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DECREASED CONTENT IN LEFT ATRIUM AND INCREASED PLASMA CONCENTRATION OF ATRIAL NATRIURETIC POLYPEPTIDE IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR STROKE-PRONE

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The atrial contents and concentrations, and the plasma concentrations of atrial natriuretic polypeptide (ANP) in spontaneously hypertensive rats (SHR) and SHR stroke-prone (SHRSP) were measured and compared with those of age-matched Wistar Kyoto rats (WKY) using a specific radioimmunoassay (RIA) for α-rat ANP (α-rANP). The contents of α-rANP-LI in the atria of SHR (19.0±0.9 μg, mean±SEM) and SHRSP (19.3±0.6 μg) were significantly lower than that of WKY (22.8±1.4 μg)($p<0.05$). The atrial concentration of α-rANP-LI was also significantly lower in SHR (248.2±11.3 ng/mg, $p<0.05$) and tended to be lower in SHRSP (272.2±12.4 ng/mg) than that of WKY (300.0±14.2 ng/mg). Furthermore, the concentrations in the left auricles of SHR and SHRSP were significantly lower than that of WKY ($p<0.01$ and $p<0.05$, respectively). In contrast, no significant difference was observed in the α-rANP-LI concentrations in the right auricles of WKY, SHR and SHRSP. Gel filtration studies coupled with RIA showed that gel filtration profiles of the extracts from the right and left auricles of WKY, SHR and SHRSP were essentially identical. The plasma α-rANP-LI levels in SHR (260±34 pg/ml) and SHRSP (319±19 pg/ml) were significantly higher than that in WKY (170±17 pg/ml)($p<0.05$ and $p<0.01$, respectively). These results suggest that the secretion of ANP from the heart is increased in SHR and SHRSP compared with WKY.

Since the discovery of potent natriuretic, diuretic and vasorelaxant activities of extracts from the rat atrium (1), multiple forms of atrial natriuretic polypeptide (ANP) have been isolated from human (2,3) and rat atrial tissues (4-9), and implicated in the control of fluid and electrolyte balance and blood pressure.

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Abbreviations: ANP; atrial natriuretic polypeptide, α,β and γ-rANP; α, β and γ-rat atrial natriuretic polypeptide, -LI; -like immunoreactivity, RIA; radioimmunoassay, HP-GPC; high performance gel permeation chromatography, WKY, Wistar Kyoto rat, SHR; spontaneously hypertensive rats, SHRSP; spontaneously hypertensive rats stroke-prone.

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Since several lines of evidence in experimental models and patients with essential hypertension suggest a close link between sodium regulation and the development of hypertension, and another saluretic substance, a circulating sodium transport inhibitor or ouabain-like compound, has been suggested as a possible contributor to the development and maintenance of hypertension (10), it is of great importance to study whether or not ANP is involved in the pathogenesis of this disorder. The content of ANP in the atrium of spontaneously hypertensive rats (SHR) is a matter of debate at present (11,12). Sonnenberg et al (11) showed the reduced cardiac content of ANP in SHR using a bioassay, whereas Winquist (12) reported that the content of ANP tended to increase in the atria of SHR compared with Wistar Kyoto rats (WKY). Recently, we developed a specific radioimmunoassay (RIA) for α-ANP(13,14), and reported that ANP is present not only in the atrium (13) but also in the brain (14,15,16) and kidney (17), and that α-ANP is released from the heart and circulates in the body as a hormone (18).

In order to elucidate the role of ANP in hypertension, we have compared the tissue contents and concentrations of α-rat ANP-like immunoreactivity (α-rANP-LI) in the atria and the plasma concentrations in SHR and SHR stroke-prone (SHRSP) with those of age-matched WKY.

MATERIALS AND METHODS

Animals

All experiments were performed on 16-week-old male SHR (324 ± 8.3g, mean ± SEM, body weight, n=10), SHRSP (273±7.5 g, n=10) and WKY (301 ± 10.4 g, n=10), the colonies of which had been maintained by selective inbreeding in our laboratory (Department of Pathology, Shimane Medical University, Izumo, Japan). The animals were housed in a temperature, humidity and light controlled room with free access to water and food (0.30% NaCl, 0.52% KCl). The experiments were performed after systolic blood pressure was measured by indirect tail cuff method.

Tissues and extraction

Hearts were removed from rats under pentobarbital anesthesia (50 mg/kg, intraperitoneal injection). The atria were immediately separated from the ventricles on ice and divided into the right and left auricles and non-auricle part of the atrium. Tissues were weighed and stored at -70°C until extraction. Table 1 shows systolic blood pressure and the tissue weights of the respective parts dissected from the heart of each strain examined. Tissue extraction was performed as previously reported (13).

Blood samples

Blood samples for determination of plasma levels of α-rANP-LI were obtained from conscious unrestrained rats as follows. Under sodium pento-

Table 1 Systolic blood pressure and tissue weights of atria and their respective parts

Strain	Systolic Blood pressure	Tissue Weight			Non-auricle part
		Atrium	Right auricle	Left auricle	
WKY	125 ± 2.6	76.2 ± 4.3	15.5 ± 0.7	13.6 ± 0.8	47.0 ± 3.9
SHR	187 ± 3.9	77.7 ± 3.8	18.5 ± 0.9	12.9 ± 0.6	47.0 ± 3.3
SHRSP	207 ± 3.8 (mmHg)	71.8 ± 3.2 (mg)	15.1 ± 0.6 (mg)	14.1 ± 0.5 (mg)	42.0 ± 2.7 (mg)

Values are mean ± SEM from 10 determinations.

barbital anesthesia (50 mg/kg, i.p.), the left femoral vein was cannulated with a polyethylene tube and the rats were allowed to recover from surgery for at least 3 days before collection of the blood. Blood samples (0.25 ml) were withdrawn into the plastic syringe from the cannula, transferred to a chilled polypropylene tube containing aprotinin (1,000 kallikrein inactivator units/ml, Ohkura Pharmaceutical Co. Kyoto, Japan) and EDTA (1mg/ml), and immediately centrifuged at 4°C. Plasma samples were stored at -20°C until RIA.

Measurement of plasma α-rANP-LI and RIA

Measurement of the plasma α-rANP-LI concentration was performed as previously reported (18). The minimum detectable amount of α-rANP was 1.5 pg/tube. The RIA for α-rANP was performed as previously reported (13,14).

High performance gel permeation chromatography (HP-GPC)

Extracts from the right and left auricles of WKY, SHR and SHRSP were subjected to HP-GPC on a TSK-GEL G2000 SW (Toyo Soda, Tokyo, Japan) column (7.5 x 600 mm) as previously reported (13).

Statistical analysis

The results were compared by Duncan's multiple rage test following one-way analysis of variance.

RESULTS

The contents and concentrations of α-rANP-LI in the atria from SHR, SHRSP and WKY are shown in Table 2. The atria from SHR and SHRSP contained comparable amounts of α-rANP-LI and the atrial contents in SHR and SHRSP were

Table 2 Contents and concentrations of α-rANP-LI in the atria of WKY,SHR and SHRSP

Strain	Content (μg)	Concentration (ng/mg)
WKY	22.8 ± 1.4	300.0 ± 14.2
SHR	19.0 ± 0.8*	248.2 ± 11.3*
SHRSP	19.3 ± 0.6*	272.2 ± 12.4

Values are mean ± SEM from 10 male rats of each strain.
Significantly different from WKY, * $P<0.05$.

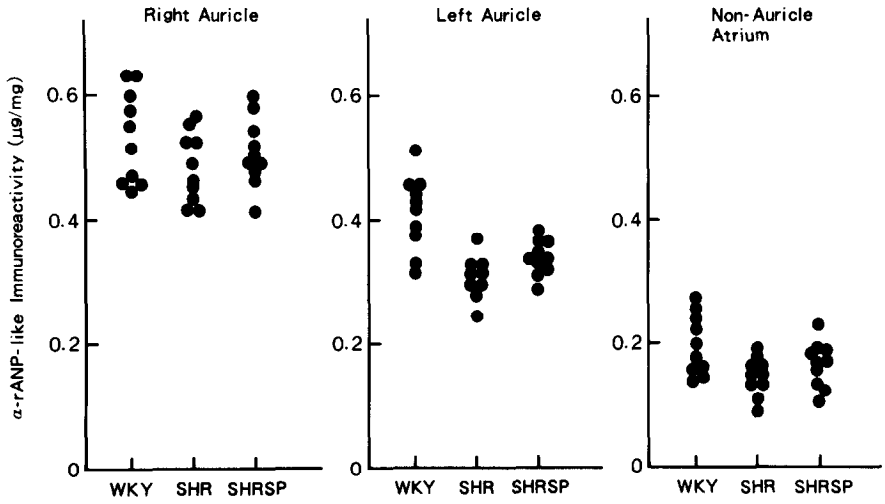

Figure 1 Individual values of α-rANP-LI concentrations in the right and left auricles and non-auricle parts of the atria of WKY, SHR and SHRSP.

significantly lower than that of WKY ($p<0.05$). The atrial concentration of α-rANP-LI was significantly lower in SHR ($p<0.05$) and tended to be lower in SHRSP than in WKY. As shown in Figure 1 and Table 3, the atrial α-rANP-LI concentration was the highest in the right auricle and the lowest in the non-auricle part for all three experimental groups. The right auricular concentrations of α-rANP-LI were similar among WKY, SHR and SHRSP. In contrast, the concentrations in the left auricles of SHR and SHRSP were significantly lower than that of WKY ($p<0.01$ and $p<0.05$, respectively). There was no significant difference between SHR and SHRSP in the left auricular concentration of α-rANP-LI. Thus, the ratios of the left to the right auricular concentration of α-rANP-LI were significantly lower both in SHR and SHRSP than that in WKY ($p<0.05$).

Table 3 Concentrations of α-rANP-LI in the right and left auricles and non-auricle parts of atria of WKY, SHR and SHRSP

Strain	Right auricle (ng/mg)	Left auricle (ng/mg)	Non-auricle part (ng/mg)	Ratio (Left/Right)
WKY	506.6 ± 25.9	408.8 ± 19.4	195.2 ± 15.6	0.82 ± 0.04
SHR	468.4 ± 23.9	304.3 ± 10.9**	145.5 ± 9.3*	0.65 ± 0.03*
SHRSP	505.9 ± 16.9	337.7 ± 8.6*	165.5 ± 11.9	0.66 ± 0.01*

Values are mean ± SEM from 10 determinations.
significantly different from WKY, * P<0.05;** P<0.01.

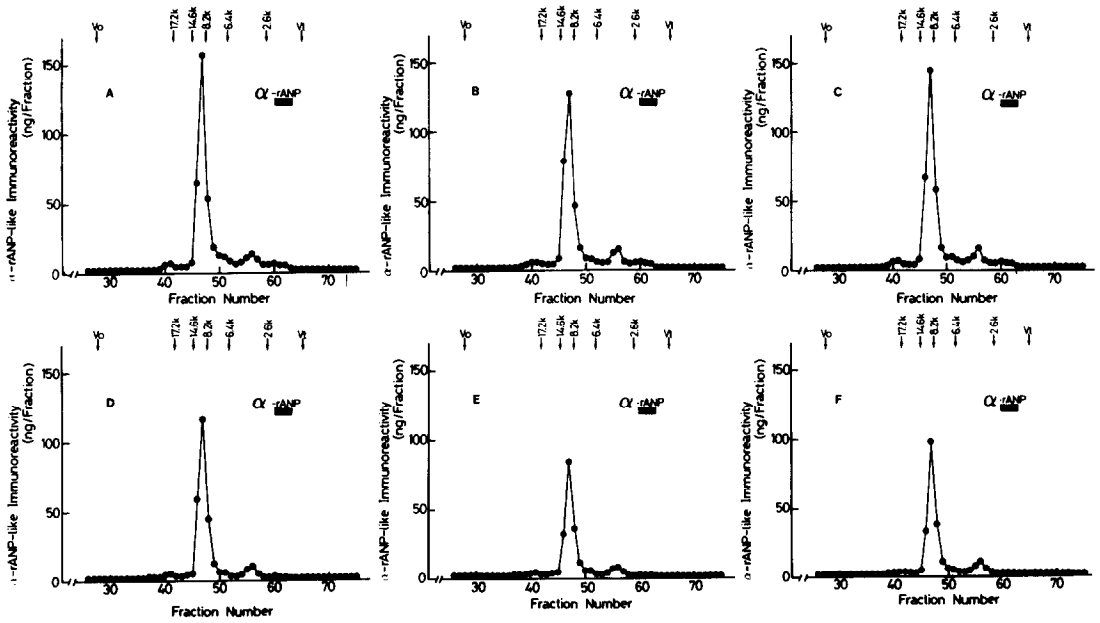

Figure 2 Gel filtration profiles of the extracts from (A) right auricle of WKY, (B) right auricle of SHR, (C) right auricle of SHRSP, (D) left auricle of WKY, (E) left auricle of SHR and (F) left auricle of SHRSP. Arrows show the elution positions of a series of myoglobins (Pharmacia, Uppsala, Sweden) with their molecular weights expressed in k daltons.

Figure 2 shows HP-GPC profiles of α-rANP-LI in the right and left auricles of WKY, SHR and SHRSP. These gel filtration patterns were almost identical, and two peaks of α-rANP-LI were evident. The major peak was eluted at the position of apparent molecular weight of 13k daltons and the minor peak was eluted at the position of apparent molecular weight of 3k-5k daltons. The former was presumed to be γ-rANP and the latter to be β-rANP. Only a relatively small amount of α-rANP-LI was found at the elution position of α-rANP.

Table 4 Plasma levels of α-rANP-LI in WKY, SHR and SHRSP

Strain		α-rANP-LI (pg/ml)
WKY	(n=7)	170 ± 17
SHR	(n=7)	260 ± 34*
SHRSP	(n=8)	319 ± 19**

Values are mean ± SEM.
Significantly different from WKY, *P < 0.05; **P < 0.01.

As shown in Table 4, the α-rANP-LI concentrations in plasma from SHR and SHRSP were significantly higher than that from WKY ($p<0.05$ and $p<0.01$, respectively). The highest plasma concentration of α-rANP-LI was seen in SHRSP.

DISCUSSION

The present study demonstrates a significant decrease of ANP levels in the atria of SHR and SHRSP. This finding is in agreement with those obtained by bioassay on SHR by Sonnenberg et al (11), although the difference between SHR and WKY in the atrial level of α-rANP-LI in this study was not as marked. The values of α-rANP-LI content and concentration in the atrium of WKY are consistent with those of Sprague-Dawley rats reported by Gutkowska et al (19) and our previous result on Wistar rats (13), indicating the validity of our method. The present study also showed that the HP-GPC profiles of the atrial extracts from the right and left auricles of the three strains are essentially identical. It is, therefore, unlikely that posttranslational processing of ANP precursor, preproANP, in the atria of SHR and SHRSP differs from that of WKY, and that the difference in the atrial α-rANP-LI level between these strains can be accounted for by the altered composition of ANP in the atria of SHR and SHRSP, although multiple forms of ANP have been isolated from rat atria so far (4-9).

Furthermore, a particularly important finding in the present study is that the α-rANP-LI levels in the left auricles of SHR and SHRSP were significantly lower than that of WKY, whereas no significant differences were observed among the levels in the right auricles of SHR, SHRSP and WKY. Previous investigations in the dog showed that atrial distension resulted in a marked increase of diuresis and natriuresis (20) and atrial stretch receptors were postulated to be involved in water and sodium regulation. Recently, the secretion of ANP induced by atrial distension has been reported in some experimental situations, suggesting that the secretion of ANP from the heart is related to atrial pressure (21,22). Since left atrial pressure is known to be elevated in SHR (23) and probably in SHRSP, such a preferential decrease of

the α-rANP-LI level in the left auricles of SHR and SHRSP may result from the increased secretion of ANP from the left atrium.

Since ANP has potent diuretic, natriuretic and vasorelaxant activities (1-9), it is considered to be an endogenous antihypertensive agent. Therefore, it is of great importance to clarify whether or not the secretion of ANP from the heart into the blood is decreased in hypertensive models such as SHR and SHRSP. To accurately determine the plasma concentration of α-rANP-LI, the blood sampling condition is critical, because our unpublished observations indicate that the plasma α-rANP-LI level is easily affected by stress or anesthesia. Recently, similar findings were reported by Horky et al (24). Therefore, we examined the plasma concentration of α-rANP-LI in conscious unrestrained rats allowed to recover from surgery for at least 3 days. The present study demonstrates that the plasma concentrations of α-rANP-LI in SHR and SHRSP are significantly increased despite the decrease in the atrial ANP content. This suggests the increased secretion of ANP from the heart into the blood in SHR and SHRSP. It has been reported that SHR and patients with essential hypertension show accelerated natriuresis upon being given a rapid intravenous infusion of saline (25). This observation suggests a state of enhanced tendency to oppose sodium and water retention in hypertensive rats and essential hypertensives (10). The increased plasma level of ANP in SHR and SHRSP observed in the present study, may be a compensatory response to the continuing need to oppose sodium and water retention in these rats. Recently, we also observed that essential hypertensives have the increased plasma ANP levels (unpublished observations).

In conclusion, the decreased ANP level in the left atrium and the increased plasma ANP concentration in SHR and SHRSP suggest that the secretion of ANP from the heart into the blood is increase in these spontaneously hypertensive rats and that atrial receptors in the left atrium may be involved in the regulation of the ANP secretion.

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