasma samples were compared with the expected concentration. Statistical significance was designated at a value of \( P < 0.05 \) (2-tailed test). Statistical analysis was performed with commercial software.

Results

Animals—Age, body weight, and echocardiographic and radiographic variables for 6 healthy dogs and 12 dogs with CHF were compared (Table 1). Median body weight of healthy dogs and CHF dogs did not differ (Table 2).

All echocardiographic measurements were made in triplicate and averaged.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dogs with CDVD</th>
<th>Dogs with DCM</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12 (6–14)</td>
<td>8 (4–13)</td>
<td>0.581</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>6.0 (3.2–38.6)</td>
<td>31.4 (24–64.5)</td>
<td>0.030</td>
</tr>
<tr>
<td>La:Ao ratio</td>
<td>2.24 (1.94–2.54)</td>
<td>1.89 (1.05–2.76)</td>
<td>0.103</td>
</tr>
<tr>
<td>LVIDd index*</td>
<td>2.13 (1.80–2.17)</td>
<td>2.05 (1.65–2.42)</td>
<td>0.636</td>
</tr>
<tr>
<td>LVIDs index*</td>
<td>1.09 (0.81–1.33)</td>
<td>1.57 (1.42–1.89)</td>
<td>0.002</td>
</tr>
<tr>
<td>FS (%)</td>
<td>48.0 (33.3–62.2)</td>
<td>23.4 (9.0–23.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>VHS</td>
<td>12.1</td>
<td>11.9</td>
<td>0.569</td>
</tr>
</tbody>
</table>

Table 1 for key.

Figure 1—Dilution linearity for ADH concentrations in a pooled plasma sample (obtained from 6 healthy dogs) spiked with 10.24 pg of ADH/mL before and after serial dilutions (1:2, 1:4, and 1:8) and measured in triplicate. Data points are plotted as mean observed concentration (with SE bars) versus expected concentration. Linear regression analysis yielded the regression equation: observed = 1.171(expected) + 0.8644.
Plasma ADH concentrations in healthy dogs and dogs with CHF—The median plasma concentration of ADH was significantly \((P = 0.005)\) higher in dogs with CHF \((6.15\ \text{pg/mL} [\text{SD}, 3.2\ \text{pg/mL}; \text{range}, 4.18\text{ to }15.47\ \text{pg/mL}])\), compared with findings for healthy dogs \((3.67\ \text{pg/mL} [\text{SD}, 0.93\ \text{pg/mL}; \text{range}, 3.49\text{ to }5.45\ \text{pg/mL}; \text{Figure 2})\). Among dogs with CHF, the median plasma ADH concentration for the dogs with CDVD \((6.71\ \text{pg/mL} [\text{SD}, 1.9\ \text{pg/mL}; \text{range}, 4.20\text{ to }9.34\ \text{pg/mL})\) was not significantly \((P = 0.53)\) different from the value for dogs with DCM \((5.85\ \text{pg/mL} [\text{SD}, 0.93\ \text{pg/mL}; \text{range}, 4.18\text{ to }15.47\ \text{pg/mL})\), compared with findings for healthy dogs \((3.67\ \text{pg/mL} [\text{SD}, 0.93\ \text{pg/mL}; \text{range}, 3.49\text{ to }5.45\ \text{pg/mL}; \text{Figure 2})\). However, compared with the value determined in healthy dogs, median plasma ADH concentration in dogs with CDVM was significantly \((P = 0.004)\) higher, whereas median plasma ADH concentration in dogs with DCM did not differ \((P = 0.51)\; \text{Figure 3}).

Discussion

In the veterinary medical literature, reports of circulating ADH concentrations in dogs in the context of cardiovascular disease are sparse. This is, at least in part, attributable to the difficulty in collecting samples and performing a radioimmunoassay, which must be carried out at regulated laboratories because of the requirement for radioactive material and the potential human health hazard. The development of a commercial EIA for ADH allows a more affordable and practical assessment of plasma ADH concentrations for most veterinary clinical laboratories. The limit of detection of the EIA kit used in the present study is 3.39 pg/mL, whereas for most radioimmunoassays, the detection limit is ≤ 1.0 pg/mL,\(^{12,13}\) indicating that the EIA kit is less sensitive for detection of ADH. In the present study, the accuracy of the kit was tested at 3 levels (low, medium, and high) of ADH concentration by spiking recovery. Accuracy was acceptable at the medium and high ADH concentrations, but ADH concentrations were slightly overestimated at the concentration closest to the reported sensitivity limit of the kit. Despite the potential inability of the EIA kit to detect very low concentrations of ADH in canine plasma samples, ADH concentrations in healthy dogs determined in the present study were comparable to those measured by radioimmunoassay in apparently normal dogs\(^{13,14}\) and humans.\(^{15}\) The limitation of this kit at lower concentrations of ADH might be overcome by altering the quantities of extracted plasma and reconstituting diluent to increase the measured ADH concentration and adjusting the final concentration result accordingly.
More than 30 years ago, it was reported that plasma ADH concentration was high in humans with CHF. Results of several subsequent studies in humans and in dogs as well as those of the study reported here have corroborated that finding. Elevated plasma ADH concentration has been found in humans with acute or chronic CHF, including individuals who were hyponatremic. The major physiologic actions of ADH include the regulation of plasma osmolality and blood pressure. The release of ADH is stimulated by several mechanisms including an increase in plasma osmolality, reduction in blood pressure or volume, and an increase in angiotensin II activity. An increase in plasma osmolality, reduction in blood pressure or volume, and an increase in angiotensin II activity. An increase in plasma osmolality of as little as 1% causes an increase in ADH synthesis and release due to the activation of osmoreceptors located within the hypothalamus. Baroreceptors located within the aortic arch, carotid sinus, and left atrium monitor blood pressure and plasma volume within the circulatory system. Depression of arterial pressure or plasma volume by 5% to 10% can trigger the release of ADH from the hypothalamus. Activation of the RAAS, well documented in dogs with CHF, also increases ADH secretion via a direct effect of angiotensin II on the hypothalamus.

Many investigators have sought to reconcile the paradox of high ADH concentration in the setting of low plasma osmolality and hypervolemia. The dogs with CHF in the present study had left atrial enlargement with larger La:Ao ratios than those found in the healthy dogs. Physiologically, left atrial dilatation should cause a depression of ADH release in response to atrial baroreceptor detection of hypervolemia, yet the dogs with CHF had high plasma ADH concentrations. A unifying hypothesis that has emerged from human studies is that atrial underfilling and unloading of associated baroreceptors is the stimulus for maintained nonosmotic ADH release. It appears that in the setting of heart failure, low cardiac output, arterial hypotension, and RAAS activation have a greater influence than do osmoreceptors on controlling the release of ADH. In addition, several studies have revealed that arterial hypotension has a more substantial effect on ADH release, compared with the effect of atrial volume receptors, in hypovolemic states. It is likely that increased ADH release in dogs with CHF is also driven by arterial hypotension, reduced cardiac output, and RAAS activation, similar to the mechanisms proposed for humans.

In the present study, neither plasma osmolality nor components of the RAAS cascade were measured and the mechanisms responsible for the observed increase in plasma ADH concentration in dogs with CHF cannot be assessed. It is possible that some of the dogs with CHF in the study of this report were hyponatremic with low plasma osmolality as a result of treatment with diuretics. However, these data were not available and, consequently, the relationship of plasma osmolality to circulating ADH concentration in dogs receiving treatment for CHF requires further investigation. Nevertheless, the aim of this initial study was not to assess the relative stimuli to ADH release in the setting of CHF but simply to assess whether a difference in plasma ADH concentrations between affected and healthy dogs could be identified by use of the ADH EIA kit. Interestingly, plasma osmolality in healthy dogs and dogs with CHF did not differ in the study by Tidholm et al, although those analyzed blood samples were collected prior to failure treatment. The relative importance of physiologic processes such as plasma osmolality, arterial pressure, or RAAS activation for circulating ADH concentrations in dogs with CHF should be investigated in future work.

Certainly, 1 difficulty in assessing circulating ADH concentrations in dogs relates to the pulsatile nature of release of many plasma hormones, including ADH. Plasma concentrations measured from a single sample yield the plasma concentration at that specific time. In a study to evaluate the secretion patterns of ADH in dogs under basal conditions, during water deprivation, and following osmotic stimulus, a pulsatile pattern was identified in each setting. This mechanism may explain some of the discrepancies between the results of studies evaluating ADH concentrations in healthy dogs. The mean plasma ADH concentration for healthy dogs reported by Tidholm et al was substantially lower (0.79 pmol/L [SD, 0.82 pmol/L] or 0.85 pg/mL [SD, 0.88 pg/mL]) than the median value determined for the healthy dogs used in the present study (3.67 pg/mL [SD, 0.93 pg/mL]). Alternatively, this observed difference might simply be due to the lower limit of detection of the radioimmunoassay used for ADH concentration measurement in the earlier study (1.1 pg/mL) versus that of the EIA kit used in the present study (3.39 pg/mL). Interestingly, the mean plasma concentrations of ADH in healthy dogs (measured via radioimmunoassays with similar detection limits) range from 0.85 pg/mL (SD, 0.88 pg/mL) to 2.4 pg/mL (SE, 0.02 pg/mL) and 3.2 pg/mL (SD, 0.7 pg/mL). This indicates that the difference in test sensitivity may not be the only cause of the observed variation. The temporal pattern of ADH secretion in dogs should be examined more closely, and the possibility that this pattern is pulsatile should be taken into consideration when designing future studies to assess circulating ADH concentrations in dogs with CHF. Such studies may require that several samples be collected at frequent intervals to address the potential pulsatile secretion in dogs with CHF. Despite the differences in absolute values, both the radioimmunoassay and EIA methods revealed significant differences in plasma ADH concentrations between healthy dogs and dogs with heart failure. Thus, the EIA kit appears to be a reasonable means of measuring ADH in clinically affected dogs and can be used as an alternative to the previously used radioimmunoassay with the described limitations.

In the study reported here, plasma ADH concentrations in dogs with CHF as a result of either CDVD or DCM were not significantly different. Interestingly, plasma ADH concentration in dogs with CHF induced by CDVD was significantly higher than the value in healthy dogs; plasma ADH concentration in dogs with CHF induced by DCM did not differ from the value in healthy dogs. This latter finding may be attributable to the smaller number of dogs with DCM, and analysis of samples from additional dogs may have led to the identification of a significant difference.
The present study had several limitations that should be considered when interpreting the results. The number of dogs with CHF was small, although a significant difference in plasma ADH concentrations between healthy dogs and dogs with CHF was detected. Inclusion of larger numbers of dogs with CDVD and DCM may have allowed more definitive discrimination of plasma ADH concentrations in relation to the different diseases and variable degrees of disease severity. Additionally, the diagnosis of CHF was based on the subjective identification of pulmonary edema in radiographic views and was not quantitatively assessed by degree of edema or measurement of pulmonary capillary wedge pressures. Lastly, plasma sodium concentration and plasma osmolality were not measured prospectively during the study. Concurrent measurement of these variables with assessment of plasma ADH concentration would provide interesting and valuable information regarding the stimuli for ADH release in dogs with CHF.

On the basis of the results of the present study, it is feasible to measure ADH concentrations in canine plasma samples with a commercially available human EIA kit. In addition, data indicated that plasma ADH concentration is significantly higher in dogs with clinical CHF compared with that in healthy dogs. The expanded accessibility and absence of required radioactive material when using the EIA kit present clear advantages over the more traditionally used radioimmunoassay for measurement of plasma ADH concentration in dogs. Further study is warranted to assess circulating ADH concentrations in dogs with various types and degrees of acquired heart disease while concurrently assessing plasma osmolality and sodium status to evaluate the relative contributions of these stimuli to high ADH concentrations. Furthermore, the potential for use of ADH antagonists in dogs with refractory or hyponatremic CHF should be investigated.

References