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SIMULTANEOUS MEASUREMENT OF ATRIAL NATRIURETIC POLYPEPTIDE (ANP) MESSENGER RNA AND ANP IN RAT HEART —— EVIDENCE FOR A PREFERENTIALLY INCREASED SYNTHESIS AND SECRETION OF ANP IN LEFT ATRIUM OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) ——

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Tissue levels of atrial natriuretic polypeptide (ANP) messenger RNA (ANPmRNA) and ANP in the rat heart were measured simultaneously. In Wistar rats, ANPmRNA of the same size (approximately 0.95 kbp) was detected in all four chambers of the rat heart. The ANPmRNA level was the highest in the right atrium, and the left atrial level was slightly lower than the right atrial level. Ventricular levels were more than two orders of magnitude lower than atrial levels. Tissue ANP concentrations of four chambers were roughly parallel to ANPmRNA levels. In spontaneously hypertensive rats (SHR) with the elevated plasma ANP level, the ANPmRNA level in the left atrium was substantially increased. The left/right ratio of atrial ANPmRNA level in SHR (150 %) was higher than that in control Wistar Kyoto rats (WKY) (90 %). In contrast, the left/right ratio of atrial ANP concentration was decreased in SHR (44 %) compared with that in WKY (84 %). The ratio of ANP to ANPmRNA levels in the left atrium of SHR was about three times smaller than that in the right atrium of SHR, and those in bilateral atria of WKY. These results indicate that the biosynthesis and secretion of ANP from the left atrium is preferentially increased in SHR. Thus, simultaneous determination of ANPmRNA and ANP levels is a refined strategy of investigation for the biosynthesis, storage and secretion of ANP.

Atrial natriuretic polypeptide (ANP) is a group of peptides with diuretic, natriuretic, and vasorelaxant activities isolated from the atrium and implicated in the regulation of fluid and electrolyte

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Abbreviations used in this paper: ANP, atrial natriuretic polypeptide; ANPmRNA, ANP messenger RNA; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats; RIA, radioimmunoassay.

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balance and blood pressure (1-4). We developed a specific radioimmunoassay (RIA) for α-ANP (5) and reported that α-ANP is secreted through the coronary sinus from the heart (6-8) and circulates in the body as a hormone (6,7). We and others also showed that the plasma ANP level is increased in patients with essential hypertension (9,10) and in the spontaneously hypertensive rats (SHR) (11-13) and SHR-stroke prone (11). In SHR, however, the tissue ANP level in the atrium was decreased, especially in the left atrium (11-13). In addition, salt loading in SHR resulted in a further decrease of the ANP level in the left atrium (14). From these findings, we speculate that the decreased ANP level in the left atrium in SHR reflects the increased ANP secretion from the left atrium (11,14).

In order to further assess the biosynthesis, storage and secretion of ANP in the heart, we measured tissue ANPmRNA and ANP levels in the heart using the Northern blot hybridization technique and RIA for ANP.

MATERIALS AND METHODS

Animals

The animals were housed in a temperature, humidity and light controlled room with free access to water and standard rat chow CA-1 (Japan CLEA, Tokyo, Japan) containing 0.50 % sodium and 0.84 % potassium.

Experiment 1: Male Slc: Wistar rats (Shizuoka, Japan) at the age of 8 weeks (n=10, 202 ± 9 g) were used. Hearts were removed from decapitated rats, immediately dissected into four parts, bilateral atria and ventricles on ice. To avoid contamination of atrial tissues, apical half of the ventricles were used for measurements of ANPmRNA and ANP in the ventricle. Tissues were weighed, frozen in liquid nitrogen, and stored at -70°C until use.

Experiment 2: Male SHR at the age of 27 weeks (n=5) and its age-matched control, WKY, (n=5) were studied. Rats were maintained in Shionogi Research Laboratories (Shionogi & Co., Osaka, Japan). Atria were removed from decapitated rats, immediately dissected into right and left atria on ice. Tissues were weighed, frozen in liquid nitrogen and stored at -70°C until use.

RNA extraction and Northern blotting analysis

RNA extraction was performed several times from pooled samples and ANPmRNA levels were measured by Northern blotting analysis as previously reported (15). ANPmRNA levels (arbitrary units) were expressed as relative levels to that in the right atrium of 8-week male Slc: Wistar rat (the level in the right atrium = 1.0 unit/μg total RNA).

Peptide extraction and Radioimmunoassay (RIA)

Extraction of ANP from cardiac tissues was carried out as previously reported (5,11). The ANP concentration in the supernatant was measured by RIA (5,6,8).

Statistical analysis

Statistical analysis was performed using Student's t test.

RESULTS

ANPmRNA and ANP in male Wistar rats

The results of Northern blot analysis of RNA from 8-week-old male Wistar rats are shown in Figure 1. Both right and left ventricles harbor a transcript of the same length, approximately 0.95 kbp, as that in atria. The radioactivity in hybridized band showed a linear relationship with the amount of RNA applied to the electrophoresis gel. The ANPmRNA levels are summarized in Table I. The ANPmRNA level in the right atrium was slightly but reproducibly higher than that of the left atrium. ANPmRNA levels in left and right ventricles were approximately 0.7 % and 0.1 % of that in the right atrium, respectively.

Tissue ANP levels are also shown in Table I. The ANP level in the left atrium was 80 % of that in the right atrium. ANP levels in left and right ventricles were 0.03 % and 0.01 % of that in the right atrium. ANP levels were roughly parallel to ANPmRNA levels. However,

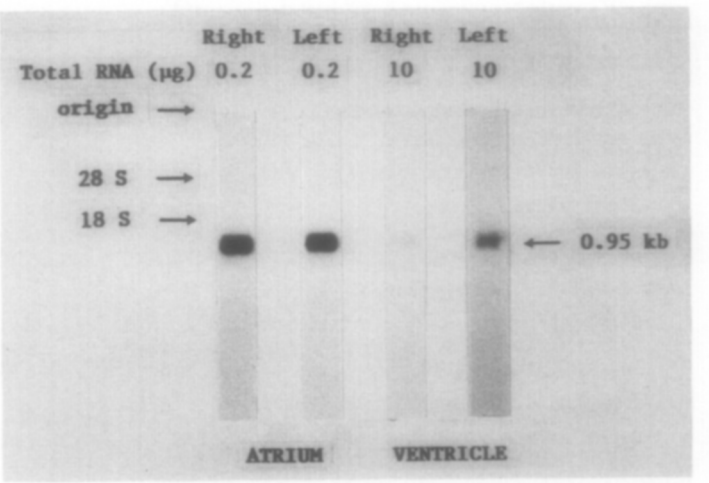

Figure 1. Northern blot analysis of ANPmRNA from hearts of 8-week-old male Wistar rats.

Table I. Tissue ANPmRNA levels and ANP concentrations in 8-week-old male Wistar rat heart

	Atrium		Ventricle	
	Right	Left	Right	Left
ANPmRNA level (unit/g tissue)	510	480	0.5	3.5
ANP concentration (μg/g tissue)	60.7±2.6	49.0±1.9	0.006±0.001	0.019±0.002
ANP/ANPmRNA (ng/unit)	120	100	12	5.4

(Mean ± SEM)

the ratio of ANP to ANPmRNA levels in the atrium was approximately one order of magnitude larger than that in the ventricle.

ANPmRNA and ANP in atria of WKY and SHR

As seen in Table II, systolic blood pressure of SHR at the age of 27 weeks was much higher than that of WKY. The results of Northern blot analysis of RNA from both atria of WKY and SHR are shown in Figure 2. ANPmRNA of the same size was found in both WKY and SHR.

In 27-week-old WKY, ANPmRNA and ANP levels in the left atrium were approximately 90 % and 84 % of those in the right atrium, respec-

Table II. Profiles and atrial levels of ANPmRNA and ANP in 27-week-old male WKY and SHR

	WKY	SHR
Body weight (g)	391 ± 11	436 ± 9*
Systolic blood pressure (mmHg)	147 ± 2	234 ± 4**
ANPmRNA level (unit/g tissue)		
Right atrium	450	410
Left atrium	410	610
Left/Right ratio (%)	90	150
ANP level (μg/g tissue)		
Right atrium	75.9 ± 5.8	85.5 ± 17.9
Left atrium	63.9 ± 12.8	37.6 ± 9.1
Left/Right ratio (%)	84 ± 5	44 ± 3*
ANP/ANPmRNA (ng/unit)		
Right atrium	170	210
Left atrium	160	62

Values of ANP are means ± SEM.
Significantly different from WKY, * $p<0.05$; ** $p<0.01$.

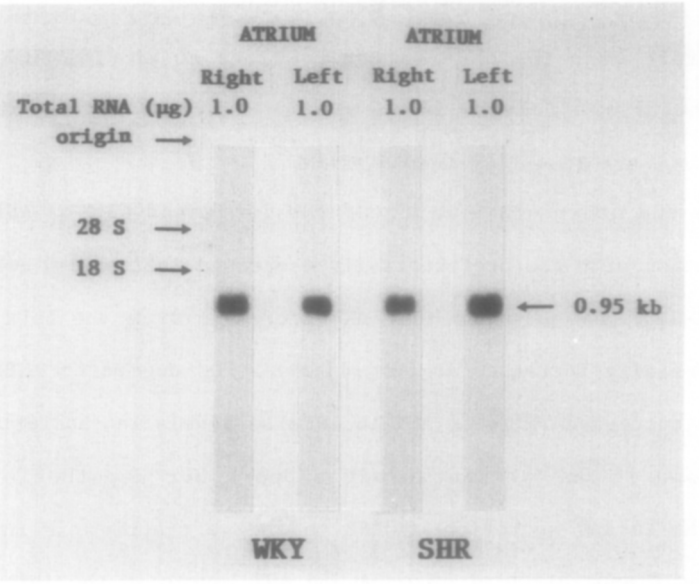

Figure 2. Northern blot analysis of atrial ANPmRNA in 27-week-old male WKY and SHR.

tively. In contrast, the ANP level in the left atrium of SHR was decreased to 44 % of that in the right atrium, whereas the ANPmRNA level in the left atrium of SHR was increased to 150 % of that in the right atrium. The ratio of ANP to ANPmRNA levels in the left atrium of SHR was about three times smaller than that in the right atrium of SHR, and those in bilateral atria of WKY.

DISCUSSION

The present study demonstrates that the ANP gene is expressed in the ventricle as well as in the atrium. The rank order of the ANPmRNA level in the normal male rat was right atrium > left atrium >> left ventricle > right ventricle. The ANP level was roughly parallel to the ANPmRNA level in each tissue as shown in Table I. These results suggest that the ANP level in each tissue is related to the ANP biosynthesis under normal conditions, and are consistent with those in recent reports (18-20), indicating the validity of our assay systems. The ANP/ANPmRNA ratio in the atrium was approximately one order of magnitude larger than that in the ventricle. This finding is also in agreement with the recent results reported by others (19-20), and

compatible with the observation by Block et al (18) that neonatal ventricular cardiocytes in culture release ANP more rapidly after biosynthesis than atrial cardiocytes.

We and others have reported the increased plasma ANP level in association with the preferentially decreased left atrial ANP level in SHR (11-14), and proposed that ANP secretion from the left atrium is preferentially increased in SHR (11,14). The augmented ANPmRNA level and the decreased ratio of ANP to ANPmRNA levels in the left atrium of SHR shown in the present study support our hypothesis that the biosynthesis and secretion of ANP in the left atrium is increased in SHR. The dissociation of ANPmRNA and ANP levels in the left atrium of SHR also indicates that measurement of either ANPmRNA or ANP level alone is not sufficient to evaluate the biosynthesis, storage and secretion of ANP.

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