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ATRIAL NATRIURETIC POLYPEPTIDE (ANP) IN HUMAN VENTRICLE INCREASED GENE EXPRESSION OF ANP IN DILATED CARDIOMYOPATHY

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Tissue levels of atrial natriuretic polypeptide (ANP) messenger RNA (ANPmRNA) and ANP in the human atrium and ventricle were measured simultaneously by the blot hybridization technique and the specific radioimmunoassay for ANP. Hearts were obtained from two patients without cardiac complications and from a patient with dilated cardiomyopathy (DCM) at autopsy. Total RNA extracted from ventricles contained a hybridizing RNA band of the same size as atrial ANPmRNA in both control and DCM hearts. The ANPmRNA level in the control ventricle was 0.2 % of that in the atrium. The ANPmRNA level in the DCM ventricle increased to about 7 % of that in the corresponding atrium and was approximately 40 times higher than that in the control ventricle, although the ANPmRNA level in the DCM atrium was comparable to that in the control atrium. The total content of ANPmRNA in the DCM ventricle reached about 30 % of that in the corresponding atrium and was much the same as that in the control atrium. The ANP level in the DCM ventricle was approximately 1.0 μg/g and much higher than that in the control ventricle (0.02 μg/g). © 1987 Academic Press, Inc.

Atrial natriuretic polypeptide (ANP) is a cardiac hormone with diuretic, natriuretic and vasorelaxant activities, and is implicated

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Abbreviations used in this paper: ANP, atrial natriuretic polypeptide; DCM, dilated cardiomyopathy; ANPmRNA, ANP messenger RNA; RIA, radioimmunoassay.

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in the control of blood pressure, body fluid and cardiovascular homeostasis (1-4).

Since ANP was isolated from human and rat atrial tissues, the atrium has been thought to be the major source of the circulating ANP (6-10). Recently, however, evidence has accumulated indicating that ANP is synthesized in various extra-atrial tissues including the ventricle (11-14) and the brain (15-18).

Patients with severe congestive heart failure including advanced dilated cardiomyopathy (DCM) show the extremely elevated plasma ANP concentration (19-21) and this elevation is mainly due to the increased ANP secretion from the heart (22,23). These results suggest that ANP synthesis is markedly enhanced in these patients.

In the present study, to clarify ANP synthesis in the human heart, we measured ANP messenger RNA (ANPmRNA) and ANP levels in atria and ventricles obtained at autopsy from two control patients and one patient with DCM using the blot hybridization technique and the specific radioimmunoassay (RIA) for ANP.

MATERIALS AND METHODS

Hearts

Hearts were excised within 3 hr after death from two patients without cardiac complications (gastric cancer and acute promyelocytic leukemia) as control and a patient with DCM (New York Heart Association class III). Two control hearts showed no pathological findings macroscopically or microscopically. The DCM heart weighed 460 g (atrium 94 g, ventricle 366 g). Four parts of atrial tissues (0.5-1.0 g) were dissected from bilateral auricles and non-auricle parts of the atrium. Ventricular tissues (0.5-1.0 g) were also dissected from both sides of free walls with special cares to avoid atrial contamination. Samples were immediately frozen in liquid nitrogen and kept at -70°C until used. Informed consents were obtained from their families. This study was approved by the ethical committee on human research of Kyoto University (No. 61-9).

Measurement of tissue ANPmRNA levels

Total RNA was extracted from each part of the heart and ANPmRNA levels were analysed by the blot hybridization technique as previously described (9). The 581-bp Sac I-Pst I fragment was used as a probe and radiolabeled (specific activity, 5.0×10^8 cpm/μg) by nick-translation with α-[^{32}P]dCTP (Amersham, International pls, Buckinghamshire, England). After autoradiography, the portion corresponding to a hybridization band was cut and its radioactivity was counted in toluene scintillator solution. ANPmRNA levels (arbitrary units) were estimated for 1 μg of total RNA and expressed as relative levels to

that in the right auricle of the control heart (the right auricular level = 1.0 unit/μg total RNA).

Measurement of tissue ANP concentrations

Extraction of ANP from cardiac tissues was carried out as previously reported (7). Tissues were boiled for 5 min in 10 volumes of 1 M acetic acid containing 20 mM HCl to abolish intrinsic proteolytic activity (7,8) and then homogenized with a polytron homogenizer. The ANP concentration was measured by RIA as previously reported (7,15, 24,25). This RIA recognizes a carboxy-terminal fragment of α-ANP, α-ANP[17-28], and is equally specific for both α-human ANP and α-rat ANP. Cross-reactivities with β-human ANP and γ-rat ANP in the RIA are 120 % and 100 % on a molar basis. The minimal detectable quantity of α-human ANP is 1 pg/tube.

RESULTS AND DISCUSSION

The results of Northern blot analysis of RNA from atrial and ventricular tissues of patients without cardiac complications are shown in Figure 1. Total RNA extracted from control ventricles contained a hybridizing RNA band of the same size (about 1000 bp) as atrial ANPmRNA. The radioactivity in the hybridized band showed a linear relationship with the amount of RNA applied to the electrophoresis gel. Average levels and total contents of tissue ANPmRNA and ANP are summarized in Table I. The ANPmRNA level in the control ventricle was 0.22 % of that in the atrium. The ANP level in the control ventricle was 20 ng/g. Thus, the simultaneous detection of ANPmRNA and ANP in the human normal ventricle clearly indicates that ANP is synthesized in the human ventricle as well as in the human atrium. This finding is compatible with recent results that rat

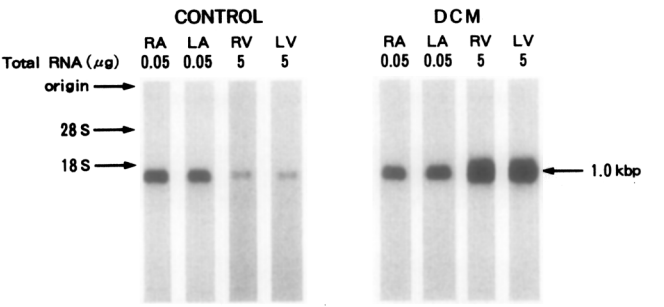

Figure 1. Northern blot hybridization analysis of total RNA from control heart (left) and DCM heart (right). Fifty ng and 5 μg of total RNA from both sides of atria and ventricles were fractionated on 2.0 % agarose gel.

Table I Tissue levels and total contents of ANPmRNA and ANP in human hearts

ANP and ANPmRNA	Control			DCM		
	Atrium	Ventricle	Ventricle/Atrium(%)	Atrium	Ventricle	Ventricle/Atrium(%)
ANPmRNA level (Units/g)	110	0.24	0.22	120	8.78	7.19
ANPmRNA content (Units)	7500	120	1.55	23000	6400	27.9
ANP level (μg/g)	38.7	0.02	0.05	84.8	1.00	1.18
ANP content (μg)	1360	4.56	0.34	8140	366	4.50

ANPmRNA levels (arbitrary units) were estimated as described in Methods.
DCM = dilated cardiomyopathy.

neonatal and adult ventricular cardiocytes produce ANP (11-14). The ventricular ANP level is one of the highest ANP levels in extra-atrial tissues including the brain (15,16).

As shown in Figure 1, ANPmRNA was also detected in both the atrium and ventricle in the DCM heart and its size was the same as that in the control atrium. Although the ANPmRNA level in the atrium of DCM was similar to that in the control atrium, the level of ANPmRNA in the DCM ventricle was approximately forty times higher than that in the control ventricle (Table I). The total ANPmRNA content in the DCM ventricle reached about 30 % of that in the DCM atrium and was about 85 % of that in the control atrium (Table I). The ventricular ANP level was 1.0 μg/g. When compared with control hearts, the DCM heart showed a fifty-fold increase in the ventricular ANP level and an eighty-fold increase in the ventricular ANP content. These findings indicate that the expression of the ANP gene is augmented in the ventricle of DCM. These findings also suggest that the ventricle of DCM is possibly an additional source of the circulating ANP, because the ANPmRNA content in the ventricle of DCM was much the same as that in the normal atrium.

We recently reported that the ANP concentration in the ventricle is more than 1 μg/g in human fetuses at 14 and 22 weeks' gestation, and decreases in parallel with fetal development, and that adult

Table II

Ratio of ANP level to ANPmRNA level in human hearts

	ANP/ANPmRNA	
	control	DCM
Atrium	0.35	0.71
Ventricle	0.08	0.11

ventricles contain less than 50 ng/g of ANP (27). Thus, the ANP level in the DCM ventricle is comparable to that in the human fetal ventricle. These results suggest that the ANP gene is re-induced in the ventricle of the patient with DCM. The precise mechanism of the augmented expression of the ANP gene in the DCM ventricle is not clear at present. However, the hypertrophic process of ventricular cardiocytes and/or other factors associated with DCM including hemodynamic changes may contribute to the increased expression of the ANP gene in the ventricle. The amount of fetal myosin-light-chain-1-like protein has been reported to be increased in the ventricle of DCM (28). Changes in myosin heavy chain or light chain isoenzymic pattern and switching of the corresponding mRNAs have been also demonstrated in animals with the hypertrophic ventricle or spontaneously hypertensive rats (29,30).

The ratio of the ANP level to the ANPmRNA level (ANP/ANPmRNA) in the ventricle was much smaller than that in the atrium of control patients and the patient with DCM (Table II). These results are consistent with the pulse-chase experiment performed by Bloch et al (11) showing that neonatal ventricular cardiocytes in culture release ANP more rapidly after synthesis than atrial cardiocytes. Therefore, our results along with those by Bloch et al (11) raise the possibility that ANP is secreted from the ventricle more rapidly and that ventricular ANP contributes to the marked elevation of the plasma ANP level in the patient with DCM. (The plasma ANP level in the patient

with DCM (3940 pg/ml) was approximately one hundred times higher than that in normal subjects (37.7 ± 4.0 pg/ml) (24).) Further studies are necessary to clarify whether or not synthesis, storage and secretion of ANP in the ventricle is different from those in the atrium (11,13, 14,26).

In conclusion, the present study demonstrates that the ANP gene is expressed in the human ventricle as well as in the atrium and that the expression of the ANP gene in the ventricle is augmented in the patient with DCM.

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