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## Clinicopathological studies on atrial and brain natriuretic peptide in canine heart disease

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In small animal medicine, there has been an increase in the number of canine patients diagnosed and treated for heart diseases. A more accurate recognition of the pathophysiology of canine heart diseases allows for a better and more appropriate therapeutic selection which may improve quality of life and reduce mortality from canine heart diseases.

In human cardiovascular medicine, cardiac hormones, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), are attractive markers to reflect the pathophysiological conditions of heart diseases. ANP in response to load of the atrium and BNP in response to load of the ventricle are secreted from atrial and ventricular cardiomyocytes, and are responsible for the regulation of body fluid homeostasis and blood pressure. In human congestive heart failure (CHF), plasma ANP and BNP levels increase in relation to the deterioration of the clinical signs, elevated atrial and ventricular pressures, and left ventricular dysfunction. ANP and BNP are therefore suggested to be indicators for the severity, the therapeutic efficacy and the prognosis of human cardiac diseases.

ANP and BNP are thought to be useful in veterinary medicine similar to human medicine, but there are limited reports describing canine ANP. Therefore, the objectives of the present

study were to investigate the pathophysiological significance of canine ANP and BNP and to assess the clinico-pathological usefulness of canine ANP and BNP.

1. Tissue distributions of ANP and BNP in healthy dogs.

Complementary DNA (cDNA) clonings of canine ANP and BNP were first carried out, and mRNA expressions of ANP and BNP were investigated in canine various tissues using RT-PCR and Northern blot analysis for the purpose of determining of tissue distributions of canine ANP and BNP.

The lengths of cDNA and amino acid sequence of canine prepro-BNP were 420 bp and 140 residues, respectively. The length of amino acid sequence of canine prepro-BNP was the longest among those of mammals so far determined. The homology of amino acid sequence between canine and the other mammalian prepro-BNP was lower in comparison with prepro-ANP.

Secondly, mRNA expression of canine ANP was detected in atrium, ventricle, central nervous system and kidney, and abundant amount of BNP mRNA was detected in the atrium. On the other hand, mRNA expression of canine BNP was detectable only in atrium, but not the ventricle and central nervous system. Thus, both ANP

and BNP were synthesized mainly in the atrium in normal dogs, and the tissue distribution of canine BNP was different from other mammals. Therefore, the pathophysiological significance of canine BNP may be suggested to be different from that of human BNP due to the discrepancy of the tissue distributions.

2. Synthesis and secretion of ANP and BNP in dogs with experimental heart diseases.

Plasma concentrations and mRNA expressions of ANP and BNP were investigated in dogs with experimental acute myocardial infarction (AMI) and mitral regurgitation (MR) to clarify their pathophysiological significances.

In AMI models of dogs, plasma ANP and BNP concentrations changed slightly between pre- and post-operation. The mRNA expression of ANP was not detectable in non-infarcted ventricle and was only slightly detected in infarcted ventricle. The mRNA expression of BNP was however detected in both infarcted and non-infarcted ventricles, and the amount of BNP mRNA in the infarcted ventricle significantly increased in comparison with that in the non-infarcted ventricle.

In MR models of dogs, plasma ANP and BNP concentrations were significantly elevated in the decompensatory heart failure group, compared with the compensatory heart failure group. And, plasma concentrations of ANP and BNP were significantly correlated with pulmonary capillary wedge pressure, an indicator of left atrial pressure. The mRNA expression of BNP was detected not only in the atrium but also in the ventricle in contrast to ANP mRNA which was only expressed in the atrium.

It is therefore suggested that the synthesis and secretion of ANP increase mainly in the atrium, but those of BNP are augmented from the atrium as well as the ventricle in canine cardiac abnormal condition attributable to AMI and MR.

3. Plasma levels of ANP and BNP in canine clinical cases with heart diseases.

Plasma ANP and BNP concentrations were measured in 4 dogs with patent ductus arteriosus (PDA) and 19 dogs with chronic MR which were diagnosed and treated at the Hokkaido University Veterinary Teaching Hospital in order to investigate the relationship between plasma ANP and BNP levels and their pathophysiological status.

In 4 canine patients with PDA, pre-operative high ANP concentrations in 3 dogs were dramatically reduced after the operation in contrast to pre-operative ANP concentration within normal range in another dog. In addition, 3 dogs with higher pre-operative ANP concentrations had enlargement of left atrium. ANP is therefore thought to be synthesized and secreted mainly from the dilated left atrium in dogs with PDA. On the other hand, post-operative changes in BNP concentrations had slight wavy curves in all PDA cases and were different from those in ANP concentrations. The synthesis and secretion of BNP are suggested to depend on the different stimulating mechanisms from those of ANP in dogs with PDA.

In 19 canine patients with chronic MR, ANP and BNP concentrations tended to increase in relation to the severity of heart failure. In addition, ANP and BNP concentrations in the dogs with decompensatory CHF were significantly elevated in comparison with those in the dogs with compensatory CHF, respectively. Thus, ANP and BNP concentrations may be indicators for the severity of canine heart failure. The magnitude of the increase in BNP concentrations was however lower than that of ANP concentration in 19 canine patients with chronic MR.

Therefore, ANP may be a convenient biochemical marker of the therapeutic efficacy in canine PDA, and both ANP and BNP are suggested to be useful biochemical indicators for the severity of heart failure in canine chronic MR.

In conclusion, both canine ANP and BNP are synthesized mainly in the atrium. In canine abnormal heart conditions, it is suggested that

the synthesis and secretion of ANP are augmented mainly in the atrium, but those of BNP increase in the atrium as well as the ventricle. In addition, ANP and BNP are likely to be useful clinicopathological markers of the severity of

heart failure and the evaluation of therapeutic efficacies in canine clinical cases with heart diseases. Further clinical investigations of canine ANP and BNP are worthwhile and warranted.

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### Generation of Reactive Oxygen Species by Neutrophils in Host Defense Mechanisms —Spectrophotometric Analyses—

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Generation of reactive oxygen species (ROS) by leukocyte is helpful for the first defense mechanism in case of bacterial infections and others, while it also induces tissue damages, and plays an important role in systemic inflammatory response syndrome, multiple organ dysfunction syndrome and multiple organ failure.

Phagocytic leukocytes include neutrophils, eosinophils, monocytes and macrophages. Among them, neutrophils and eosinophils, both of which contain specific peroxidases in exceptionally high concentration, exhibit the strongest microbicidal function. Generation of ROS by neutrophils consists of two step reactions. They are the superoxide anion ( $O_2^-$ ) generation derived from reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-catalyzing reaction, and the following disinfection and decomposition mediated by myeloperoxidase (MPO). In this mechanism, the drop of bactericidal activity may be considered as a possible result of defect and abnormality. However, the most useful ROS in disinfection have not been determined. The fact that the defect of NADPH oxidase deficiency,

chronic granulomatous disease (CGD), leads to clinically severe infectious disease, whereas MPO deficiency, usually, but not always, tends to indicate no symptoms of infection shows the complexity of the ROS generation system in vivo.

To realize physiological production of singlet molecular oxygen ( $^1O_2$ ) in porcine MPO-hydrogen peroxide ( $H_2O_2$ )-chloride ion ( $Cl^-$ ) system, the research group I am working with have developed a novel detection technique in the chapter 1 of this thesis.

Neutrophils kill ingested microorganisms by releasing microbicidal proteins from cytoplasmic granules and by generating  $O_2^-$  and other ROS into the intracellular phagosomal compartment. The formation of  $O_2^-$  is catalyzed by a membrane-associated NADPH oxidase, and in the subsequent reactions  $O_2^-$  is dismutated to  $H_2O_2$ . All mammalian phagocytes have the oxidative metabolism. Following phagocytosis, the membranes of azurophilic granules fuse with the membrane of the phagocytic vacuole, which phagolysosomes release MPO into the vacuole containing the ingested microorganisms. The