Increased Kidney Xanthine Oxidoreductase Activity in Salt-Induced Experimental Hypertension

Juha Laakso, Eero Mervaala, Jaakko-Juhani Himberg, Terttu-Liisa Teräväinen, Heikki Karppanen, Heikki Vapaatalo, Risto Lapatto

Abstract—Clinical and experimental studies have established an association between high sodium intake and arterial hypertension. The renal mechanisms resulting in impaired sodium excretion in hypertension-prone subjects are not clear. In hypertension-prone rats, high blood pressure results in increased renal mass and hemodynamic changes, both of which may alter renal oxygen distribution. Xanthine oxidoreductase (XOR) oxidizes ATP metabolites hypoxanthine and xanthine to urate. Because XOR is induced by hypoxia, we assessed kidney XOR activity in 2 models of salt-sensitive hypertension, spontaneously hypertensive rats (SHR) and Dahl salt-sensitive (Dahl S) rats. Increasing sodium intake from basal (0.08%) to high (2.56% wt/dry wt in the diet) increased renal XOR activity dose-dependently from 68±8 to 143±21 mU/mg protein in the Dahl S (P<0.05) but not in Dahl salt-resistant (Dahl R) rats. On basal and high sodium diets, SHR had higher renal XOR activity (101±10 and 134±26 mU/mg protein, respectively) than normotensive Wistar-Kyoto rats (55±2 and 58±6 mU/mg protein, P<0.05). Sodium restriction (0.02% wt/wt) downregulated kidney XOR activity in both Dahl S and R rats by nearly 40%. In SHR, allopurinol treatment totally inhibited renal XOR activity, but neither systolic blood pressure nor renal mass changed. The results suggest that renal XOR induction is a consequence of increased salt intake or the resulting hypertension. However, further studies on renal XOR activity during the development of hypertension are needed to assess the importance of XOR in the pathophysiology of arterial hypertension. (Hypertension. 1998;32:902-906.)

Key Words: hypertension, essential ■ xanthine oxidoreductase ■ rats, inbred SHR ■ rats, Dahl ■ hypertrophy ■ sodium ■ allopurinol

In salt-sensitive rats, increasing salt intake results in marked hypertension and left ventricular and renal enlargement within weeks. The genetically related salt-insensitive rats develop these harmful, sodium-induced effects to a much lesser extent.1-3 Kidney transplants derived from normotensive rats have been shown to decrease permanently the systolic blood pressure of an adult salt-sensitive recipient and also abolish the predisposition to hypertension in young animals.4,5 These observations, based on studies on the spontaneously hypertensive rat (SHR) and its normotensive control Wistar-Kyoto rat (WKY) as well as the Dahl salt-sensitive (Dahl S) and salt-resistant (Dahl R) rat, indicate the crucial importance of the kidney in the pathogenesis of hypertension. Mechanisms resulting in an impaired excretion of sodium in relation to renal perfusion pressure in salt-sensitive rat strains have not been fully worked out.6

In clinical studies, the association of high serum urate levels with the development of essential hypertension has been known for decades, but the underlying mechanisms are still unknown.7,8 A significant association of plasma urate and xanthine oxidoreductase (XOR) activity with mean arterial blood pressure has been observed in clinical studies among normotensive individuals.9 Urate is produced in purine catabolism from the ATP degradation products xanthine and hypoxanthine by XOR, which occurs as xanthine dehydrogenase (XDH, EC 1.1.1.204) and xanthine oxidase (XO, EC 1.2.3.2).10 In humans, urate is the end metabolite, whereas in rats urate is further oxidized to allantoin. Urate is one of the most potent water-soluble antioxidants.

In pathological renal enlargement, the diffusion distances for oxygen are increased, which may result in hypoxia in most susceptible areas. As hypoxia upregulates XOR synthesis,11 tissue XOR determination may be used as an indicator of oxygen deprivation.

In the present study we demonstrate an increased kidney XOR activity in 2 models of essential hypertension (SHR and Dahl S rats). An increased sodium intake produced a dose-dependent kidney XOR induction only in the salt-sensitive rats. To further assess the importance of XOR in the pathophysiology of arterial hypertension, we studied...
the effects of XOR inhibition by allopurinol in SHR. These findings may be important in delineating the mechanisms behind salt sensitivity and hypertension-induced target organ damage.

Methods

Animals

Three-week-old Dahl salt-sensitive (SS/Jr) and salt-resistant (SR/Jr) male rats were assigned to 4 weight-matched subgroups of each strain to be kept for 7 weeks on diets differing in sodium content. In a separate experiment, 12 8-week-old SHR Okamoto rats and 10 WKY rats were assigned to 2 groups, respectively, to be kept for 8 weeks on basal or high sodium diets. An additional group of 6 matched SHR receiving high sodium diet containing allopurinol (117 mg/kg was included. The rats were obtained from Harlan Sprague-Dawley Inc (Indiana). Diets containing 0.02, 0.08, 1.28, and 2.56% of sodium (low, basal, moderately high, and high sodium diets) were prepared by mixing NaCl to a low sodium laboratory chow (TD90228, Harlan Teklad) and for the SHR experiments to a standard laboratory chow. The rats were obtained from Harlan Sprague-Dawley Inc (Indiana). Diets containing 0.02, 0.08, 1.28, and 2.56% of sodium (low, basal, moderately high, and high sodium diets) were prepared by mixing NaCl to a low sodium laboratory chow (TD90228, Harlan Teklad) and for the SHR experiments to a standard laboratory chow. At the end of the experiments, the rats were anesthetized with a mixture of CO2 (70%) and O2 (30%) and decapitated. The kidneys were removed and washed with ice-cold saline, frozen in liquid nitrogen, and kept at −70°C (SHR experiment) or lyophilized and kept at −20°C (Dahl experiment). The procedures and protocols of the study followed our institutional guidelines and were approved by the Animal Experimentation Committee of the Institute of Biomedicine.

Blood Pressure Measurement

The systolic blood pressures of unanesthetized rats were measured with a tail-cuff pressure analyzer (Apollo-2AB blood pressure analyzer, model 179-2AB, IITC Life Science). The analog signals obtained were converted to digital values by an on-line microprocessor. Before the measurements the rats were warmed for 10 to 15 minutes at 28°C to make the pulsations of the tail artery detectable. Values for systolic blood pressure and heart rate were obtained by averaging results from 3 to 5 measurements. To minimize stress-induced fluctuations in blood pressure, all measurements were taken randomly by the same person at the same time of day (9 to 12 AM).

Biochemical Determinations

Frozen kidneys were homogenized with sodium phosphate buffer, pH 7.4, containing 0.25 mol/L sucrose, 1.0 mmol/L EDTA, 1.0 mmol/L DTT, 0.5 mmol/L PMSF, 1.0 μmol/L leupeptin, and 1.0 μmol/L peptatin A. Homogenates were centrifuged (100 000g, 60 minutes), and supernatants were passed through a gel filtration column (Bio-Gel P-10, Bio-Rad). Protein fraction was supplemented with 0.5 mmol/L DTT and 0.2 mmol/L EDTA. XO and XDH were measured fluorometrically. Intra-assay imprecision of the total activity was not >5%. Protein determinations were carried out with the Biuret method. One unit of XOR, XDH, or XO is defined as 1 U=1 μmol isoxanthopterin formed per minute.

Statistical Analyses

ANOVA was applied to test the statistical significance for strain and diet effects followed by Duncan’s multiple comparison test for comparison of pairs of groups. Student’s t test was used where indicated. Linear regression analysis was used to calculate correlation coefficients. SPSS/PC statistical software (SPSS Inc) was used for computations.

Results

Renal XOR and Sodium Intake in Dahl Rats

Dahl S and R rats kept for 7 weeks on a basal sodium diet (0.08% NaCl wt/wt of chow) showed no difference in renal XDH, XO, or XOR (Table 1). No differences were found in systolic blood pressure between Dahl S and R rats kept on the basal diet. The Dahl S rats had higher body weights, while their absolute and relative kidney weights were not different from those of Dahl R rats on the same diet (Table 2).
Increased sodium intake (1.28% and 2.56% sodium) resulted in increased kidney XDH and XOR activity in Dahl S rats in a dose-dependent manner (\( P<0.001 \), ANOVA). In contrast, in the Dahl R rats increased sodium intake had no effect on kidney XDH, XO, or XOR activity. The Dahl S rats on moderately high and high sodium diets had elevated systolic blood pressure and increased absolute and relative kidney weights. High sodium diet resulted in growth retardation in Dahl S but not in Dahl R rats. Sodium restriction to 0.02% resulted in decreased kidney XO and XOR activity both in Dahl S and R rats compared with the groups on low (0.08%) sodium diet.

Renal XOR and Sodium Intake in SHR
SHR kept for 8 weeks on a basal sodium diet (0.08%) had markedly higher kidney XOR activities than WKY (Table 1). The SHR on the basal sodium diet had markedly higher systolic blood pressure (Table 2) compared with the WKY. The absolute and relative kidney weights were similar in SHR and WKY. Increased sodium intake (2.56% sodium wt/wt) produced increased kidney XDH and XOR of SHR. Increasing dietary sodium intake from 0.08% to 2.56% elevated systolic blood pressure and relative kidney weight in SHR but not in WKY (Table 2).

Interrelationships Between Blood Pressure, Renal Mass, and XOR
XDH, XO, and XOR revealed highly significant positive correlations with relative kidney weight in Dahl S but not in the Dahl R rats kept on different levels of sodium intake (Table 3). In Dahl R rats, XO had a negative correlation with relative kidney weight. In the Dahl S rats, blood pressure correlated positively with kidney XDH and XOR activities. A similar pattern of correlations was also found in the SHR experiment.

Inhibition of XOR in SHR by Allopurinol
The chosen allopurinol dose (10 mg · kg\(^{-1} \) · d\(^{-1} \)) totally blocked kidney XOR activity in the SHR (Table 1). The allopurinol treatment resulted in slightly decreased growth. Systolic blood pressure, kidney weight, and relative kidney weight did not differ significantly between SHR groups on high sodium diet treated in the presence or absence of allopurinol (Table 2).

Discussion
The present findings of increased kidney XOR activities in 2 different rat models of hypertension may reflect the intrinsic

### TABLE 2. Effects of Different Sodium Intake Levels on Body Weight, Renal Mass, and Blood Pressure in Different Salt-Sensitive and -Resistant Rat Strains

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Sodium Content in Diet, % (wt/wt)</th>
<th>Rats, n</th>
<th>Body Weight, g</th>
<th>Kidney Weight, g</th>
<th>Relative Kidney Weight, g/kg</th>
<th>Systolic Blood Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl R</td>
<td>0.02</td>
<td>7</td>
<td>112±4*</td>
<td>1.00±0.04*</td>
<td>8.9±0.2*</td>
<td>110±3</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>6</td>
<td>267±7</td>
<td>2.02±0.06</td>
<td>7.3±0.1</td>
<td>115±3</td>
</tr>
<tr>
<td></td>
<td>1.28</td>
<td>7</td>
<td>272±4</td>
<td>2.24±0.03</td>
<td>8.2±0.1</td>
<td>123±3</td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>6</td>
<td>280±8</td>
<td>2.54±0.08*</td>
<td>9.0±0.1</td>
<td>130±3</td>
</tr>
<tr>
<td>Dahl S</td>
<td>0.02</td>
<td>6</td>
<td>155±12†</td>
<td>1.10±0.08*</td>
<td>7.1±0.2†</td>
<td>124±2</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>10</td>
<td>309±5†</td>
<td>2.13±0.05</td>
<td>6.9±0.1</td>
<td>128±2</td>
</tr>
<tr>
<td></td>
<td>1.28</td>
<td>10</td>
<td>302±8</td>
<td>2.67±0.09†</td>
<td>8.9±0.3†</td>
<td>177±7†</td>
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<tr>
<td></td>
<td>2.56</td>
<td>6</td>
<td>210±13†</td>
<td>2.60±0.17*</td>
<td>12.5±0.7†</td>
<td>201±3†</td>
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<tr>
<td>SHR experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>WKY</td>
<td>0.08</td>
<td>6</td>
<td>287±9</td>
<td>1.93±0.08</td>
<td>6.7±0.1</td>
<td>137±7</td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>6</td>
<td>273±9</td>
<td>2.07±0.04</td>
<td>7.6±0.1</td>
<td>167±3</td>
</tr>
<tr>
<td>SHR</td>
<td>0.08</td>
<td>5</td>
<td>328±9†</td>
<td>1.93±0.05</td>
<td>5.9±0.1</td>
<td>204±11†</td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>5</td>
<td>273±9*</td>
<td>2.07±0.04</td>
<td>8.6±1.4†</td>
<td>241±13†</td>
</tr>
<tr>
<td>SHR treated with allopurinol</td>
<td>2.56</td>
<td>6</td>
<td>272±12‡</td>
<td>2.02±0.09</td>
<td>6.4±0.1</td>
<td>225±11</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Significance of the differences (ANOVA followed by Duncan’s multiple comparison test) has been denoted as follows: * \( P<0.05 \) compared with control group of same strain kept on 0.08% sodium diet; † \( P<0.05 \) compared with corresponding salt-resistant strain kept on respective diet; ‡ \( P<0.05 \) (Student’s t test) compared with SHR on high sodium diet without allopurinol.

### TABLE 3. Correlations of Kidney XO, XDH, and XOR Activities Between Systolic Blood Pressure and Relative Kidney Weight in Different Salt-Sensitive and -Resistant Rat Strains

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Variable</th>
<th>XO</th>
<th>XDH</th>
<th>XOR</th>
<th>XO/XOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl R</td>
<td>Systolic BP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Relative KW</td>
<td>-0.53*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Dahl S</td>
<td>Systolic BP</td>
<td>+0.62*</td>
<td>+0.47†</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relative KW</td>
<td>+0.52†</td>
<td>+0.56*</td>
<td>+0.59*</td>
<td>NS</td>
</tr>
<tr>
<td>SHR experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>Systolic BP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Relative KW</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SHR</td>
<td>Systolic BP</td>
<td>+0.78†</td>
<td>+0.79†</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Relative KW</td>
<td>+0.96*</td>
<td>+0.93*</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

BP indicates blood pressure; KW, kidney weight. Significance of the correlations has been denoted as follows: * \( P<0.001 \); † \( P<0.01 \); ‡ \( P<0.05 \).
metabolic differences of these strains or may be a consequence of increased sodium intake or the resulting hypertension. Dahl S rats kept on a basal sodium diet (0.08%) had systolic blood pressure levels not different from those kept on the sodium-restricted diet, while their kidney XOR was twice as high, indicating that systolic blood pressure is not the sole factor contributing to changes in kidney XOR activity. Statistically, kidney XDH and XOR activities revealed a close correlation with systolic blood pressure in both the Dahl S and the SHR.

Abnormal kidney function has a pivotal role in the predisposition to sodium-induced experimental hypertension. The kidney is also one of the main target organs of harmful effects of hypertension. Sodium-induced increase in renal mass has been described in both the presence and absence of increased systolic blood pressure. In contrast to compensatory renal growth, sodium-induced renal growth seems to be due mainly to renal hyperplasia in Dahl S rats. The renal enlargement in Dahl R rats is more due to hypertrophy than hyperplasia. Relative kidney weight provides only a rough estimate of renal enlargement.

Although the regulation of XOR has remained obscure, it is known that hypoxia upregulates XOR at the transcriptional level. The presence of hypoxia, at least in the most susceptible areas of the kidney, is thus one possible factor that contributes to the observed strain- and hypertension-related increases in kidney XOR activity. The 2 kidneys represent 1% of body weight in the rat and receive 20% of the resting cardiac output. Renal outer medulla and cortex are, however, remarkably susceptible to hypoxia because of effective oxygen shunting in the kidneys. The medullary thick ascending limb has high Na⁺/K⁺-ATPase activity and oxygen demand. Sodium-induced increases in renal mass may increase the diffusion distances for oxygen and result in lowered oxygen availability. Hemodynamic changes in the kidney, eg, increased vascular resistance, during the development of hypertension might also contribute to altered oxygen tension in the kidney.

Lowered oxygen tension in the kidney has not been reported in association with experimental hypertension, although decreased kidney NAD⁺/NADH ratios found in several rodent models of hypertension, including salt-induced hypertension, support this possibility. During the development of hypertension, isolated tubular cells from SHR show 15% to 25% higher oxygen consumption and slightly lower ATP levels than cells from WKY even when hypertension is treated with drugs. The SHR kidney may therefore be more susceptible to hypoxia than WKY kidney during the excetration of excess sodium. Increased oxygen tension results in a decrease in XOR synthesis. Sodium depletion drastically decreases workload and oxygen consumption in the kidney and could thereby contribute to the decreases in kidney XOR activity observed in the present study. Sodium depletion did not result in the present study in strain-related changes in renal XDH, XO, or XOR activities.

In short-term experimental studies, inhibition of XOR by allopurinol has been reported to result in a transient decrease of systolic blood pressure. In the long-term experiments, allopurinol has been shown to be more nephrotoxic in SHR than in WKY without any effect on blood pressure. Interestingly, allopurinol was reported to lower serum urate levels in SHR but not in WKY, which indicates an altered purine metabolism of the SHR strain and supports the present findings.

In the present study, prolonged administration of 10 mg · kg⁻¹ · d⁻¹ allopurinol totally inhibited renal XOR activity but failed to lower blood pressure of SHR, which is in agreement with previous studies. To avoid nephrotoxicity, we used a 10-fold lower dose of allopurinol. In a different experimental setting, Suzuki et al found in their concurrent study that abolishing XOR activity by tungsten-enriched molybdenum-deficient diet reduced SHR blood pressure to levels similar to those in WKY. While allopurinol and molybdenum-deficient diet inhibit XOR activity, both treatments also have other metabolic effects. Therefore, further studies are needed to clarify the role of XOR in the pathogenesis of hypertension.

XO has been suggested to be the most important enzyme producing superoxide anion in vascular endothelium. XO inhibitors have been shown to inhibit superoxide anion production. Superoxide anion may contribute to increased degradation of nitric oxide, resulting in increased vascular tone. Scavenging superoxide by exogenous superoxide dismutase has been reported to result in a fall of blood pressure in SHR but not in WKY. In the present study, the sodium-induced changes in kidney XO activity in Dahl S rats were not dose-dependent. Therefore, the role of XO in the pathogenesis and maintenance of hypertension may be smaller than might be expected from the previous studies.

The XO/XOR ratio has also been suggested to be an indicator of tissue hypoxia, although hypoxia-induced change in XO/XOR ratio has not been a constant finding. Conversion of XDH to XO has been shown to occur also as an artifact during extraction of the enzyme. In the present study, we did not find any strain-related differences in the XO/XOR ratio. Interestingly, the significant dietary sodium-induced changes in XO/XOR ratio in both Dahl S and R rats were very similar. The different XO activity levels found in the Dahl and SHR experiments may be an artifact due to different handling of the specimens.

The present findings of sodium-induced renal XOR activity seem to support the view of sodium-induced hypoxia in the kidney. Long-term inhibition of XOR by allopurinol failed to decrease blood pressure in SHR, which also indicates that the increased renal XOR activity is likely a consequence of increased blood pressure rather than a factor contributing to it. However, as an enzyme capable of producing harmful reactive oxygen species, it might play a role in end-organ damage in arterial hypertension. Further studies on kidney XOR during the development of essential hypertension will be required to determine whether increased renal XOR activities precede or are a consequence of the development of hypertension in these models.

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