NOTE Internal Medicine

Gene Expression of Adrenomedullin in Canine Normal Tissues and Diseased Hearts

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ABSTRACT. The purposes of this study were to determine the tissue distribution of canine adrenomedullin (AM) and to determine whether increased canine AM mRNA expression is associated with congestive heart failure (CHF) due to mitral regurgitation (MR). Canine AM mRNA expression was detectable in various normal tissues, including cardiovascular tissues. In addition, the AM mRNA expression in the left atrium of dogs with MR was significantly higher than that in normal subjects. In conclusion, AM is a potential neurohumoral factor in dogs with CHF due to MR.

KEY WORDS: adrenomedullin, canine, congestive heart failure.

Adrenomedullin (AM), originally isolated from human pheochromocytoma, is a potent endogenous vasorelaxing, and natriuretic peptide [2, 8, 10, 11]. AM is produced by the proteolytic cleavage of its precursor, which has 52 amino acids and a COOH-terminal amide structure, the common structure in the calcitonin gene-related peptide superfamily [2, 3, 6, 10, 11].

In humans, AM has been demonstrated to be synthesized and secreted mainly by endothelial cells, vascular smooth muscle cells and fibroblasts [2–4, 6, 18, 19]. However, it has been demonstrated that the expression level of AM mRNA is highest in atrial tissues; moreover, the circulatory AM concentration increases in certain cardiovascular pathological conditions, including pulmonary hypertension, congestive heart failure (CHF), dilated cardiomyopathy and myocardial infarction [2, 3, 5, 6, 14, 17]. Therefore, AM is thought to play an important role in circulatory regulation, similar to the roles of the atrial natriuretic (ANP) and brain natriuretic peptides (BNP).

The complementary DNA (cDNA) sequence of canine AM has been determined [15], and its distribution in the heart and kidney has also been investigated [9]. In addition, the plasma and cardiac tissue concentrations of canine AM have been demonstrated to increase in canine subjects in whom heart failure was experimentally induced by rapid ventricular pacing [7, 8]. To our knowledge, however, there are no reports on the synthesis of canine AM in tissues other than those of the heart and kidney or on the mRNA expression of AM in the cardiac tissues of dogs with CHF due to mitral regurgitation (MR).

The purposes of this study were to determine the tissue distribution of canine AM and to investigate the mRNA expression of AM in the canine myocardium of subjects with MR.

Two healthy Beagles and 5 privately owned dogs with CHF due to MR (1 Shih Tzu, 2 Maltese, and 2 mongrels) were used in this study. The Beagles were confirmed to be healthy on the basis of physical examination, complete blood count and serum biochemical profiling. They were sacrificed by an intravenous overdose of sodium pentobarbital and potassium chloride. To determine the normal distribution of mRNA, we then collected the following 22 tissue samples from the Beagles: left atrium (LA), right atrium (RA), left ventricle (LV), right ventricle (RV), lung, liver, spleen, renal cortex, renal medulla, adrenal gland, stomach, aorta, urinary bladder, ovary, uterus, skeletal muscle, lymph node, small intestine, cerebrum, medulla oblongata, mammary gland and salivary gland.

The 5 canine patients were referred to the Animal Medical Center of Nihon University. Each dog was definitively diagnosed with CHF attributable to MR. Autopsies were performed with the consent of each owner. One of the dogs died from hepatic failure induced by hepatocellular carcinoma, and the other dogs died from advanced CHF. In the dog that died from hepatic failure, autopsy revealed no indications of metastasis of the carcinoma in the cardiac tissues. LA, RA, LV and RV specimens were obtained from each dog; the specimens were dissected into approximately 1-cm³ cubes, frozen by immersion in liquid nitrogen and stored at −80°C until use.

All the dogs were cared for according to the principles outlined in the Guide for the Care and Use of Laboratory Animals approved by the College of Bioresource Sciences, Nihon University.

Total RNA was extracted from 22 tissue samples from the 2 normal dogs and from myocardial samples from the dogs with MR. The first-strand cDNA was synthesized using 2 μg of the total RNA as a template. Reverse transcription was performed using reverse transcriptase obtained from the Moloney murine leukemia virus (M-MLV Reverse Transcriptase; Invitrogen Corp., Tokyo,
To amplify the cDNA fragments of canine AM, we designed oligonucleotide primers (Table 1) on the basis of known canine AM sequences (GenBank/EMBL/DDBJ accession no. AF045773); the same approach was used to amplify the canine ANP and BNP cDNA fragments (Table 1) [1]. The polymerase chain reaction (PCR) assay was performed with each primer pair using commercially available Taq polymerase (Blend Taq; Toyobo Corp., Osaka, Japan). The thermal profile consisted of predenaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s and extension at 72°C for 1 min and a final extension procedure at 72°C for 10 min. Each PCR product was electrophoresed through 2.0% agarose gel and stained with ethidium bromide for visualization.

The PCR products were quantified on the basis of the intensity of SYBR Green I. Real-time PCR was carried out using a Light Cycler TM (Roche Diagnostics, Basel, Switzerland) with SYBR® PrimeScript® RT-PCR Kit (Takara Bio Inc., Shiga, Japan), according to the manufacturer’s instructions. The canine AM gene was amplified using specific oligonucleotide primers (Table 1). The PCR protocol was as follows: initial denaturation at 95°C for 10 s followed by 40 cycles of denaturation at 95°C for 5 s and annealing at 64°C for 20 s. The expression level of AM mRNA was normalized by dividing the copy number of the mRNA of each gene by that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Data were expressed as means ± standard deviation (SD). The Mann-Whitney test was used to statistically compare the mRNA expression levels in the normal and diseased cardiac tissues. Differences were considered to be statistically significant at $p<0.05$.

Expression of the mRNA of AM was detectable in all the tissues examined, and the expression was relatively strong in the cardiac tissues. Expression of ANP mRNA was observed in a broad spectrum of tissues. In contrast, expression of BNP mRNA was restricted to the heart and aorta (Fig. 1).

Table 1. Primer sequences used in this study

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Orientation</th>
<th>Primer sequence</th>
</tr>
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<tbody>
<tr>
<td>AM</td>
<td>Forward</td>
<td>5’-GCCACCTCTGTGTTTCTCAC-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TGTCACTGCTGTTGGACTGGTA-3’</td>
</tr>
<tr>
<td>ANP</td>
<td>Forward</td>
<td>5’-ATGGGGCTCCCACATCGCCCGAAGCT-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TTTTCCTCCCTGCGACATTACTTCA3’</td>
</tr>
<tr>
<td>BNP</td>
<td>Forward</td>
<td>5’-TTCCTGCTCTCCTGTTGTTGC-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TTGGGGCTGCGTAGCTTCTTCT-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward</td>
<td>5’-GTGATGCTGCTGCTGAGTATG-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-GTGATGGCATGGACTGTGG-3’</td>
</tr>
</tbody>
</table>

Fig. 1. PCR detection of AM, ANP and BNP mRNA expression in various tissues from 2 healthy dogs. The expression of GAPDH served as an internal control. Expression of canine AM and ANP was detected in various normal tissues. The band sizes of AM, ANP, BNP and GAPDH were 481, 472, 481 and 243 bp, respectively.

The AM mRNA expression level in the LA of the dogs with MR was significantly elevated compared with those in the LA, LV, RA and RV in the normal dogs (Fig. 2). However, the AM mRNA expression levels in the LV, RA, and RV of the dogs with MR were not significantly different from those of the normal subjects.

The mRNA expression of canine AM was found to be detectable in 22 normal tissues, including those from the heart, adrenal gland, aorta, liver and spleen. In addition, comparatively weaker mRNA expression was detected in the lung and medulla oblongata. The relative intensities of AM mRNA expression in the canine tissues were rather dif-
different from those in human or porcine tissues. A previous study that investigated the tissue distribution of human AM using northern blotting [3, 13] reported the following observations: the AM mRNA transcript was detected most strongly in pheochromocytoma tissue followed by the adrenal medulla, ventricle and lung; the transcript was detected to a lesser degree in the kidney, while the level in the pancreas was even lower; and finally, the transcripts could not be detected in the brain, liver, intestine and spleen. In a study that investigated AM mRNA expression in porcine tissues using northern blotting [12], AM mRNA expression was detected most strongly in the adrenal medulla followed by the lung and kidney and was weakly detected in the intestine, spleen and thyroid. In similar studies using rat tissues [3, 16], the AM mRNA expression level was the strongest in the lung followed by the adrenal glands, heart, spleen, kidney, duodenum and the submandibular glands. Expression was weak in the liver and brain and undetectable in the pancreas and testis. These results suggest that the patterns of AM gene expression differ among mammalian species. Because AM mRNA, like ANP, is synthesized in various tissues, the production of AM might be regulated by circulatory homeostasis.

In the present study, the AM mRNA expression was found to be elevated in the cardiac tissues of dogs with MR. In particular, the AM mRNA expression in the LA of dogs with MR was significantly higher than that of the normal subjects, whereas the AM mRNA expression in the LV of dogs with MR was not significantly different from that of the normal subjects. However, it has been reported that ventricular AM expression is activated in dogs by the progression of experimental CHF induced by rapid ventricular pacing [8]. The differences between the results of our study and those of the previous report might be attributed to the different pathophysiological conditions of the cardiac tissues. The rapid ventricular pacing used in the previous study could have induced myocardial damage in the ventricle, eventually resulting in CHF. In contrast, in our study, MR caused a volume overload in the LA and LV, thereby aggravating CHF. It is therefore suggested that the AM expression pattern in the atrium and ventricle is associated with the different pathophysiological conditions in canine CHF. In addition, the results of our study and those of the previous report may support the hypothesis that AM, together with other neurohumoral factors, plays a compensatory role in regulation of intravascular volume and circulatory homeostasis in cardiac pathological conditions.

In conclusion, expression of AM mRNA was detectable in various normal canine tissues. Moreover, cardiac synthesis of AM was found to be elevated in dogs with CHF. Further studies on canine AM are required to clarify its pathophysiological role in canine heart diseases.

REFERENCES