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Tissue-Specific Regulation of Angiotensinogen and Angiotensin II Receptor Gene Expression in Deoxycorticosterone Acetate-Salt Hypertensive Rats

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Molecular regulation of the renin-angiotensin system (RAS) was investigated in deoxycorticosterone acetate (DOCA)-salt hypertension. The expression of renin, angiotensinogen and angiotensin II receptor genes in the kidney and liver was determined by Northern blot analysis in rats which were made DOCA-salt hypertensive over the period of 2 or 4 weeks. Along with the hypertension, renin mRNA was decreased in the remnant kidney. The expression of angiotensinogen gene was not significantly altered in the kidney, but was significantly decreased in the liver. The expression of angiotensin II receptor gene was increased in the kidney, while it remained unaltered in the liver. The duration of hypertension did not affect the altered gene expression. It is suggested that the components of RAS are transcriptionally regulated in DOCA-salt hypertension in a tissue-specific manner.

Key Words: Northern blot analysis, Renin, Angiotensinogen, Angiotensin II receptor, Deoxycorticosterone acetate-salt hypertension

INTRODUCTION

The renin-angiotensin system (RAS) consists of a successive cleavage of angiotensinogen by renin and angiotensin converting enzyme, which results in the formation of angiotensin II. Angiotensin II in turn binds to its specific receptors, mediating the most of the known biological effects of RAS.

Among the various components of RAS, renin represents the rate-limiting factor in the catalytic formation of angiotensin II. Therefore, the transcriptional regulation of renin has been of particular scientific interest for the past 10 years. However, an increasing body of evidence suggests a specific regulation of the other components of RAS such as angiotensinogen and angiotensin II receptor.

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The hepatic expression of angiotensinogen gene was significantly increased in two-kidney, one clip (2K1C) rats along with contemporary changes of the plasma angiotensinogen level (Morishita et al, 1991). However, the renal expression of angiotensin II receptor was either upregulated (Modrall et al, 1995) or diminished after clipping the unilateral renal artery (Haefliger et al, 1995). These observations may represent the molecular regulation of RAS when its biological activity is activated.

RAS components may also be altered when its overall activity is diminished. Angiotensinogen mRNA levels were reduced in rats on a high-salt diet (Singh et al, 1996), and angiotensin II receptor gene expression was inversely related to salt intake (Sechi et al, 1996; Nickenig et al, 1998). However, little has been known on the molecular changes of RAS in hypertension associated with an attenuated activity of the system.

The present study was aimed at investigating the molecular regulation of RAS in deoxycorticosterone acetate (DOCA)-salt hypertension that is characterized by an expansion of extracellular body fluid

and diminished activity of RAS. The expression of renin, angiotensinogen and angiotensin II receptor genes was examined by Northern blot analysis in rats with DOCA-salt hypertension.

METHODS

Development of hypertension

Male Sprague-Dawley rats were used. They were kept in accordance with the Institutional Guidelines for Experimental Animal Care and Use. To develop DOCA-salt hypertension, the rats weighing 150~200 g were subcutaneously implanted with silicone rubber containing DOCA (200 mg/kg) under light ketamine anesthesia, one week after they had been unilaterally nephrectomized. They were given 1.0% saline to drink thereafter. Control group was also unilaterally nephrectomized and given saline to drink, but was not implanted with DOCA. They were used 2 or 4 weeks after inducing the hypertension.

On the day of experiment, systolic blood pressure was indirectly measured by the tail-cuff method without anesthesia. The trunk blood was then collected by decapitation in a conscious state. The plasma was measured of its renin concentration in the presence of an excessive renin substrate using the commercial radioimmunoassay kit (New England Nuclear; Boston, MA, USA).

RNA extraction

Following the decapitation, kidneys and livers were rapidly removed, frozen in liquid nitrogen, and stored at −70°C until used. Total RNAs were isolated using Ultraspec™ RNA isolation system (Biotecx Laboratories; Houston, TX, USA) according to the single-step method of Chomczynski & Sacchi (1987). The frozen tissue was homogenized with RNA reagent. The homogenate was incubated at 4°C for 5 min to permit complete dissociation of nucleoprotein complexes. Next, 0.2 ml of chloroform per ml of RNA reagent was added to the homogenate. The mixture was vigorously vortexed for 15 sec, and kept on ice for 5 min. It was then centrifuged at 12,000 g and 4°C for 15 min. The upper aqueous phase was transferred into a tube while the interphase containing DNA and protein was maintained undisturbed. An equal volume of isopropanol was added to the aqueous phase. The mixture was kept at 4°C for at least 10 min, and centrifuged again at 12,000 g and 4°C for 15 min. The RNA pellet was then washed twice with prechilled 75% ethanol, air-dried, and resuspended in DEPC-treated water. The concentration of RNA was measured by spectrophotometry at 260 nm. The integrity of the RNA and equal loading were tested by comparing 28 S and 18 S rRNA intensities in ethidium bromide-stained gels.

Northern blot analysis

For Northern blotting, RNA samples were resolved by electrophoresis through 8% formaldehyde-1.4% agarose denaturing gel buffered with 20 mM 3-N-morpholinopropanesulfonic acid and 1 mM ethylenediaminetetraacetic acid, pH 7.4. The RNAs were then transferred overnight from the gel to Nylon membrane (Boehringer Mannheim; Mannheim, Germany) in 20×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate). The membrane was baked under vacuum at 80°C for 2 h.

To determine renin and angiotensinogen mRNA, the blots were probed using ^{32}P-labeled RNA transcribed from the plasmids containing cDNA sequences for renin (pREN44.ceb) and angiotensinogen (pRang6) genes which were linearized with BamH1 and EcoRI, respectively, using T7 RNA polymerase. To detect angiotensin II receptor mRNA, 2-kb insert was isolated by digesting a full length rat angiotensin II type 1A receptor cDNA template with Hind III and Xho I, and radiolabeled by the method of nick translation (Promega; Madison, WI, USA). The membranes were prehybridized in a shaking water bath for 4 h, at 65°C for renin and angiotensinogen, and at 42°C for angiotensin II receptor in a solution containing 50% formamide, 4×SSC, 100 g/ml sheared and denatured salmon sperm DNA, 4×Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 50 mM sodium phosphate, pH 6.5. Hybridization was made in the above solution containing approximately 5×10^6 cpm/ml of ^{32}P-labeled RNA or DNA for 16~20 h at 65°C or 42°C. After hybridization, the membranes were washed four times for 15 min each at room temperature in washing solution I (2×SSC and 0.1% SDS) and three times for 45 min each at 65°C or 42°C in washing solution II (0.1×SSC and 0.1% SDS). They were then dried under the lamp for 30 min and exposed to X-ray films (O-MAT™; Eastman Kodak; Rochester, NY, USA). mRNA levels were

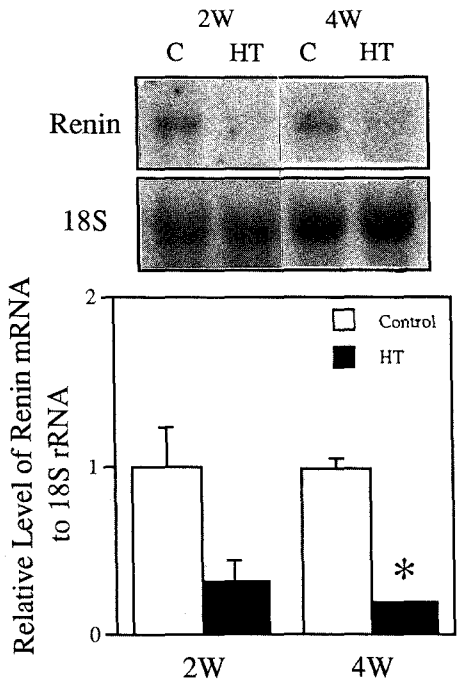

Fig. 1. Autoradiograms showing Northern blot analysis for renin mRNA and 18 S rRNA in kidneys of DOCA-salt rats at 2 and 4 weeks after DOCA implantation and drinking of 1% NaCl. Bar graphs represent relative renin mRNA levels (n=3 each). *$p<0.05$, compared with control.

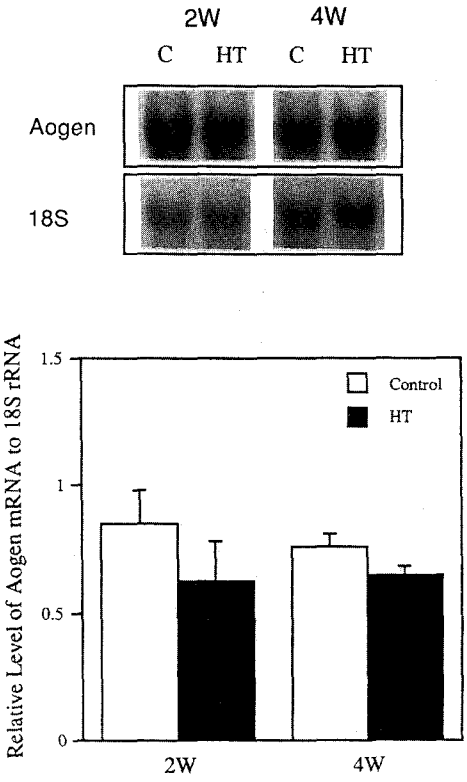

Fig. 2. Representative Northern blot autoradiograms and bar graphs showing relative levels of angiotensinogen mRNA in the kidney. Each column depicts mean±SEM (n=3).

determined by densitometric analysis of autoradiograms, which were normalized with 18 S rRNA.

All data were expressed as mean±SEM. For statistical analysis of differences between the groups, unpaired *t* test was used.

RESULTS

Systolic blood pressure measured on the experimental day was significantly higher in the experimental group than in the control: 169±6 (n=10) vs 120±3 mmHg (n=11) at week 2 ($p<0.01$), and 187±4 (n=10) vs 124±2 mmHg (n=12) at week 4 ($p<0.01$). On the contrary, plasma renin concentration was significantly lower in the experimental group than in the control at both weeks 2 and 4: 3.8±0.9 vs 10.7±2.4 ngAI/ml/h at week 2 ($p<0.05$, n=6 each), and 3.0±0.2 vs 8.3±2.0 ngAI/ml/h at week 4 ($p<0.05$, n=6 each).

The renin mRNA in the remnant kidney was decreased by 55~80% in the experimental group compared with that in the control (Fig. 1). The expression of angiotensinogen gene in the kidney was not significantly different between the groups (Fig. 2). In the liver, however, the angiotensinogen mRNA was significantly decreased by 45~60% in the experimental group (Fig. 3).

The expression of angiotensin II receptor (type 1A) gene was increased by 40~45% in the kidney concomitantly with the hypertension (Fig. 4), while it remained unaltered in the liver (Fig. 5). The duration of hypertension, either 2 or 4 weeks, did not affect the altered gene expression.

DISCUSSION

Previous studies showed decreases of renin gene expression in response to sodium loading, angiotensin II, or DOCA (Morris, 1992). We also observed a suppressed renin gene expression in the remnant kidney along with concomitant changes of plasma renin concentration in DOCA-salt hypertension. Therefore, the

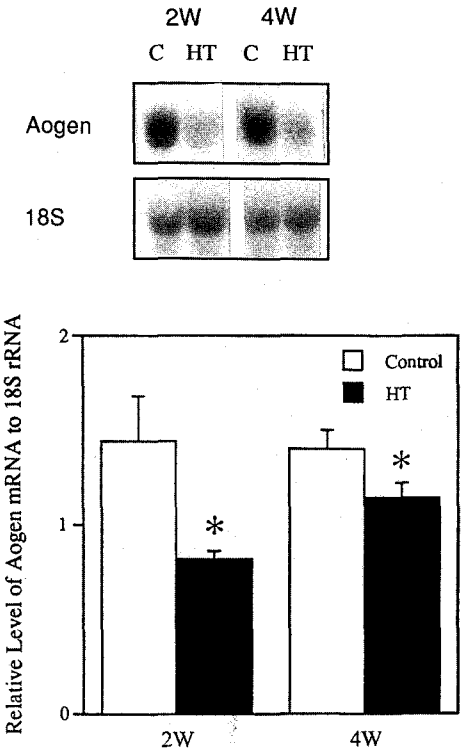

Fig. 3. Representative Northern blot autoradiograms and bar graphs showing relative levels of angiotensinogen mRNA in the liver. Each column depicts mean ± SEM (n=3). *$p < 0.05$, compared with control.

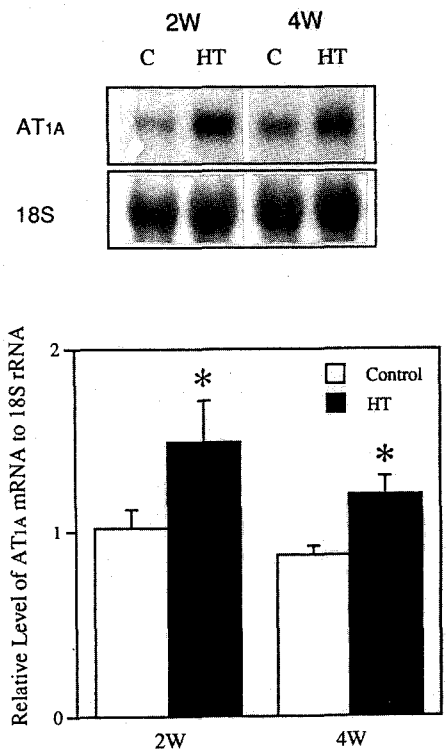

Fig. 4. Representative Northern blot autoradiograms and bar graphs showing relative levels of angiotensin II receptor mRNA in the kidney. Each column depicts mean ± SEM (n=3). *$p < 0.05$, compared with control.

decrease of plasma renin concentration may be attributed to a decreased synthesis and release from the kidney. The suppressed expression of renin genes in the remnant kidney may in turn be attributed to the increased blood pressure, such as in the contralateral non-clipped kidney in 2K1C hypertension (Morishita et al, 1991; Haefliger et al, 1995).

Although renin has been considered to be a rate-limiting step in angiotensin II production, recent evidence suggests the critical significance of angiotensinogen in determining the overall activity of RAS. Hepatic angiotensinogen mRNA levels are increased after clipping the unilateral renal artery in association with contemporary changes of plasma angiotensinogen (Morishita et al, 1991). Furthermore, the hepatic angiotensinogen gene expression was found to be increased by exogenous administration of angiotensin II (Nakamura et al, 1990; Kohara et al, 1992; Schunkert et al, 1992).

In this context, a decreased expression of angiotensinogen gene would be expected in DOCA-salt hypertension. Indeed, DOCA-salt hypertension was associated with significant decreases of angiotensinogen mRNA levels in the liver in the present study. The decreased expression of angiotensinogen gene may be attributed to the lack of angiotensin II stimulation as a consequence of the suppressed renin in this model of hypertension.

However, no significant changes were noted in the expression of angiotensinogen gene in the kidney. Tissue-specific regulation of angiotensinogen expression has been in fact variously demonstrated. Sodium depletion stimulates the angiotensinogen expression in the kidney, heart, aorta, and adrenal, but not in the liver and adipose tissue (Ingelfinger et al, 1986). In contrast, dexamethasone increases hepatic, adipose, and renal tissue levels of angiotensinogen mRNA but not aortic smooth muscle levels (Kalinyak & Perlman, 1987). Moreover, angiotensin II increased angiotensinogen formation in the isolated perfused liver (Nasjletti & Mason, 1973), while it did not affect angiotensinogen mRNA levels in the kidney (Sechi et al, 1996). The tissue-specificity may have important physiological implications. For instance, the

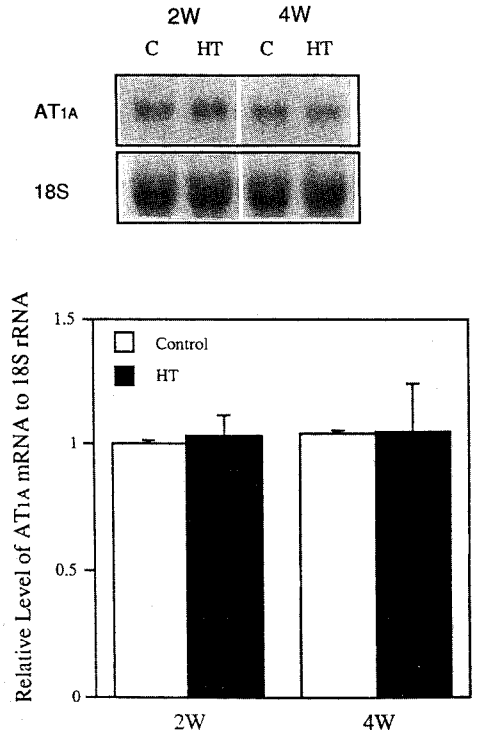

Fig. 5. Representative Northern blot autoradiograms and bar graphs showing relative levels of angiotensin II receptor mRNA in the liver. Each column depicts mean ±SEM (n=3).

decreased renal renin concerts with the suppressed hepatic angiotensinogen in diminishing the activity of RAS in DOCA-salt hypertension.

The expression of angiotensin II receptor gene is also subjected to change. An enhanced activity of RAS causes downregulation of the receptor, whereas a decreased activity results in upregulation (Wilson et al, 1989). More recently, the negative feedback regulation exerted by angiotensin II on the expression of angiotensin II receptor gene has been demonstrated (Naville et al, 1993; Tufro-McReddie et al, 1993). It is likely that the local tissue angiotensin II plays a tonic inhibitory role in the regulation of angiotensin II receptors in situ. The speculation may be in line with a recent finding in which angiotensin II binding was decreased in the kidney following the chronic infusion with angiotensin II (Sechi et al, 1996).

One may thus expect an increase of angiotensin II receptor gene expression in DOCA-salt hypertension. We indeed observed an increase of angiotensin II receptor mRNA levels in the kidney. However, the expression of angiotensin II receptor was not changed in the liver. It has been reported that dietary sodium intake may modulate the expression of angiotensin II receptor genes in a tissue-specific manner (Sechi et al, 1996). In the present study, however, the high sodium intake does not completely explain the tissue-specific regulation, since the control group was also on a high sodium diet. The tissue-specificity is more likely to be linked to the high blood pressure per se. Since we failed to observe a significant difference in the altered gene expression as a function of the duration of hypertension, either 2 or 4 weeks, however, a more prolonged study may be necessary to differentiate the effects of high salt intake and high blood pressure.

In 2K1C hypertension, binding studies revealed a reduction of glomerular angiotensin II receptors in the clipped kidney (Wilkes et al, 1988). Furthermore, a causal relationship between the circulating activity of renin (and hence angiotensin II) and expression of angiotensin II receptor genes in the clipped kidney has been suggested by an inverse correlation existing between them (Haefliger et al, 1995). Conversely, one may hypothesize that an upregulation of angiotensin II receptor expression has a physiological implication that leads to an enhanced vasoconstriction, decreased urinary sodium excretion and, eventually, elevated blood pressure. The detailed mechanisms involved in the regulation of RAS genes have to be further investigated.

To summarize, our study demonstrated that the components of RAS were transcriptionally regulated in DOCA-salt hypertension in a tissue-specific manner, which may be causally related with the maintenance of high blood pressure.

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