Gene Expression of 11β-Hydroxysteroid Dehydrogenase in the Mesenteric Arteries of Genetically Hypertensive Rats

Yoshiyu Takeda, Isamu Miyamori, Takashi Yoneda, Kazuhiro Iki, Haruhiko Hatakeyama, Ryoyu Takeda

Abstract

11β-Hydroxysteroid dehydrogenase (11β-HSD) modulates the access of corticosteroids to their receptors and plays an important role in controlling blood pressure. We determined 11β-HSD activity and mRNA levels in the mesenteric arteries of genetically hypertensive rats, the Dahl salt-sensitive hypertensive rat, and compared them with Dahl salt-resistant and Sprague-Dawley rats. 11β-HSD activity was significantly decreased in the mesenteric arteries of 8-week-old Dahl salt-sensitive hypertensive rats (11.4±1.4%) compared with Dahl salt-resistant rats (17.4±1.4%) or Sprague-Dawley rats (18.0±1.5%) of the same age (P<.05). There were no significant differences in 11β-HSD activity between 4-week-old Dahl salt-sensitive hypertensive and Dahl salt-resistant rats of the same age (15.3±1.3% and 15.1±1.9%, respectively). The concentration of 11β-HSD mRNA in the mesenteric arteries of 8-week-old Dahl salt-sensitive hypertensive rats was significantly lower than in Dahl salt-resistant or Sprague-Dawley rats of the same age (P<.05). There were no significant differences in the concentration of 11β-HSD mRNA in the mesenteric arteries of 4-week-old Dahl salt-sensitive hypertensive rats, Dahl salt-resistant rats, and Sprague-Dawley rats. These results indicate that 11β-HSD in the vascular wall may play a role in the pathogenesis of hypertension in this rat model. (Hypertension. 1994;23:577-580.)

Key Words • hydroxysteroid dehydrogenases • RNA, messenger • rats, inbred • norepinephrine • adrenal cortex hormones

The bioactivity of 11β-hydroxysteroid dehydrogenase (11β-HSD) has been identified in homogenates not only from rat liver and kidney but also from rat aorta and mesenteric artery. A physiological role for 11β-HSD has recently been suggested in the kidney, where it protects mineralocorticoid receptors from exposure to corticosterone or cortisol, thereby allowing preferential access for aldosterone. A deficiency of 11β-HSD, either congenital or when the enzyme is inhibited by licorice or carbenoxolone, leads to the activation of mineralocorticoid receptors by corticosterone or cortisol, ultimately causing sodium retention and hypertension. Thus, 11β-HSD modulates the access of corticosteroids to their receptors and plays an important role in the control of blood pressure. Glucocorticoid and mineralocorticoid receptors exist in the vasculature, and corticosteroid hormones play an important role in the control of blood pressure by modulating vascular smooth muscle tone. We conducted this study to compare 11β-HSD gene expression and activity in the mesenteric arteries of genetically hypertensive rats: Dahl Iwai salt-sensitive (DS) rats, Dahl Iwai salt-resistant (DR) rats, and Sprague-Dawley (SD) rats.

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Methods

Male DS, DR (Eisai Supply, Eisai Animal Research Center), and SD rats (Nippon Charles River), 3 to 4 weeks old, were initially fed a diet low in salt (0.45% sodium chloride) that was purchased from Nippon Charles River. Both DS and DR rats were fed a high sodium chow (7%) for 4 weeks (n=12 in each group). SD rats were also fed a high sodium chow (7%) for 4 weeks (n=10). Blood pressure was determined by the tail-cuff method. Plasma concentrations of corticosterone and aldosterone were estimated by radioimmunoassay after extraction with a Sep-Pak C18 cartridge. With the animal under pentobarbital anesthesia, the mesenteric artery was immediately excised by the method described by McGregor with minor modifications, as we previously reported. Briefly, isolated artery was perfused with Krebs-Ringer solution, pH 7.4, at a temperature of 37°C and oxygenated with a 95% O2-5% CO2 gas mixture at a constant flow rate of 3 mL/min. Perfusion pressure was constantly monitored and recorded by means of a pressure transducer connected to a polygraph (RM 600, Nihon-Koden). After 30 minutes of equilibration, 200 μmol/L nicotinamide-adenine dinucleotide phosphate (NADPH), and 200 μmol/L nicotine-adrenaline in methanol/water (40% to 100% for 60 minutes) at a flow rate of 1.5
mL/min. The retention times of B and A were 43 and 35 minutes, respectively. The eluate fractions corresponding to B and A were collected by a fraction collector. Tritium-labeled steroids were counted in a liquid scintillation counter, and the percentage conversion of \[^{3}H\]B to \[^{3}H\]A was calculated. After the perfusate experiments, the mesenteric artery was homogenized in 10 mL Krebs-Ringer buffer solution in a tissue grinder. Protein assay was done by the method of Bradford. 10

The time course of 11/3-HSD activity in isolated perfused mesenteric preparations was examined up to 5 hours. 11/3-HSD activity was found to be stable up to 3 hours (data not shown).

To establish whether significant 11/3-reductase conversion of A back to B occurred under these conditions, we performed another pair of experiments as above but using \[^{3}H\]A (purified from supernatant of liver homogenate after incubation with \[^{3}H\]B) as substrate.

Rat mesenteric arteries were removed immediately after decapitation. The tissue was promptly weighed, frozen in liquid nitrogen, and stored at -80°C before use. Total RNA from rat mesenteric arteries was prepared with selective precipitation in LiCl (3 mol/L)/urea (6 mol/L), and poly(A)^+ RNA was purified by chromatography on oligo(dT) cellulose as previously described. 12 Poly(A)^+ RNAs (5 μg per lane) from rat mesenteric arteries were separated by formaldehyde/agarose gel electrophoresis, transferred to a nylon membrane (Hybond-N+, Amersham Japan), and hybridized with \[^{3}P\]-labeled cDNA probes. The filters were washed and radioactive bands were detected by autoradiography. The 11/3-HSD mRNA in the mesenteric arteries from DS rats fed a high salt diet was significantly lowered compared with 4- or 8-week-old rats. The concentration of 11/3-HSD mRNA in 8-week-old DS rats compared with DR (17.4±1.4%) or SD (18.0±1.5%) rats of the same age (P<.05) (Fig 2). There were no significant differences in these parameters among 4-week-old DS, DR, and SD rats of the same age (15.3±1.3%, 15.1±1.9%, and 16.9±1.6%, respectively, Fig 2). The apparent K_m of 11/3-HSD in our experiments was not different from that reported by Monder and Lakshmi. 14 There were no significant differences among DS, DR, and SD rats in the protein concentrations of vessels used in these experiments (data not shown). Experiments to measure the conversion of A to B showed that under these perfusion conditions there was no significant 11/3-reductase activity in any of the groups studied. Fig 3 shows the results of Northern blot analysis and 11/3-HSD mRNA concentrations in the mesenteric arteries of DS, DR, and SD rats. The concentration of 11/3-HSD mRNA in the mesenteric arteries of 8-week-old DS rats was significantly lower compared with 4- or 8-week-old DR or SD rats (P<.05) (Fig 4).

**Discussion**

Our study showed that the isolated perfused mesenteric vasculature from DS rats fed a high salt diet was supersensitive to a bolus norepinephrine injection compared with DR or SD rats. The mesenteric artery from

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**Table:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>4-Week-Old</th>
<th>8-Week-Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR (n=12)</td>
<td>DS (n=12)</td>
</tr>
<tr>
<td></td>
<td>DR (n=12)</td>
<td>DS (n=12)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>118±3</td>
<td>128±3</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>97±6</td>
<td>108±3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>384±16</td>
<td>424±7</td>
</tr>
<tr>
<td>p-Aldosterone, nmol/L</td>
<td>0.42±0.06</td>
<td>0.38±0.04</td>
</tr>
<tr>
<td>p-Corticosterone, nmol/L</td>
<td>43±9</td>
<td>36±8</td>
</tr>
</tbody>
</table>

DR indicates Dahl Iwai salt-resistant rats; DS, Dahl Iwai salt-sensitive rats; SD, Sprague-Dawley rats; SBP, systolic blood pressure; bpm, beats per minute; and p, plasma. Values are mean±SEM.

*P<.05 vs DR or SD rats.

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**Fig 1:** Bar graph shows vasoconstrictor effect of norepinephrine (1.2x10^-4 mol/L) in mesenteric vascular beds of Dahl salt-sensitive (DS), Dahl salt-resistant (DR), and Sprague-Dawley (SD) rats. Perfusion pressure after bolus injection of norepinephrine in 8-week-old DS rats increased significantly compared with DR or SD rats of the same age (P<.05).
prehypertensive DS rats did not show an increased sensitivity to norepinephrine. Kong et al. have demonstrated that hypernoradrenergic innervation is absent in this animal model (DS rats) before the development of salt-induced hypertension. Local administration of glucocorticoids into the vasculature in vivo or into isolated vessels confirmed their permissive effect in potentiating vasoactive responses to catecholamines. With regard to the mechanism or mechanisms responsible for the permissive effect of glucocorticoids, it is interesting to refer to the findings of Takeda et al. that inhibition of 11β-HSD by the licorice derivative glycyrrhetinic acid in human skin potentiated the vasoconstrictor response to cortisol, a response known to be a glucocorticoid receptor-mediated phenomenon. Thus, 11β-HSD may modulate the access of cortisol to vascular glucocorticoid receptors. Walker et al. reported that 11β-dehydrogenase is defective in some patients with essential hypertension. Stewart et al. demonstrated that both hepatic 11β-HSD mRNA levels and activity were reduced in the hypertensive Bianchi-Milan rat. In the present study, both the concentration of 11β-HSD mRNA and the activity in the mesenteric arteries paralleled the levels of mRNA expression, suggesting that the tissue-specific inhibition of 11β-HSD in renal collecting duct cells, which does not convert A to B, has also been reported.

The decreased 11β-HSD activity in 8-week-old DS rats was not improved after treatment of hypertension (data not shown). This change in 11β-HSD activity in DS rats does not seem to be merely secondary to hypertension. Further investigation using an F2 population study is necessary to determine whether inheritance of the DS 11β-HSD gene cosegregates with hypertension.

We were unable to demonstrate significant reductase activity. Walker et al. reported that reduction of [3H]A to [3H]B is not detected in the incubation of the homogenates of mesenteric artery or aorta. A new isoform of 11β-HSD in renal collecting duct cells, which does not convert A to B, has also been reported.

The levels of 11β-HSD activity in the mesenteric artery paralleled the levels of mRNA expression, suggesting that the tissue-specific inhibition of 11β-HSD in the hypertensive rat occurs at a pretranslational level. We found no significant differences of 11β-HSD activity and the expression of mRNA in the mesenteric arteries between SD rats fed a low sodium and high sodium diet. In DS rats, sodium may affect 11β-HSD activity.

In summary, a decrease of 11β-HSD activity in small resistance vessels could play a role in mediating a change in peripheral resistance by altering tissue levels of active glucocorticoid.

References


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