0013-7227/00/$03.00/0
Endocrinology

Vol. 141, No. 5
Printed in U.S.A.

Sodium-Induced Cardiac Aldosterone Synthesis Causes Cardiac Hypertrophy

YOSHIYU TAKEDA, TAKASHI YONEDA, MASASHI DEMURA, ISAMU MIYAMORI, AND HIROSHI MABUCHI
Second Department of Internal Medicine (Y.T., T.Y., M.D., H.M), Department of Health Sciences (Y.T.), School of Medicine, Kanazawa University, Kanazawa 920, Japan
Third Department of Internal Medicine (I.M.), Fukui Medical School, Fukui 910-11, Japan

Abstract. High sodium intake causes cardiac hypertrophy independently of increases in blood pressure. Aldosterone is synthesized in extraadrenal tissues such as blood vessels, brain, and heart. Effects of 8 weeks of high sodium intake on cardiac aldosterone synthesis, as well as cardiac structure, mass, and aldosterone production, levels of mRNA coding for aldosterone synthase (CYP11B2) and the angiotensin II AT 1 receptor, were studied in normotensive Wistar-Kyoto (WKY) rats. Isolated rat hearts were perfused for 2 hr, and the perfusate was analyzed by high-performance liquid chromatography and mass spectrometry. Aldosterone synthase activity was estimated from the conversion of [^{14}C]deoxycorticosterone to [^{14}C]aldosterone. Levels of mRNA for CYP11B2 and AT 1 receptor were determined by competitive polymerase chain reactions. A high sodium intake for 8 weeks produced left ventricular hypertrophy without elevation of blood pressure. Plasma aldosterone concentrations and plasma renin concentrations were decreased by high sodium intake. Aldosterone production, activity of aldosterone synthase, and expression of mRNA for CYP11B2 and AT 1 receptor were increased in hearts of rats with high sodium intake. These results suggest that high sodium intake increases cardiac aldosterone synthesis, which may contribute to cardiac hypertrophy independently of the circulating renin-angiotensin-aldosterone system.

Left ventricular hypertrophy (LVH) is an established risk factor for cardiovascular morbidity and mortality. In animal experiments, high dietary salt leads to LVH without significant elevation of blood pressure (1), and clinical studies have shown that high salt intake is a powerful and independent determinant of LVH (2).

Aldosterone receptors are present in vascular smooth muscle cells, cardiac myocytes, endo-cardial endothelial cells, and cardiac fibroblasts (3). Peripheral infusion of aldosterone in rats causes cardiac hypertrophy and cardiac fibrosis without increasing blood pressure (4). In cultured neonatal rat fibroblasts or cardiomyocytes, aldosterone increases synthesis of collagen as well as other proteins (5).

Physiologically significant amounts of mineralocorticoids have been believed to be synthesized only in the adrenal cortex. However, extraadrenal steroid 21-hydroxylation and 11ß-hydroxylation occur in a variety of fetal and adult human tissues as illustrated by conversion of circulating progesterone to deoxycorticosterone (DOC) and DOC sulfate in extra-adrenal sites (6). As another example, expression of steroid 21-hydroxylase and 11ß-hydroxylase by a benign testicular Leydig cell tumor has been described (7). We have reported that aldosterone, synthesized in the vasculature under partially control by angiotensin II (Ang II), participates in the development of vascular hypertrophy that correlates with Ang II concentration (8, 9). Aldosterone synthesis also has been reported to occur in brain and heart (10, 11).

Ang II is one of a growing number of peptide hormones that have been implicated in regulation of cellular growth and cardiocyte hypertrophy. Evidence of an endogenous renin-angiotensin system in the heart includes demonstration of mRNAs for angiotensinogen and renin (12), as well as for angiotensin I converting enzyme (ACE) (13) and Ang II receptors (14). Upregulation of left ventricular angiotensinogen and ACE mRNAs has been described in association with cardiac hypertrophy induced by pressure overload suggesting that a cardiac renin-angiotensin system may be activated in cardiac hypertrophy (15).

Taken together, these data indicate that in addition to the circulating renin-angiotensin-aldosterone system (RAAS) a cardiac RAAS may contribute to cardiac hypertrophy. High sodium intake induces cardiac hypertrophy in spite of lowering the activity of the circulating RAAS. To clarify the effects of high sodium intake on the cardiac RAAS and cardiac structure, we studied changes in cardiac mass, aldosterone production by the heart, and cardiac levels of mRNA for the aldosterone synthase (CYP11B2) and type-I Ang II receptor (AT_1R) genes in normotensive Wistar-Kyoto (WKY) rats after 8 weeks of high sodium intake.

Methods

Animals

Studies were conducted using 3-week-old male WKY/Izm rats (69±1g, n=32) donated by the Disease Model Cooperative Research Association (Kyoto, Japan) and were approved by the Institutional Animal Care and Use Committee of Kanazawa University. All animals were housed in metabolic cages and had free access to tap water (n=16) or 0.9% NaCl solution as drinking water for 8 weeks (n=16) as well as standard rat chow (Na, 0.1 mmol/g; K, 0.24 mmol/g; Nippon Charles River, Kanagawa, Japan). Animals were maintained in an environment with a constant temperature and 12-h light-dark cycles in the Animal Care Facility at Kanazawa University. Blood pressure was determined by the plethysmographic tail-cuff method and blood was collected from the tail vein as previously reported (8). Plasma concentrations of aldosterone were measured as described previously (8). Plasma renin concentration (PRC) was measured as the rate of Ang I generation in the presence of excess rat angiotensinogen with the use of method of Menard and Catt (16). All procedures were performed according to the guidelines of the US NIH Guide for Use of Laboratory Animals and were approved by the Animal Research Committee of Kanazawa University (permit no . 28078).

Received 12/07/99.

Isolated Perfused Heart

Rats were heparinized and anesthetized with pentobarbital (100 mg/kg ip). Once the rat was deeply anesthetized, the heart was removed via sternectomy and placed in ice-cold Krebs-Ringer solution. The heart was cannulated immediately via the aorta for retrograde perfusion in a Langendorff apparatus under constant pressure (90 mm Hg) with a modified Krebs-Henseleit solution (17). The solution was aerated with 95 % O_2/5% CO_2 and adjusted to a pH 7.4. Studies were initiated after a 20-min equilibration perfusion.

Measurements of aldosterone in the perfusate

After 20 min of equilibration, perfusate was collected for 2 h. The perfusate was extracted with a Sep-pak C18 cartridge (Waters, Milford) and subjected to reversed-phase high-performance liquid chromatography (HPLC) using methanol/water (40%/100%) as the mobile phase at a flow rate of 1.5 mL/min for 60 min. The retention times of 18-hydroxycorticosterone, aldosterone, corticosterone, deoxycorticosterone (DOC), progesterone, and pregnenolone were 35, 32, 43, 49, 55, and 59 min, respectively. The fraction corresponding to synthetic aldosterone was collected and analyzed by gas chromatography/mass spectrometry (GC/MS). The applied GC temperature was 90°C with a rising rate of 4°C / min. The MS conditions were electro-impact ionization (70 eV) and a linear upscan m/z of 70 to 750 in a 2-sec scanning period. After derivatization as described elesewhere (8), the samples were dissolved in hexane and injected into the GC/MS apperatus. Perfusate with tritiated aldosterone (3000 cpm, Amersham Japan, Tokyo, Japan) added to determine recovery, was extracted with a Sep-pak C18 cartridge prior to HPLC separation as described above. The aldosterone concentration in the perfusate was measured using an RIA after HPLC separation. The sensitivity of the assay was 5 fmol/tube. The overall recovery was 70-80%. The interassay variation was 11.6% and intraassay variation was 9.0%. At the end of the experiment, the heart was homogenized in 10 mL Krebs-Ringer buffer solution using a tissue grinder. Protein was assayed as described previously (8).

Measurements of aldosterone synthase activity in the heart

Aldosterone synthase activity was estimated by conversion of[^{14}C]DOC to [^{14}C]aldosterone (8). Diced heart tissues were homogenized in 2 mL of Krebs buffer (without BSA) by three 10-sec bursts in an Ystral homogenizer and the homogenate was assayed colorimetrically for protein. Steroid conversion rates were assayed in triplicate by the addition of homogenate (1 mg protein) to 1 mL of buffer containing 0.5 μmol/L [^{14}C] DOC (0.001 μCi), sucrose (250 mmol/L), isocitrate (25 μmol/L), and Tris-HCl (50 mmol/L, pH 7.4). Following 120 min of incubation at 37°C, the incubation medium was extracted with a Sep-pak C18 cartridge. The steroids were separated by reversed-phase HPLC. Radioactivity in the fraction corresponding to DOC and aldosterone was counted using a liquid scintillation counter.

Quantitation of CYP11B2 and AT1R mRNA in the heart

Eight rats from each group were used for quantitation of CYP11B2 and AT1R mRNA in the heart. After the rat was anesthetized, the heart was removed immediately after decapitation. The tissue was weighed promptly, frozen in liquid nitrogen, and stored at –80°C until analysis. Total RNA from rat hearts was isolated with guanidium thiocyanate followed by centrifugation in a cesium chloride solution. RNA competitors mimicking CYP11B2 and AT1R were synthesized using a competitive RNA transcription kit (Takara Shuzo, Tokyo, Japan). Quantitative RT-PCR assays for CYP11B2 and AT1R mRNA were performed using a competitive PCR method as previously reported (18). Sequences for the sense and antisense primers for CYP11B2 (19) and AT1R have been described previously (20). For Southern blotting analysis, the RT-PCR products were electrophoresed in 10-μL aliquots on a 3% agarose gel and transferred to nylon membranes. Hybridization was performed as previously reportetd (21). The expression of renin mRNA of adrenal gland and kidney was measured as reported previously (22).

Data are expressed as the means ± SEM. Differences were assessed by one-way ANOVA and multiple comparison tests. Statistical significance was accepted for a value for $p < .05$.

Results

Figure1 shows the HPLC elution profile of immuno-reactive aldosterone in the cardiac perfusate. One aliquot with a retention time of 43 min (corresponding to aldosterone) was injected into the GC/MS system. The mass spectrum of aldosterone isolated from the rat hearts was identical to that o f synthetic aldosterone (data not shown).

High sodium intake did not significantly increase plasma sodium concentration (data not shown). Table 1 summarizes the data for body weight, systolic blood pressure, the left ventricular weight/body weight ratio (LV/BW), PRC, plasma aldosterone concentration, and AT 1R mRNA levels in the heart. The blood pressure did not differ between the two groups. The LV/BW ratio was significantly increased in rats with high sodium intake ($p<.05$). Plasma aldosterone concentration and PRC were significantly lower with high sodium intake than with the normal-sodium diet ($p<.05$). High sodium intake significantly increased cardiac AT 1R mRNA levels ($p<.05$).

Upper panel of Figure 2 shows the data of Southern blotting of RT-PCR products of CYP11B2 mRNA. The expression of CYP11B2 mRNA in heart is about 1/100 of that in adrenal gland. Lower panel of Figure 2 illustrates that increasing concentrations of each competitive template for CYP11B2 from 0 to 100 × 10^3 mRNA molecules progressively inhibited the amplification endogenous CYP11B2 mRNA in the heart or adrenal gland. When PCR was carried out in the absence of reverse transcription, no bands were seen at 297 bp (data not shown).

Figure 3 summarizes the amount of aldosterone synthesized, the activity of aldosterone synthase, and the concentrations of CYP11B2 mRNA in the heart for each experimental group. Cardiac aldosterone production in rats with high sodium intake was significantly increased compared with that o f controls (19 ± 2.5, 39 ± 2.2 pmol/mg/h, $p<.05$). Aldosterone synthase activity in hearts was significantly increased by high sodium intake (14 ± 2 .1 vs 29 ± 2.9 %, $p<.05$). Expression of CYP 11B2 mRNA in the heart was significantly increased by high sodium intake (2.3 fold increase; $p<.05$). High sodium intake significantly decreased renin mRNA expression in the adrenal gland and kidney, which was parallel to PRC (data not shown) .

Discussion

The present results demonstrate a functioning system involving local aldosterone synthesis in the heart. Cardiac hypertrophy is an important and independent predictor of cardiac morbidity and mortality. However, the factors responsible for hypertrophy are poorly understood. In this experiment, high salt intake caused cardiac hypertrophy without significantly elevating the blood pressure. Histologically, perivascular/interstitial fibrosis in the heart was obserbed (data not shown). In recent years evidence has accumulated that high sodium intake might increase cardiovascular morbidity and mortality via effects on cardiac structure that are independent of increased pressure load (23).

Increasingly, the RAAS is being implicated in development of cardiac hypertrophy. Treatment with ACE inhibitors or AT_1 receptor antagonists can bring about regression of cardiac hypertrophy and fibrosis in animal models of hypertension as well as in hypertensive patients (24, 25), while chronic mineralocorticoid excess induces cardiac hypertrophy in rats and in humans (26). Treatment with spironolactone, an antagonist of aldosterone, prevents

TABLE 1. Effects of sodium intake on body weight, systolic blood pressure, left ventricular weight, plasma renin concentration, plasma aldosterone concentration, and AT 1 receptor mRNA levels in the heart

	BW (g)	SBP (mmHg)	LV (mg/B W)	PRC (ng/mL/h)	P-aldo (pmol/L)	AT 1R mRNA (mRNA molecules/μg total RNA)
Control (n=16)	335 ± 3	112 ± 4	2.40 ± 0.08	5.9 ± 0.5	102 ± 20	0.75 × 10^6
High sodium intake (n=16)	326 ± 3	124 ± 6	2.90 ± 0.10*	0.7 ± 0.15*	48 ± 9*	1.65 × 10^{6*}

Value are mean ± SEM. BW, body weight; SBP, systolic blood pressure; LV, left ventricular weight; PRC, plasma renin concnetration; P-aldo, plasma aldosterone; AT 1R, type-I angiotensin II receptor. *p<.05 vs control.

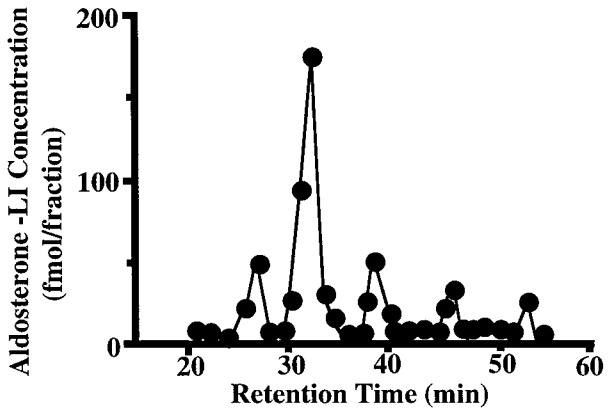

Fig 1. The high-performance liquid chromatographic elution profiles of immunoreactive aldosterone (aldosterone LI) in the perfusate of rat hearts.

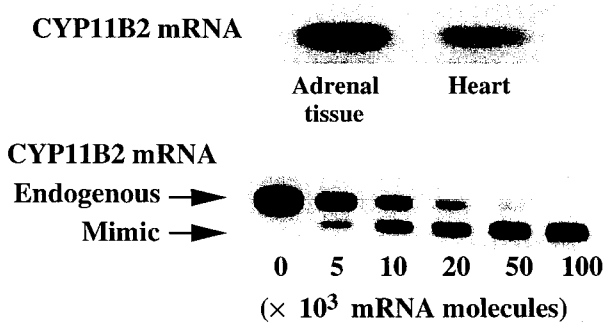

Fig. 2. The expression of CYP11B2 mRNA using Southern blotting of reverse-transcriptase polymerase chain reaction in rat normal adrenal tissue and heart (upper panel). Lower panel shows the analysis of relative changes in CYP11B2 mRNA concentrations by competitive polymerase chain reaction.

myocardial fibrosis in rats (27). Pitt et al.(28) recently have reported that spironolactone reduces the risk of both morbidity and death among patients with severe heart failure.

The capacity of cardiac tissues to synthesize renin is still being debated (29). However, expression of angiotensinogen and ACE mRNA are detected in the heart (12). Local angiotensin formation in the heart may contribute to the pathogenesis of cardiac hypertrophy, congestive heart failure, and tissue remodeling (30). We have reported that rat and human blood vessels produce aldosterone (8, 18). Vascular aldosterone production is controlled by the renin-angiotensin system at the transcriptional level (8) and

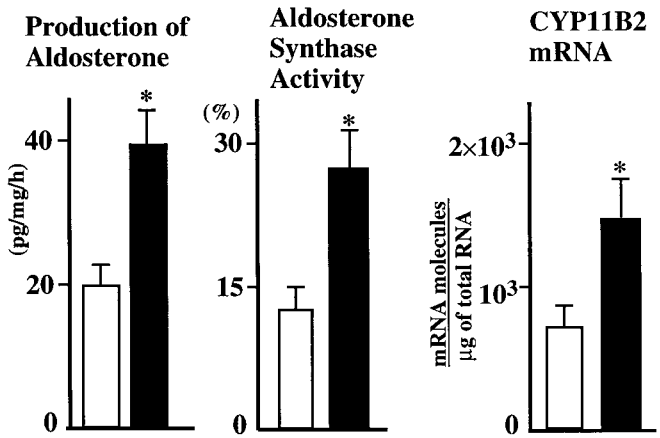

Fig 3. Effects of high sodium intake on cardiac aldosterone production, aldosterone synthase activity, andCYP 11B2 mRNA levels. Open column indicates control rats and closed column indicates rats with high sodium intake. *p<.05vs control.

participates in the development of hypertension in rats (19). Studies in our laboratory and others have shown that the heart can produce aldosterone and its production is controlled by renin-angiotensin system. Silvestre et al.(31) have reported that myocardial infarction activates myocardial aldosterone synthesis. We have found that cardiac aldosterone was increased in hypertensive hearts of rats (unpublished data). In our experiments, high salt intake decreased PRC and plasma aldosterone concentration, so circulating RAS may not be concerned with the cardiac hypertrophy induced by high salt intake. In contrast to components of the circulating RAAS, cardiac aldosterone synthesis and AT1R mRNA levels were increased by high salt intake. Boddi et al. (32) have reported that Ang II formation by human forearm vascular tissue was increased by high sodium diet while PRA was decreased.

High sodium intake, then, increases cardiac aldosterone synthesis, which may contribute to the cardiac hypertrophy independently of the circulating RAAS.

Acknowledgements

This work was supported in part by Grants-in-Aid for Sceintific Research from the Ministry of Education, Science and Culture, Japan and Yamanouchi Foundation for Research on Metabolic Disorders.

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