**Plasma Atrial and Brain Natriuretic Peptide Levels in Dogs with Congestive Heart Failure**

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**ABSTRACT.** Plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) were measured in 6 dogs with experimental mitral regurgitation (MR) and 19 canine patients with asymptomatic and symptomatic congestive heart failure (CHF). In dogs with experimental MR, ANP and BNP concentrations were significantly correlated with pulmonary capillary wedge pressure (PCWP) (ANP; r=0.852, P=0.0004, BNP; r=0.832, P=0.0008). ANP level was shown to have a predominant effect on PCWP in comparison with BNP using multiple regression analysis. In canine patients with asymptomatic and symptomatic CHF, ANP and BNP concentrations were significantly different among the heart failure classes according to the New York Heart Association functional classification (ANP, P0=0.0165, BNP, P0=0.0005). In addition, ANP and BNP levels in dogs with decompensated heart failure (n=10) significantly increased in comparison with those in dogs with compensated heart failure (n=9). There was however no correlation between ANP and BNP levels in each heart failure class. In conclusion, plasma ANP and BNP levels may become predictors of PCWP and the severity of heart failure in dogs with MR, although further investigations on ANP and BNP levels in more clinical cases are required. — KEY WORDS: atrial natriuretic peptide, brain natriuretic peptide, canine, heart failure, pulmonary capillary wedge pressure.

**Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are cardiac hormones responsible for the regulation of body fluid homeostasis and blood pressure [18]. The synthesis and secretion of ANP and BNP have demonstrated to augment in human abnormal heart conditions [19, 21]. Plasma ANP and BNP concentrations are therefore good indicators for the severity, the prognosis and the left ventricular (LV) dysfunction in human acute myocardial infarction (AMI) and congestive heart failure (CHF) [12, 19]. Additionally, ANP and BNP levels were shown to increase in proportion to pulmonary capillary wedge pressure (PCWP) in human patients with CHF [13, 21]. It has however been demonstrated that ANP is predominantly of atrial origin in contrast to BNP primarily from ventricle [20] and has a different secretion pattern from BNP in human heart diseases [21]. These observations suggest different pathophysiological roles of ANP and BNP in the cardiovascular regulation. In veterinary medicine, ANP concentration was reported to increase in the dogs with mitral regurgitation (MR) [5, 6, 15], heartworm disease [14] and CHF [16, 17]. ANP concentration was suggested to be an indicator for cardiac decompensation but not left atrial (LA) and LV enlargement [5]. ANP level has not yet been demonstrated to increase in relation to PCWP as an indicator of pulmonary edema in dogs with MR which are frequently observed in small animal clinics. On the other hand, BNP concentration has not yet been demonstrated in canine clinical cases.

The objective of the present study was to determine whether plasma levels of canineANP and BNP become useful markers for PCWP and severity of heart failure.

**MATERIALS AND METHODS**

**Relationship between PCWP and plasma levels of ANP and BNP in dogs with experimental MR**

**Animals:** Six healthy mongrel dogs, 5 males and 1 female, weighing between 6.5 and 12.0 kg, were used for the surgical creation of MR.

**Methods:** Anesthesia was induced with sodium thiopental (25 mg/kg, IV) and maintained with isoflurane (1.3%) and oxygen (1.0 l/min). A 5-Fr balloon catheter was introduced into a 6-Fr sheath placed in the jugular vein and advanced into the pulmonary artery. PCWP was recorded by a polygraph (Bedside Monitor BSM-8502; Nihon Kohden Co., Tokyo, Japan). A 6-Fr 28 cm-long sheath was introduced into the exposed carotid artery and placed into the LV. A biopompe was inserted into the long sheath, and the mitral valvular chordae tendineae was then cut by it to cause significant MR under transesophageal echocardiographic and fluoroscopic guidance, and the surgical site was closed. One week after the operation, a second operation was repeated similarly to the first operation. Postoperative antibiotic (ampicillin 20 mg/kg, PO, BID, for 3 days), and when needed, diuretic (furosemide 2.0–4.0 mg/kg, PO, BID) were given. Two weeks after the second operation, PCWP was recorded in each dog under the general anesthesia as mentioned above.

Blood samples were collected from the dogs before the first operation and 2 weeks after the second operation. In addition, the clinical signs of heart failure and physical examination in the dogs with experimental MR were evaluated to be divided into the heart failure classes according to the New York Heart Association (NYHA)
functional classification.

These dogs were operated and cared for according to the principles outlined in the guide for the care and use of laboratory animals approved by the Graduate School of Veterinary Medicine, Hokkaido University.

Relationship between the heart failure classification and plasma ANP and BNP levels

Animals: Nineteen privately owned dogs (14 males and 5 females, median age of 11-year-old) were diagnosed as chronic MR due to physical examination, thoracic radiography, electrocardiography and/or echocardiography at an initial examination in the Hokkaido University Veterinary Teaching Hospital (Table 1). Of these canine patients with asymptomatic and symptomatic CHF, 17 patients and the others were suspicious of chronic valvular disease (CVD) and dilated cardiomyopathy (DCM), respectively. The case histories relating to the underlying heart diseases were also ascertained by careful interviews with the owners.

Nineteen healthy mongrel dogs without any abnormal cardiac conditions diagnosed by physical examination, thoracic radiography and echocardiography were used to establish the normal plasma levels of ANP and BNP.

Blood collection was also performed in the patients with chronic MR and normal subjects at the initial examination.

Classification of heart failure: According to NYHA functional classification, the heart failure class of each dog was categorized on the basis of the history of clinical signs of heart failure and the results of physical examination (Table 1).

Assays of plasma ANP and BNP concentrations: Blood was drawn from the jugular vein into 2-ml tubes (Vacutainer®; Becton Dickinson Co., Franklin Lakes, N.J., U.S.A.) containing ethylenediaminetetraacetic acid. The blood was centrifuged at 4°C at 1,500 × g for 10 min. Plasma was separated from the blood and was stored at -30°C until assay. ANP concentration was measured by immunoradiometric assay for human α-ANP (Shionoria-ANP® provided by courtesy of Shionogi Co., Osaka, Japan) according to the manufacturing instruction.

For the measurement of BNP concentration, plasma samples were purified by passing through C_{18}-octadecyl silica cartridges (Sep-Pak plus®, Waters Co., Milford, M.A., U.S.A.). Sixty percent acetonitrile in 1% trifluoroacetic acid was used as an elution solvent. The eluant was evaporated by blowing nitrogen gas and lyophilization. The dissolution of the evaporated sample was made by the radioimmunoassay buffer. The recovery rate was 64 ± 2.9% (mean ± SD; n=9). BNP concentration was measured by radioimmunoassay for canine BNP-32 (Peninsula Laboratories Inc., Belmont, C.A., U.S.A.) according to the manufacturing instruction.

Statistical analysis: All data were expressed as mean ± SE. The dogs with experimental MR were classified into compensated (NYHA class I) and decompensated (NYHA class III and IV) groups. Comparisons of PCWP and plasma levels of ANP and BNP between the dogs in compensated and decompensated groups were assessed using Mann-

Table 1. Breed, age, sex, weight, clinical diagnosis, and heart failure classification in dogs with chronic mitral regurgitation

<table>
<thead>
<tr>
<th>No.</th>
<th>Breed</th>
<th>Age a)</th>
<th>Sex b)</th>
<th>BW c)</th>
<th>Diagnosis d)</th>
<th>NYHA e)</th>
<th>Medicine f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shetland sheepdog</td>
<td>9</td>
<td>F</td>
<td>7.3</td>
<td>CVD</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Shetland sheepdog</td>
<td>13</td>
<td>M</td>
<td>17.4</td>
<td>CVD</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Mongrel dog</td>
<td>15</td>
<td>M</td>
<td>28.0</td>
<td>CVD</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Cocker spaniel</td>
<td>13</td>
<td>M</td>
<td>14.0</td>
<td>CVD</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Maltese</td>
<td>10</td>
<td>F</td>
<td>3.8</td>
<td>CVD</td>
<td>I</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Poodles</td>
<td>16</td>
<td>F</td>
<td>1.7</td>
<td>CVD</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>Shetland sheepdog</td>
<td>14</td>
<td>M</td>
<td>8.4</td>
<td>CVD</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Maltese</td>
<td>11</td>
<td>M</td>
<td>5.9</td>
<td>CVD</td>
<td>II</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>Pomeranian</td>
<td>7</td>
<td>F</td>
<td>3.8</td>
<td>CVD</td>
<td>II</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Shih tzu</td>
<td>10</td>
<td>M</td>
<td>5.5</td>
<td>CVD</td>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>Shih tzu</td>
<td>11</td>
<td>M</td>
<td>5.8</td>
<td>CVD</td>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>Pomeranian</td>
<td>11</td>
<td>M</td>
<td>3.9</td>
<td>CVD</td>
<td>III</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Maltese</td>
<td>11</td>
<td>M</td>
<td>3.6</td>
<td>CVD</td>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>Japanese spaniel</td>
<td>11</td>
<td>M</td>
<td>4.5</td>
<td>CVD</td>
<td>III</td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>Great Pyrenees</td>
<td>5</td>
<td>M</td>
<td>60.0</td>
<td>DCM</td>
<td>IV</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>Shetland sheepdog</td>
<td>8</td>
<td>M</td>
<td>13.9</td>
<td>DCM</td>
<td>IV</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>Mongrel dog</td>
<td>12</td>
<td>M</td>
<td>13.3</td>
<td>CVD</td>
<td>IV</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>Maltese</td>
<td>12</td>
<td>F</td>
<td>3.1</td>
<td>CVD</td>
<td>IV</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>Maltese</td>
<td>17</td>
<td>M</td>
<td>1.8</td>
<td>CVD</td>
<td>IV</td>
<td>+</td>
</tr>
</tbody>
</table>

a) Age; year-old.
b) Sex; M: male, F: female.
c) Body weight; kg.
d) Clinical diagnosis; CVD: chronic valvular disease, DCM: dilated cardiomyopathy.
e) New York Heart Association classification.
f) Medical management; –: without medical management, +: with medical management.
PLASMA NP LEVELS IN CANINE HEART FAILURE

and BNP levels were significantly correlated with PCWP compared with BNP concentration ($\text{PCWP = } 23.5 + 0.12A + 0.43B$; ANP level and BNP level), although only ANP level had a significant effect on PCWP ($r^2=0.725, P=0.0004$).

Relationship between the heart failure classification and plasma levels of ANP and BNP: ANP and BNP concentrations of each heart failure class are summarized in Table 2. ANP levels were shown to have a significant difference among the heart failure classes ($P=0.0165$) as shown in Fig. 2. ANP level tended to increase in proportion to the severity of heart failure, although there was no difference between the ANP levels of NYHA class I and II. ANP concentration increased significantly in NYHA class IV in comparison with class 0 ($P<0.05$), but not classes I, II and III. In addition, ANP levels in two dogs with DCM (244.5 and 208.0 pg/ml) appeared to increase slightly in comparison to three dogs with CVD in NYHA IV (188.3 ± 117.5 pg/ml). ANP level in the decompensated group was significantly elevated in comparison with that in the compensated group ($P=0.0337$).

There was a significant difference of BNP levels among the heart failure classes ($P=0.0005$) as shown in Fig. 2. As the increment in NYHA heart failure classification, BNP level gradually elevated. BNP level in NYHA class IV significantly increased in comparison with NYHA class 0 ($P<0.01$), but not classes I, II and III. BNP concentration in NYHA class III was also significantly elevated in comparison with only class 0 ($P<0.05$). Similar to ANP, BNP level in the decompensated group increased significantly in comparison to the compensated group ($P=0.0011$). Unlike ANP, plasma levels of BNP in two dogs with DCM (100.2 and 93.4 pg/ml) were similar to three dogs with CVD in NYHA IV (93.6 ± 17.0 pg/ml).

Correlation between ANP and BNP concentrations in dogs with chronic MR was significant as shown in Fig. 3 ($P=0.006$). There was however no significant correlation between ANP and BNP concentrations in each NYHA class.

RESULTS

Relationship between PCWP and plasma ANP and BNP levels: After the second experimental MR operation, PCWP (33.3 ± 3.5 mmHg) and plasma levels of ANP (140.0 ± 20.1 pg/ml) and BNP (87.7 ± 1.0 pg/ml) in three dogs with decompensated heart failure increased significantly in comparison with PCWP (9.0 ± 2.0 mmHg) and plasma levels of ANP (20.0 ± 4.3 pg/ml) and BNP (71.6 ± 8.6 pg/ml) in three dogs with compensated heart failure, respectively (PCWP; $P=0.0463$, ANP; $P=0.0495$, BNP; $P=0.0495$).

ANP concentration had a significant correlation with PCWP ($r=0.852, P=0.0004$) as shown in Fig. 1. ANP concentrations of the 6 dogs were distributed between 6.7 and 163.3 pg/ml. There was a significant correlation between BNP concentration and PCWP ($r=0.832, P=0.0008$) as shown in Fig. 1. Unlike ANP level, there was a little distribution of BNP concentrations in the 6 dogs (distribution range between 54.5 and 89.3 pg/ml).

In the result of the multiple regression analysis, ANP concentration had a predominant effect on PCWP ($F=6.527$) compared with BNP concentration ($F=4.885$). Both ANP and BNP levels were significantly correlated with PCWP ($r^2=0.822, P=0.0004$) in the following regression equation; $\text{PCWP = } -23.5 + 0.12A + 0.43B$ (A; ANP level and B; BNP level), although only ANP level had a significant effect on PCWP ($r^2=0.725, P=0.0004$).

DISCUSSION

In the present study, we demonstrated that plasma levels of ANP and BNP significantly increased in dogs with experimental and chronic MR in relation to PCWP and the severity of heart failure. Then, ANP and BNP levels in dogs with experimental and chronic MR were augmented significantly in the decompensated group, as compared with the compensated group.

In human medicine, it was demonstrated that ANP concentration had a good correlation with PCWP in the patient with CHF [13], and ANP and BNP levels significantly correlated with PCWP in the patients with DCM [21]. In veterinary medicine, ANP levels was demonstrated to be correlated with the right atrial pressure in the dogs with experimentally induced mild heartworm disease [14]. In our study, good correlations between PCWP and plasma ANP and BNP levels were also observed in dogs with experimental MR. Plasma ANP and BNP concentrations are therefore likely to become non-invasive parameters for the estimation of PCWP in canine patients with spontaneous MR. Especially, ANP level was demonstrated to have more effect on PCWP than BNP level using multiple regression analysis. This observation was thought to be attributable to the different incremental magnitude of the synthesis between canine ANP and BNP due to the stimulation of atrial pressure. In human, it has been demonstrated that ANP is mainly synthesized and secreted from the atrium and BNP mainly from the ventricle [11, 20]. In normal dogs, canine ANP is suggested to be synthesized mainly in the atrium rather than the ventricle in
healthy dogs in contrast to human BNP [2, 9]. Therefore, the main origin of the secretion of both ANP and BNP in dogs with experimental MR is thought to be the LA wall due to volume-overload by MR. In our study, the synthesis and secretion of canine ANP is thought to be stimulated more strongly by increased LA pressure in comparison with BNP.

A previous study demonstrated that ANP concentration was significantly correlated with left atrium-aortic root ratio and LV end-diastolic dimension using two-dimentional echocardiography [5]. In our study, MR was caused by the surgical resection of mitral valvular chordae tendineae, and ANP and BNP concentrations were measured 2 weeks after the operation. One major limitation of this study was the discrepancy between spontaneous and experimental MR. In addition, PCWP does not always reflect the clinical signs of heart failure in dogs with chronic spontaneous MR. This may be due to several factors relating to complicated intervention of underlying heart disease, age and activity of compensatory neurohumoral system. However, canine CHF patients with high PCWP may show greater possibility of deterioration of the clinical signs of heart failure. Clinical applications of ANP and BNP concentrations as parameters of PCWP in canine patients with chronic spontaneous MR
Fig. 3. Relationship between ANP and BNP in dogs with asymptomatic and symptomatic CHF. Generally, significant correlation between ANP and BNP levels was observed (P<0.0001). There was however no correlation in each NYHA class I, II, III and IV. For keys see for Figs. 1 and 2.

Fig. 2. Plasma levels of ANP and BNP in canine patients with asymptomatic and symptomatic congestive heart failure (CHF) grouped according to New York heart association (NYHA) functional classification. Significant difference of ANP level was observed between NYHA class 0 and IV. BNP levels in NYHA class III and IV significantly increased in comparison with that in NYHA class 0. For keys see for Fig. 1.

Table 2. Plasma ANP and BNP levels in dogs with asymptomatic and symptomatic congestive heart failure

<table>
<thead>
<tr>
<th>Classa</th>
<th>n</th>
<th>Plasma ANP level (pg/ml)</th>
<th>Plasma BNP level (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean SE median range</td>
<td>mean SE median range</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>19</td>
<td>16.0 3.5 9.0 2.1–50.6</td>
<td>36.8 4.0 26.1 20.5–66.3</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>44.6 31.9 5.0 4.4–169.2</td>
<td>43.8 3.0 43.6 34.4–51.5</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>39.6 34.5 6.9 1.6–142.9</td>
<td>50.4 5.2 52.0 36.6–61.1</td>
</tr>
<tr>
<td>III</td>
<td>5</td>
<td>85.5 23.3 108.3 4.8–132.9</td>
<td>70.7 7.1 74.2 46.3–88.2</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>203.5 65.3 208.0 33.9–418.9</td>
<td>94.9 9.4 98.8 61.8–120.1</td>
</tr>
</tbody>
</table>

a) New York Heart Association functional classification.

are of great worth to be investigated in future canine clinics.

In the present results, the synthesis and secretion of ANP and BNP augmented in relation to the severity of the canine heart failure. In human medicine, ANP concentration was demonstrated to provide the prognostic information in chronic heart failure [3] and the assessment of the severity of CHF [7]. In veterinary medicine, the previous study using the Cavalier King Charles Spaniels with chronic MR showed that ANP concentration significantly increased in the NYHA class III and IV compared with class 0, I and II
that were not significantly different [5]. In our study, ANP concentration in the decompensated group was also significantly elevated in comparison with that in the compensated group. Thus, ANP concentration may provide useful information relating not only PCWP but also the activation of compensatory neurohumoral system in dogs with CHF. Another study has however demonstrated that the activation of renin-angiotensin-aldosterone system was not observed in spite of the increased pro-ANP concentration in the Cavalier King Charles Spaniels with early decompensated MR [6]. It is therefore essential to clarify what interactions between ANP and compensatory neurohumoral system change in the progression of canine CHF.

On the other hand, BNP concentration has not yet been reported in canine patients with heart diseases. In human medicine, it has been demonstrated that BNP level provide predominantly important and independent prognostic information after AMI in comparison with ANP level [1, 12]. In addition, BNP concentration was shown to become a more powerful indicator of LV systolic and diastolic functions and LV hypertrophy than N- and C-terminal ANP [19]. In veterinary medicine, BNP concentration is also thought to have a possibility to provide many beneficial information for veterinary cardiologists. In the present study, BNP level significantly increased in proportion to the severity of heart failure in the dogs with spontaneous CHF. However, the magnitude of the elevation of BNP concentration (mean level; NYHA class 0: class IV=1:2.6, maximum=120.1 pg/ml) was less than that of ANP (mean level; NYHA class 0: class IV=1:12.7, maximum=418.9 pg/ml). In contrast, BNP level was demonstrated to augment markedly at the peak level of more than 300 pg/ml in human patients after AMI [10]. In the previous studies with experimental canine CHF model produced by ventricular rapid pacing, BNP concentration was shown to increase significantly but great less than ANP concentration [8] and to be hardly elevated [4]. Those observations indicate that responsiveness of canine BNP for the heart failure and/or the sensitivity of the assay of canine BNP are lower than those of canine ANP. The present method for radioimmuno-assay of canine BNP needs the extraction procedure from plasma. More sensitive and specific assay of canine BNP must be established similar to ANP for the purpose of further investigation that BNP concentration is associated with prognosis of canine heart diseases.

In this study, effects of underlying cardiac disease, aging, breed, treatment, and periods which showed clinical signs of heart failure on ANP and BNP levels could not be determined due to limited clinical cases. And, it was demonstrated that the increase in ANP level was not always parallel to the BNP level in each heart failure class. The effects of these factors need to be known due to their potential influences in ANP and BNP levels.

Further investigations are required to fully establish the clinical means of measuring plasma ANP and BNP concentrations as a new diagnostic technique in providing additional information in adjunct to the conventional examinations (physical examination, thoracic radiography, electrocardiography and echocardiography) in order to better evaluate the conditions of canine heart diseases.

In conclusion, plasma ANP and BNP levels increased significantly in relation to PCWP and the severity of heart failure in dogs with experimental MR and spontaneous chronic MR. ANP and BNP levels may therefore become predictors of PCWP and the severity of heart failure in dogs with chronic MR.

REFERENCES


