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ATRIAL NATRIURETIC POLYPEPTIDE (ANP) IN THE DEVELOPMENT OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND STROKE-PRONE SHR (SHRSP)

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In order to investigate the pathophysiological role of atrial natriuretic polypeptide (ANP) in genetic hypertensive rats, the atrial content and plasma concentration of ANP were measured by a sensitive radioimmunoassay (RIA) for rat ANP in 5-, 10- and 20-week-old spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) and compared to age-matched Wistar Kyoto rats (WKY). Atrial content of immunoreactive ANP (ir-ANP) tended to be higher in SHR and was already significantly higher in SHRSP than in WKY at 5 weeks of age. Atrial content in the hypertensive strains became significantly higher than in WKY when hypertension developed at 10 and 20 weeks. On the other hand, plasma ir-ANP in SHR was significantly lower than in WKY at 5 weeks, however, it became significantly higher in both SHR and SHRSP than in WKY at 10 and 20 weeks. These findings suggest that ANP release may increase to compensate for the elevation of blood pressure in SHR and SHRSP and that biosynthesis of ANP may be concomitantly stimulated, resulting in an increase in atrial ANP.

Following the finding of natriuretic and hypotensive activities in rat atrial extract by de Bold et al. (1), several atrial natriuretic polypeptides (ANPs) have been isolated from mammalian atria (2-6). Potent natriuretic and hypotensive effects have been also observed when these polypeptides were administered to experimental animals (3,4,7). Atrial granularities or natriuretic activities in atrial extract were reported to be changed in water deprivation and salt loading (8,9), and in genetic hypertensive rats (10). These observations suggest that ANP may play a role not only in water and electrolyte balance but also in blood pressure regulation. Recently, developed radioimmunoassay (RIA) for ANP revealed that plasma concentration of ANP increased in various types of hypertensive rats, however, atrial content of ANP has been reported to decrease or even to increase (11-15). Thus, its pathophysiological role in the development of hypertension is still unknown. To answer this question, it is necessary to

ABBREVIATIONS: ANP, atrial natriuretic polypeptide; α - and γ -rANP, α - and γ -rat ANP; ir-ANP, immunoreactive ANP; RIA, radioimmunoassay; RP-HPLC, reverse phase high-performance liquid chromatography; WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; SHRSP, stoke-prone SHR; SBP, systolic blood pressure.

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measure both atrial and plasma concentration of ANP according to the developement of hypertension. In the present study, therefore, we measured the atrial content and plasma concentration of ANP in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) at three different ages, using a specific RIA for rat ANP (rANP), and compared with those from Wistar Kyoto rats (WKY).

MATERIALS AND METHODS

Animals and extractions

Five-, 10- and 20-week-old male WKY, SHR and SHRSP were used (n = 11 to 12). These colonies of rat were kindly provided from Prof. Okamoto (Kinki University School of Medicine, Osaka, Japan) and maintained by selective mating in our laboratory. All rats were housed in a light and temperature controlled room with a standard rat chow containing 0.85g/100g of NaCl (Nihon CLEA CE-2, Tokyo, Japan) and tap water *ad libitum*. After systolic blood pressure (SBP) was measured indirectly by the tail-cuff method, the rats were sacrificed by decapitation. Blood was collected with aprotinin (500 KIU/ml) and EDTA-2Na (1 mg/ml) and centrifuged at 2,000 xg for 10 min. Plasma was stored at -20°C until assayed. Atrial tissue was carefully removed after decapitation and homogenized in 20 volumes of 0.1N acetic acid containing 1 % Triton X-100 by a polytron mixer for 60 s. After boiling for 3 min to inactivate proteases, the homogenate was centrifuged at 25,000 xg for 30 min, then the supernatant was stored at -20°C until RIA. Plasma sampling and extraction procedure were performed at 4°C.

RIA and RP-HPLC procedure

The atrial content and plasma concentration of ANP were measured using a specific RIA for rANP as described previously (16,17). Anti-α-rANP antiserum used in this RIA detects the ringed structure of α-rANP with equal specificity to γ-rANP. Standard α-rANP or the unknown sample were incubated with anti-α-rANP antiserum diluent for 12 h, and tracer solution (18,000-20,000 cpm of ^{125}I-α-rANP) was added. After incubation for 36 h, anti-rabbit IgG goat serum diluent was added. Two days later, reaction tubes were centrifuged at 2,000 xg for 30 min, and the radioactivity of the precipitate was measured by a gamma counter. All assay procedures were performed at 4°C. Immunoreactive ANP (ir-ANP) in the atrial extract was characterized by reverse phase high-performance liquid chromatography (RP-HPLC). A linear gradient of acetonitrile was performed from 10% to 60% in 0.1% trifluoroacetic acid for 40 min. Immunoreactivity of each fraction was measured by RIA.

Statistical analysis

One-way analysis of variance followed by *t*-test was used for comparison among the three strains at identical ages. Linear regression was evaluated by the least-squares method. Data were presented as means ± S.E.M., and a level of $P < 0.05$ was considered to be significant.

RESULTS

SBP, body weight, atrial weight and atrial/body weight ratio (A/B ratio) in the 9 groups studied are summarized in Table 1. In SHR and SHRSP, SBP was already elevated at 5 weeks compared to WKY, and became markedly elevated at

Table 1. Systolic blood pressure (SBP), body weight and atrial/body weight ratio (A/B ratio) in each strain

	Age of rats (weeks)	SBP (mmHg)	Body weight (g)	Atrial weight (mg)	A/B ratio (mg/g)
WKY	5 (n = 11)	102.7 ± 4.0	93.0 ± 3.6	27.1 ± 1.1	0.292 ± 0.009
	10 (n = 12)	129.2 ± 2.4	247.2 ± 8.5	44.1 ± 1.9	0.179 ± 0.006
	20 (n = 12)	123.8 ± 2.7	365.3 ± 4.6	70.2 ± 2.3	0.192 ± 0.007
SHR	5 (n = 12)	116.8 ± 3.9**	108.8 ± 3.3**	30.3 ± 1.7	0.278 ± 0.012
	10 (n = 12)	156.8 ± 2.9**	216.5 ± 4.1**	37.4 ± 1.1**	0.174 ± 0.006
	20 (n = 12)	177.3 ± 4.1**	271.0 ± 4.4**	51.3 ± 1.6**	0.189 ± 0.005
SHRSP	5 (n = 12)	124.0 ± 2.1**	105.0 ± 2.7*	29.0 ± 1.2	0.276 ± 0.008
	10 (n = 12)	203.2 ± 3.0**	211.5 ± 3.5**	36.8 ± 1.1**	0.174 ± 0.005
	20 (n = 12)	218.8 ± 5.2**	232.5 ± 10.7**	50.1 ± 2.4**	0.216 ± 0.006**

*P < 0.05,**P < 0.01; compared to WKY at identical age.

10 and 20 weeks. There was no significant difference in the A/B ratio among the three strains, expect in the 20-week-old SHRSP.

Atrial and plasma ir-ANP in 5-, 10- and 20-week-old rats are shown in Figure 1A and 1B, respectively. The atiral ir-ANP tended to be higher in SHR and was significantly higher in SHRSP than in WKY at 5 weeks. Concurrent with the development of hypertension, ir-ANP was more abundant in SHR and SHRSP than in WKY at both 10 and 20 weeks. On the other hand, plasma ir-ANP in 5-week-old SHR was significantly lower than in WKY, while there was no significant difference between SHRSP and WKY. As hypertension developed, however, plasma ir-ANP in SHR and SHRSP became significantly higher than in WKY at 10 and 20 weeks.

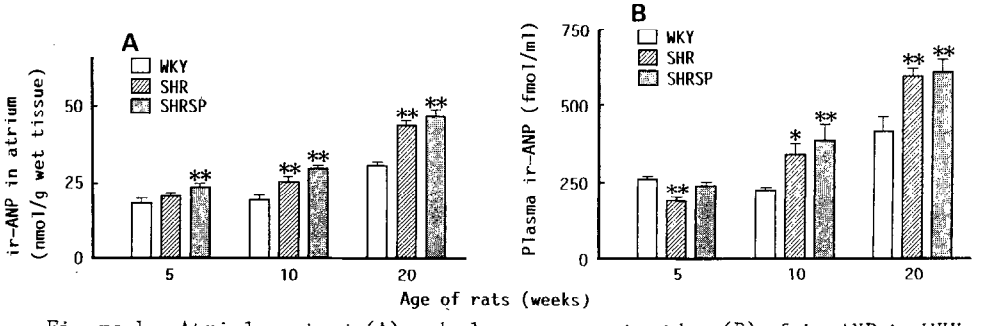


Figure 1. Atrial content (A) and plasma concentration (B) of ir-ANP in WKY, SHR and SHRSP at 5, 10 and 20 weeks. *P < 0.05,**P < 0.01, compared to WKY.

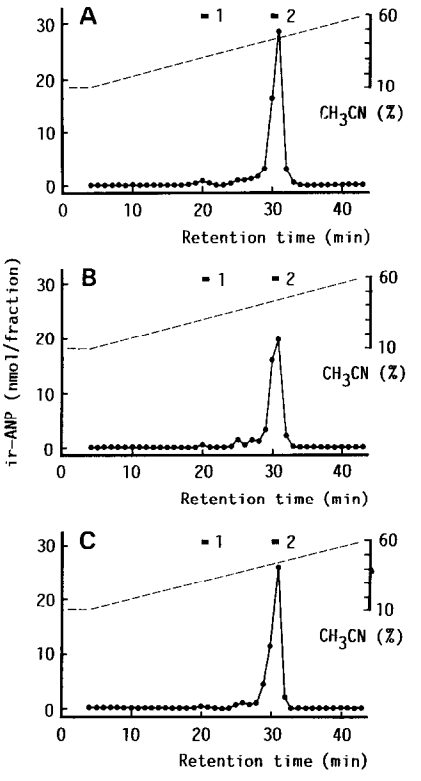


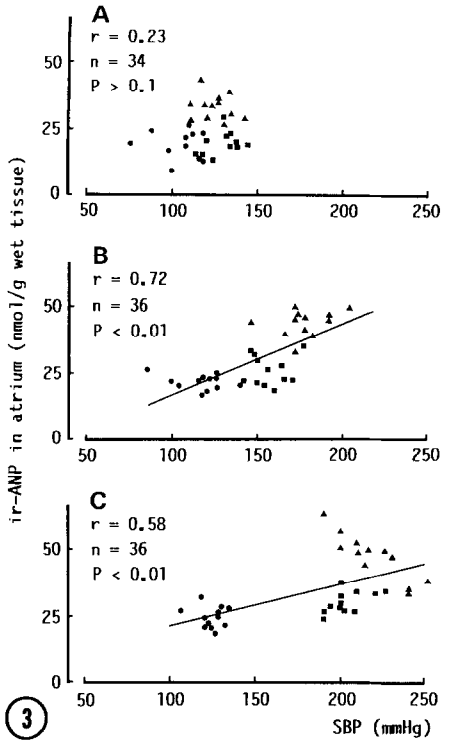
Figure 2. Characterization of ir-ANP in atrium of WKY, SHR and SHRSP by RP-HPLC. Closed squares (■) indicate the eluted positions of α - (1) and γ -rANP (2).

Analysis of atrial extract by RP-HPLC revealed that a major immunoreactive component appeared at the eluted position of γ -rANP, a 126 amino acid residue (6,16). It was identical in the three strains of rat (Figure 2).

Correlations between SBP and ir-ANP in atrium and plasma are shown in Figure 3 and 4, respectively. Both the atrial and plasma ir-ANP had significant positive correlations with SBP in SHR and SHRSP, while such correlation was not observed in WKY.

DISCUSSION

It was demonstrated that the plasma concentration of ir-ANP was more increased in SHR and SHRSP than in WKY as hypertension developed. Significant correlation between SBP and plasma ir-ANP was observed in the hypertensive strains, while there was no such correlation in WKY. The increase in plasma ir-ANP may result from an increase in ANP release, as indicated by previous observations (12-15). Several factors stimulating ANP release have been reported: a rise in left or right atrial pressure (18,19), acute volume expansion (20), acute sodium load (17) and α -adrenergic agonist (19,21).



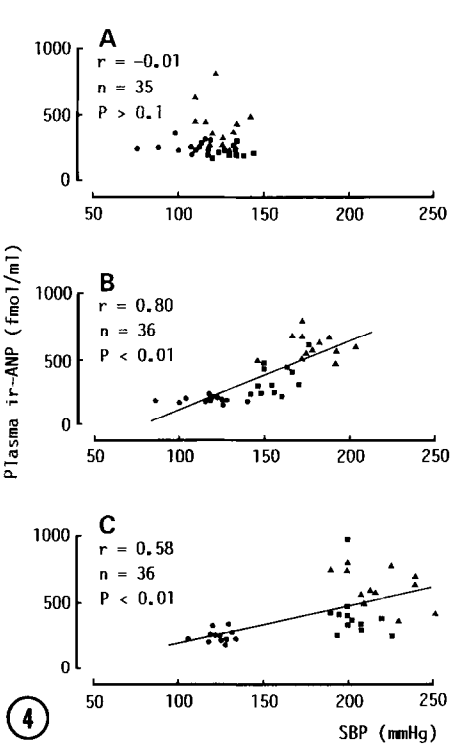


Figure 3. Correlation between SBP and ir-ANP in atrium in WKY (A), SHR (B) and SHRSP (C). ●= 5-, ■= 10- and ▲= 20-week-old rats.

Figure 4. Correlation between SBP and plasma ir-ANP in WKY (A), SHR (B) and SHRSP (C). ●= 5-, ■= 10- and ▲= 20-week-old rats.

Noresson et al. (22) reported that left atrial pressure in SHR was elevated to prevent the reduction of stroke volume involved with left ventricular hypertrophy. The same mechanism may be considered in SHRSP. Thus, an increase in left atrial pressure may be an important factor stimulating ANP release in SHR and SHRSP. It is unknown whether α-adrenergic agonist stimulates ANP release directly or indirectly (19,21), however, an increase in sympathetic nerve activity (23) may be another factor stimulating ANP release in SHR. Unexpectedly, the plasma concentration of ir-ANP was significatly lower in the early hypertensive state of 5-week-old SHR than in WKY. Genetic abnormalities in ANP release may be involved in the young of SHR, however, such a mechanism could not be clarified in the present study.

The atrial content of ir-ANP was more increased in SHR and SHRSP than in WKY according to the development of hypertension as well as plasma ir-ANP. There was a significant positive correlation between SBP and atrial content of ir-ANP in SHR and SHRSP, but not in WKY. The storage form of ANP in rat atrium has been reported to be γ-rANP, a 126 amino acid residue (6,16). Our RP-HPLC analysis of atrial extracts revealed that a single immunoreactive peak appeared at the eluted position of γ-rANP, being identical among the three

strains of rat. Consequently, it is unlikely that the processing of ANP precursor or varying enzymatic cleavages in hypertensive rats may differ from those in normotensive rats, although several molecular forms of ANP have been isolated from rat atrium (2,4-6).

Xie et al. (12) reported that the atrial content of ANP in SHR was more abundant than in WKY, similar to our observation. Gutkowska et al. (13) showed that atrial ANP in SHR was higher than in WKY at the prehypertensive stage. However, there are several reports that the atrial content of ANP was lower in SHR than in WKY (10,14,15). It is still controversial whether atrial ANP is increased or decreased in SHR.

ANP in the atrium was reported to increase following salt repletion after drinking 1% NaCl solution (24,25), and to decrease in water deprived (9,25) and DOCA-salt hypertensive rats (11). Decreased messenger-RNA for ANP precursor in the case of water deprivation (26) suggests a decrease in the biosynthesis of ANP. In contrast, ANP release may increase in salt repleted (24) or DOCA-salt hypertensive rats (11), which may indicate an increased demand for ANP. At the same time, biosynthesis of ANP may increase in response to the demand for ANP, which favors an increase in atrial storage of ANP in salt repleted rats (24,25). In the case of DOCA-salt hypertensive rats, decreased atrial storage may result from the increase in ANP release (11). Ackermann and Irizawa (27) found that fucose uptake into the atrium, a possible indicator of the rate of formation of atrial specific granules, was more rapid in DOCA-salt treated rats than in sodium-deficient rats, suggesting that the turnover of specific granules may increase. Hence, it is likely that atiral content of ANP might alter according to the degree of release and biosynthesis.

In summary, our present data showing that atrial and plasma ANP increase in SHR and SHRSP suggest a compensatory increase in biosynthesis and release in correlation with the development of hypertension. Thus, the ANP system may help to regulate blood pressure in genetic hypertensive rats against the development of hypertension.

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