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Pathophysiological roles of vascular 11β-hydroxysteroid dehydrogenase and aldosterone☆

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Abstract

Mineralocorticoid receptor (MR) binding is tightly regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSDII) which selectively metabolizes glucocorticoids to inactive metabolites, thus allowing for MR activation by aldosterone. To examine whether this enzyme is involved in the pathophysiology of salt-sensitive hypertension, 11β-HSDII activity and messenger RNA (mRNA) levels were determined in blood vessels of Dahl Iwai salt-sensitive (DS) and salt-resistant (DR) rats. Decreased 11β-HSDII activity and mRNA levels in mesenteric arteries were observed in 8-week-old DS rats on a high-salt diet, indicating that 11β-HSDII may play a significant role in salt sensitivity and hypertension. It has been suggested that mineralocorticoids act on blood vessels, leading to increased vasoreactivity and peripheral resistance. We present direct evidence that blood vessels are aldosteronogenic. The production of aldosterone in blood vessels was compared between stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar-Kyoto (WKY) rats. Vascular aldosterone and CYP11B2 mRNA levels were significantly increased in 2-week-old SHRSP versus WKY rats. However, the vascular aldosterone levels in 4- and 9-week-old SHRSP and WKY rats were similar. High sodium intake further increased both blood pressure and vascular aldosterone synthesis in the SHRSPs. Both the local renin–angiotensin–aldosterone system (RAAS) and the vascular 11β-HSDII level are critically important in the pathophysiology of cardiovascular disorders.

Keywords: Aldosterone; Hypertension; Sodium; Blood vessel; Hydroxysteroid dehydrogenase

1. Introduction

Blood vessels are target tissues for glucocorticoids and mineralocorticoids. There has been increasing evidence that mineralocorticoids, acting directly on peripheral vascular tissue, cause hypertension. The concentration of cortisol, the physiological glucocorticoid in humans, exceeds the circulating level of the mineralocorticoid aldosterone by 1000-fold. The mineralocorticoid receptor (MR) is known to possess a similar in vitro affinity for cortisol and aldosterone. The mineralocorticoid target tissues metabolize glucocorticoids to less active compounds, utilizing the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), thus protecting the cytosolic MR (Fig. 1).

Biochemical studies have revealed that there are two isoforms of 11β-HSD, a NAD^+-dependent form (11β-HSDII) and a $NADP^+$-dependent form (11β-HSDI). 11β-HSDII is found in tissues with high levels of MR activity such as kidney, placenta and colon [1]. Both 11β-HSDI and 11β-HSDII are expressed in the vascular wall, although the tissue distribution has not yet been fully characterized. Both the expression of messenger RNA (mRNA) and the enzyme activity of 11β-HSDI and 11β-HSDII have been demonstrated in the vascular smooth muscle cells (VSMC) and in endothelial cells. Glucocorticoid receptors (GR) and MR are expressed in blood vessels, and corticosteroids have diverse actions on vascular function.

The normal activity of 11β-HSDII is essential to prevent the mineralocorticoid activity of cortisol. Therefore, a deficiency of the 11β-HSD isoforms, whether congenital or resulting from inhibition of this enzyme by administration of licorice or carbenoxolone, leads to the activation of MRs by glucocorticoids. This in turn results in sodium retention and hypertension. A syndrome characterized by an apparent mineralocorticoid excess has been reported, and it is associated with several mutations of 11β-HSDII gene [2,3]. Transgenic mice deficient for 11β-HSDI do not show signs of mineralocorticoid excess [4], while 11β-HSDII gene knockout mice have hypertension and renal dysfunction [5].

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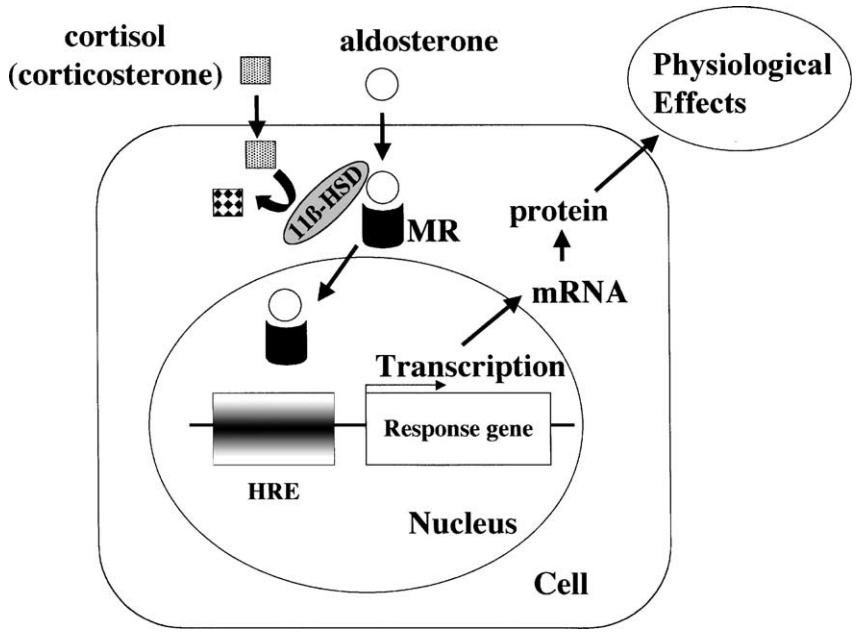

Fig. 1. 1β-Hydroxysteroid dehydrogenase (11β-HSD) functions as a protecting mechanism preventing cortisol binding to the mineralocorticoid receptor. MR, mineralocorticoid receptor; HRE, hormone responsive element.

2. Vascular 11β-HSDII in salt-sensitive hypertension

Excess sodium intake is intimately involved in the pathogenesis of hypertension. In large populations, significant correlations between levels of salt intake, blood pressure, and the frequency of hypertension have been reported. Patients with essential hypertension do not have overt signs of mineralocorticoid excess; however, more subtle changes such as a positive correlation between blood pressure and serum sodium levels or a negative correlation with potassium levels suggest a corticosteroid influence [6]. There is also growing evidence for decreased 11β-HSDII activity in essential hypertension [7]. Since steroid hormones modulate renal sodium retention, it is likely that variations in 11β-HSDII activity are responsible for the sensitivity of blood pressure to dietary salt. Ferrari and Krozowski have reported that impaired activity of 11β-HSDII is associated with an increased susceptibility of blood pressure to salt load [8].

Dahl Iwai salt-sensitive (DS) rats are widely used to study genetic determinants of salt-sensitive hypertension. In this strain, supplemental dietary sodium increases blood pressure, whereas in the Dahl Iwai salt-resistant (DR) strain, supplemental dietary sodium has little or no effect on blood pressure. There are several reports describing abnormalities of the renin–angiotensin system, adrenal steroids, and the sympathetic nerve system in DS rats. To clarify the mechanism of salt-induced hypertension in DS rats, vascular 11β-HSDII activity and the gene expression of 11β-HSDII mRNA in mesenteric arteries of DS and DR rats were compared.

2.1. Materials and methods

Male DS and DR rats aged 3–4 weeks, were initially fed a standard chow (0.45%). Both DS and DR rats were fed high sodium chow (7%) for 4 weeks. The 11β-HSDII activity in mesenteric arteries was determined by measuring the rate at which [^{3}H]-corticosterone (B) was converted to [^{3}H]-11-dehydrocorticosterone (A) at 37 °C over 30 min. The reaction mixture contained 20 μl of the microsomal fraction of mesenteric arteries (250 μg protein), 10 μl of 1.12×10^{-8} mol/l [^{3}H]-B, 250 μmol/l of NAD^+, and 60 nmol/l of B as previously reported [9]. The reaction was stopped by adding an equal volume of ethyl acetate. Metabolites of B were separated by HPLC as previously reported [9]. Quantification of 11β-HSDII mRNA in the mesenteric artery was performed using the competitive PCR method as previously reported [10]. The sequences of sense and antisense primers for 11β-HSDII were 5′-ACTCCGTGGCC-TGAGACG-3′ and 5′-TTCAAGTCCACCACACAG-3′, respectively, as previously described [10].

2.2. Results

The blood pressure of 8-week-old DS rats (228 ± 17 mmHg) significantly exceeded that of DR rats (106 ± 4 mmHg) ($P < 0.05$). There were no significant differences in blood pressure between the 4-week-old DS and DR rats. Plasma corticosterone and aldosterone concentrations did not differ in either 4- or 8-week-old DS versus DR rats. Fig. 2 shows the vascular 11β-HSDII activity and mRNA levels in DS and DR rats. The 11β-HSDII activity and the

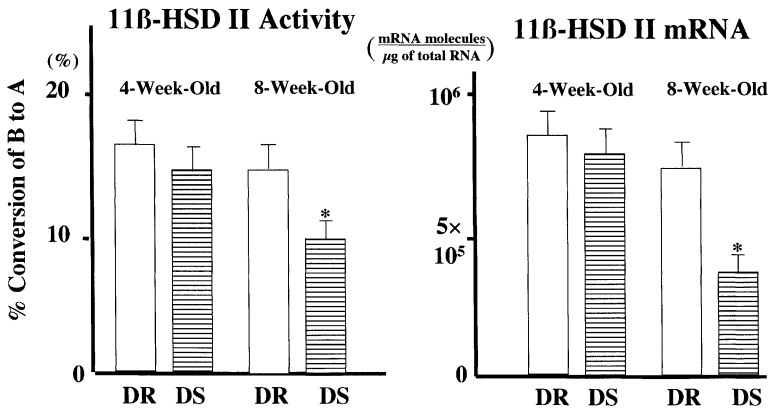

Fig. 2. High sodium intake significantly decreased both vascular 11β-HSDII activity and 11β-HSDII mRNA levels in DS rats. 11β-HSDII, 11β-hydroxysteroid dehydrogenase; DR, Dahl salt-resistant rat; DS, Dahl salt-sensitive rat. The symbol (∗) indicates $P < 0.05$ for DS vs. DR. B, corticosterone; A, 11-dehydrocorticosterone.

expression of mRNA in mesenteric arteries of 8-week-old DS rats fed a high-salt diet was significantly lower than that of DR rats on a similar diet.

2.3. Discussion

We have reported substantially greater vascular responses to noradrenalin in 8-week-old DS rats compared to DR rats. Ruschitzka et al. [11] have reported that glycyrrhizic acid (GA)-induced hypertensive rats showed impaired nitric oxide-mediated vasodilatation. Since GA is a non-specific 11β-HSD inhibitor, it is not clear which 11β-HSD isozyme was responsible for these effects. Recently Hadoke et al. [12] reported endothelial cell dysfunction in mice after a transgenic knockout of type 2, but not type 1 11β-HSD. There has been increasing evidence that mineralocorticoids, acting on peripheral vascular tissue, cause hypertension. Tobian and Redleaf [13] have proposed that aldosterone affects the salt and water balance in vascular cells, thereby influencing vessel lumen size. Virdis et al. [14] have reported that both aldosterone and angiotensin II (Ang II) increased both vascular NADPH oxidase activity and vascular hypertrophy, while these increases were prevented by spironolactone. Aldosterone mediates some of the Ang II-induced vascular effects in hypertension, in part via associated increases in oxidative stress. I summarize these data in Fig. 3 that decreased vascular 11β-HSDII activity led to an increased local glucocorticoid concentration, resulting in activation of the glucocorticoid receptor and in endothelial dysfunction. On the other hand, increased vascular glucocorticoid levels also activates the mineralocorticoid receptor, thus increasing the oxidative stress. Activation of both receptors may cause salt-induced hypertension and vascular injury.

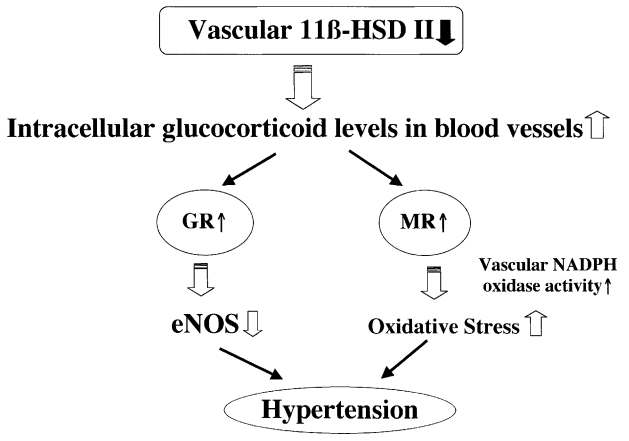

Fig. 3. Pathophysiological roles of 11β-hydroxysteroid dehydrogenase in vascular cells.

3. Vascular aldosterone synthesis in genetically hypertensive rats

Classical studies have shown that the mineralocorticoid aldosterone is involved in the regulation of sodium and water homeostasis and thus participates in the regulation of blood pressure. Aldosterone is involved in vascular smooth muscle hypertrophy and can cause vascular matrix impairment and endothelial dysfunction. Kornel presented evidence that glucocorticoids and mineralocorticoids control contractility of VSMC [15]. This control is exerted via glucocorticoid and mineralocorticoid receptors on the VSMC. We have detected MR mRNA in VSMC [16], and addition of aldosterone increased the incorporation of tritiated leucine into these VSMC. This incorporation was inhibited by a specific aldosterone antagonist [17]. We have also reported that aldosterone synthesized in the vasculature is partly controlled by Ang II, and aldosterone participates in the development of vascular hypertrophy that correlates with Ang II concentration (Fig. 4).

The hypertensive strain of stroke-prone spontaneously hypertensive rats (SHRSP) was first established in 1974 by Okamoto et al. [18] through the selective inbreeding of spontaneously hypertensive rats (SHR). In male SHRSP, high-salt intake is associated with development of malignant

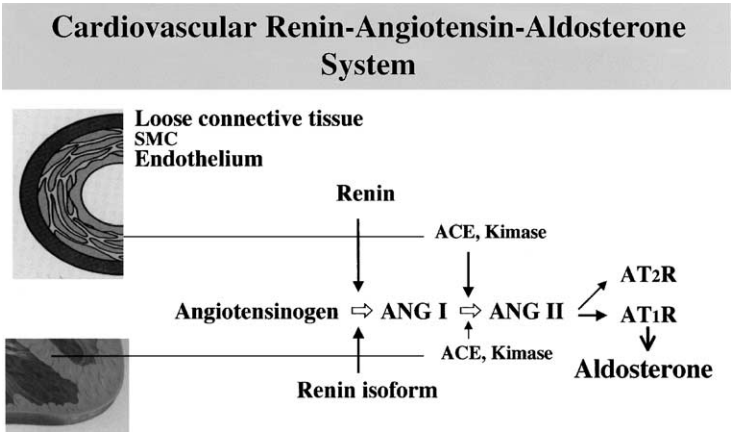

Fig. 4. Cardiovascular renin–angiotensin–aldosterone system. Angiotensin II is synthesized locally in various tissue, including the heart and blood vessels. Aldosterone synthesis in the cardiovascular tissues is reported in not only rodents but also in human.

hypertension, severe vascular injury, and cardiac hypertrophy [19]. The molecular mechanisms underlying the rapid vascular damage related to salt intake observed in this genetic model of malignant hypertension are not fully understood. The present study was undertaken to determine whether a high-salt intake increases cardiovascular aldosterone synthesis in SHRSP.

3.1. Materials and methods

Four-week-old SHRSP/Izm were housed in metabolic cages with free access to tap water or 0.9% NaCl solution as drinking water for 4 weeks as well as standard rat chow. Eight rats from each group were used for experiments involving mesenteric arterial perfusion. After 20 min of equilibration, the perfusate was collected for 2 h. The aldosterone concentration in the perfusate was measured using RIA after HPLC separation. Eight rats from each group were used for quantification of CYP11B2 and Ang II receptor (AT1R) mRNA in blood vessels. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assays for CYP11B2 and AT1R mRNA were performed using a competitive PCR method as previously described [16].

3.2. Results

Fig. 5 summarizes the effects of high sodium intake in SHRSP on blood pressure, plasma aldosterone concentration and vascular aldosterone synthesis. High sodium intake resulted in significant ($P < 0.05$) increases in blood pressure and vascular aldosterone production, which went up by factors of 1.3 and 2.8, respectively. Plasma aldosterone concentrations were lowered in SHRSP with a high sodium intake, compared with rats on a diet with normal sodium levels. High sodium intake increased the arterial expression of CYP 11B2 mRNA and AT1R mRNA by factors of 3.2 and 2.9, respectively ($P < 0.05$).

3.3. Discussion

In this study, the concentrations of circulating aldosterone and PRA were decreased by high-salt intake, while vascular aldosterone levels were increased in salt-loaded SHRSP. Increased levels of AT1R mRNA in the arteries of salt-loaded SHRSP were also observed. These results suggest that high-salt intake activates the vascular local renin–angiotensin–aldosterone system (RAAS) despite a depressed systemic RAAS. Boddi et al. [20] also reported that Ang II formation by human forearm vascular tissue was increased by a high sodium diet while PRA was decreased.

Aldosterone receptors are present in cardiac myocytes as well as in blood vessels. Peripheral infusion of aldosterone in rats with a high sodium intake causes cardiac hypertrophy and fibrosis without increasing their blood pressure [21]. There is increasing evidence supporting the existence of a cardiac RAAS that promotes local angiotensin and aldosterone formation. This localized angiotensin and aldosterone may contribute to the pathogenesis of cardiac hypertrophy, tissue remodeling, and congestive heart failure [22]. Ang II is an important regulator of adrenal mineralocorticoid synthesis and secretion. We have reported that chronic treatment

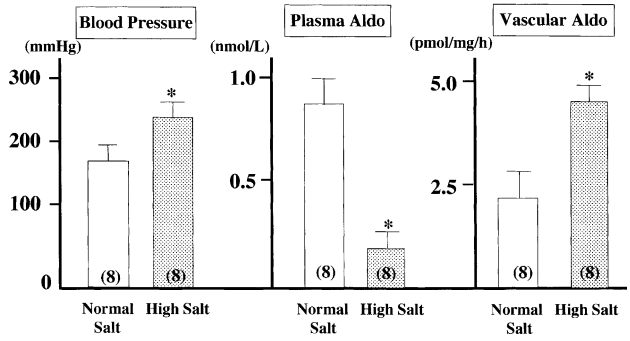

Fig. 5. High sodium intake increased blood pressure and decreased plasma aldosterone concentration, but increased vascular aldosterone synthesis in SHRSP.

with Ang II caused increases in cardiac aldosterone synthesis, aldosterone synthase activity, and expression of CYP11B2 mRNA [23]. These results suggest that cardiac aldosterone synthesis is controlled, in part, by the RAS at the transcriptional level. Silvestre et al. [24] have also reported that Ang II, as well as low sodium and high potassium diets, increased myocardial production of aldosterone in rats. A low sodium/high potassium diet increases aldosterone synthesis in the adrenal gland. We reported previously that high potassium levels led to increases in vascular aldosterone synthesis [25]. A low sodium diet increases the plasma concentration of Ang II, and this may have stimulated the cardiac aldosterone synthesis noted in Silvestre's experiments.

4. Conclusion

We have demonstrated here that a high-salt intake leads to decreased PRA and plasma aldosterone concentrations; thus the circulating RAAS may not be involved in the vascular and cardiac remodeling that are induced by high-salt intake. In contrast to effects on levels of circulating RAAS components, cardiovascular aldosterone synthesis and AT1R mRNA levels were increased by high-salt intake. High sodium intake thus increases cardiovascular aldosterone synthesis, which may well contribute to the cardiac hypertrophy and vascular injury observed in salt-loaded SHRSP, and this effect is independent of the circulating RAAS. Thus, the vascular 11β-HSDII and aldosterone are of critical importance in the pathophysiology of cardiovascular disorders.

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