LEVEL AND MOLECULAR FORM OF ATRIAL NATRIURETIC PEPTIDE (ANP) IN BOVINE HEART FAILURE

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Introduction

It has been demonstrated that ANP is released from the mammalian hearts, and also reported that α-hANP was consisted of 28 amino acid residues, and its primitive structure was completely the same in the dog, cattle and pig (Gutkowska et al., 1987). In humans, β-ANP appears in the plasma and atrium of patients with heart failure (Akimoto et al., 1988; Marumo et al., 1988). The appearance of β-ANP has never reported in diseased cattle. Present paper describes that appearance of β- and γ-ANP in cattle with dilated cardiomyopathy (DCM) and bacterial endocarditis (BEC), as representatives of cardiac failure.

Materials and Methods

Used cattle

Twenty control, 7 BEC and 4 DCM cattle were used, respectively. Cardiac catheterization was made to obtain right atrial pressure (PRA) and left ventricular end diastolic pressure (LVEDP). In BEC and DCM cattle, mild and severe heart failure were detected.

Collection of samples

Blood samples were obtained from jugular vein. Right atrium was excised at autopsy, from 3 control, 3 BEC, and 4 DCM cattle, respectively. The tissue sample was homogenized with 0.1 M acetic acid. The plasma and the extracted supernatant samples were stored at −80°C.

Radioimmunoassay

ANP levels of plasma and tissue samples were measured by radioimmunoassay developed for measurement of human ANP (Marumo et al., 1986). It was proved applicable to measure bovine ANP (Takemura et al., 1989). The RIA with the antiserum recognized the carboxylterminal end segment of ANP and α-ANP (25-27). The assay variances, recovery, and nonspecific binding of [^{125}I]α-hANP were in an ordinary basis.

Gel permeation chromatography

Molecular forms of plasma ANP and the collected tissue were analyzed by gel permeation chromatography (GPC). A sample was applied to a Sephadex G-75 column and eluted with acetic acid. Average recovery was 85 %.

Statistical analysis

Data obtained were expressed as mean ± SEM. For examining statistical significance, the Wilcoxon's non-paired test was used.

Results and Discussion

Cardiac catheterization

In control, BEC and DCM, PRA were 2.5 ± 0.45, 15.5 ± 1.68 and 48.8 ± 4.54 mmHg, respectively. The latter two was significantly increased than that of control ($p < 0.01$). PRA of DCM was significantly increased compared to that of BEC ($p < 0.05$). LVEDP were 6.5 ± 0.58, 22.2 ± 2.72 and 46.1 ± 3.05 mmHg in control, BEC and DCM, respectively, indicating their of cardiac disease were significantly severer when compared to control, and the LVEDP of DCM was significantly higher than that of BEC. These results suggest that the heart failure of DCM was much severer than that of BEC in the present study.

Plasma ANP level

Plasma ANP level in control was 14.5 ± 1.84 pmol/l, similar to that of humans (Marumo et al., 1986). In BEC and DCM, plasma ANP levels were 20.6 ± 3.45 and 73.3 ± 16.02 pmol/l, respectively. These values were significantly higher than that of control ($p < 0.01$). The ANP level

of DCM was extremely higher than that of BEC. These results suggest that release of ANP was accelerated, especially in DCM cattle. Plasma ANP level was significantly correlated with PRA and LVEDP, respectively ($p < 0.01$). These results suggest that ANP release was stimulated by increase in atrial pressure, because increase in LVEDP reflects the increased after load, i.e., increased left atrial pressure.

Right atrial ANP content

Tissue ANP content in BEC (0.09 ± 0.006) was similar to that of control (0.11 ± 0.01), while that of DCM (0.29 ± 0.08 μg/g) was higher than those of control and BEC ($p < 0.05$). Therefore, it was considered that there was no change in ANP synthesis, and in the molecular form of plasma ANP in BEC cattle, while plasma ANP level was increased.

GPC profiles

GPC profiles of plasma and right atrium in control, BEC and DCM, were shown in the table 1 and figure 1 respectively. In control plasma and the atrium, a peak of α-ANP and 2 peaks of α- and γ-ANP were observed, respectively. Similar findings were also obtained in those of BEC. In DCM plasma, however, peaks of β- and/or γ-ANP were observed in addition to the α-ANP peak. β-ANP was observed in 3 cases of DCM. In these cases, the peaks of β-ANP was also seen in the atrial tissue. It is the first case to report that β- and/or γ-ANP appeared in the plasma of the extra human species with cardiac failure. It was considered that β-ANP was synthesized in the cardiocyte, and released to circulation. In the present study, DCM was the only case that β-ANP was detected, suggesting they have much severe heart failure.

TABLE 1. MOLECULAR FORMS OF ANP PLASMA AND RIGHT ATRIUM

No.	Disease	Plasma	Atrium
1	Control	α	α, γ
2	:	α	α, γ
3	:	α	α, γ
4	:	α	α, γ
5	:	α	α, γ
6	BEC	α	α, γ
7	:	α	α, γ
8	:	α	α, γ
9	:	α	α, γ
10	:	α	α, γ
11	:	α	α, γ
12	:	α	α, γ
13	DCM	α, β	α, β, γ
14	:	α, γ	α, γ
15	:	α, β	α, β, γ
16	:	α, β, γ	α, β, γ

BEC : bacterial endocarditis
DCM: dilated cardiomyopathy

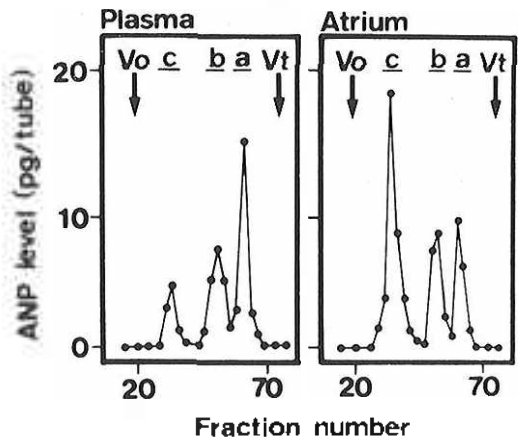

Figure 1. Gel permeation chromatographic profiles. α-, β- and γ-ANP were eluted at position a, b and c, respectively. Arrows represents the elution positions of void volume (Vo) and total volume (Vt).

In conclusion, the present study revealed that the secretion and synthesis of ANP was stimulated by increased atrial pressure, and that synthetic process was altered to release β- and γ-ANP in the cattle with severe heart failure.

(Key Words: Atrial Natriuretic Peptide, Cattle, Heart Failure)

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