AT₁ receptor regulation in salt-sensitive hypertension

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Strehlow, Kerstin, Georg Nickenig, Jörg Roeling, Sven Wassmann, Oliver Zolk, Andreas Knorr, and Michael Böhm. AT₁ receptor regulation in salt-sensitive hypertension. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1701–H1707, 1999.—The molecular events governing salt-sensitive hypertension are currently unknown. Because the renin-ANG system plays a central role in blood pressure regulation and electrolyte balance, it may be closely involved in the phenomenon of salt sensitivity. Therefore, we examined the effect of a high-salt diet (8%) and a low-salt diet (0.4%) on AT₁ receptor expression in aorta, brain, and kidney of Dahl S (salt-sensitive) and Dahl R (salt-resistant) rats by means of radioligand binding assays and quantitative PCR. NaCl diet at 8% led to a significant increase of blood pressure in Dahl S but not in Dahl R rats. High-sodium intake caused a profound decrease of ANG II-induced aortic vasoinconstriction in both Dahl R and Dahl S rats. The underlying mechanism was a downregulation of aortic AT₁ receptor density and AT₁ receptor mRNA. AT₁ receptor mRNA was downregulated to 57.8% in Dahl R and 59.0% in Dahl S rats by an 8% NaCl diet compared with a 0.4% NaCl diet (P < 0.05). There was a similar decrease in aortic AT₁ receptor density. Additionally, AT₁ receptor mRNA was also downregulated in the kidney but upregulated the brain of Dahl R and S rats on a high-salt diet. Thus high NaCl intake causes organ-specific AT₁ receptor regulation in Dahl R and in Dahl S rats despite the differential blood pressure regulation in these animal models in response to a high-salt diet. These findings suggest that the regulation of vascular AT₁ receptors is influenced by numerous factors such as the renin-ANG system and obviously by various other events that are currently only partly understood.

EPIDEMIOLOGICAL DATA have suggested a correlation of dietary salt to blood pressure regulation and to the prevalence and progression of essential hypertension. Nevertheless, several interventional studies have failed to establish a reproducible relationship between alterations in sodium intake and blood pressure. Therefore, the salt-blood pressure theory has remained the subject of ongoing controversy (11, 14, 20, 35). In this context, the genetic rat model of salt-induced hypertension introduced by Dahl et al. (9, 10) was of particular interest and helped to gain further insight into the molecular mechanisms of hypertension. Although the salt-resistant Dahl rat strain (Dahl R) displays no elevated blood pressure upon salt diet, the salt-sensitive Dahl rats (Dahl S) develop a fulminating hypertension in response to enhanced salt intake resulting in a short life span (9, 10). ANG-converting enzyme (ACE) inhibitors and ANG II type 1 (AT₁) receptor antagonists increase the life expectancy of these animals, suggesting that the renin-ANG system is involved in the development of hypertension in this particular animal model (16, 17, 19). The AT₁ receptor is a G protein-coupled receptor expressed in various tissues that mediates many effects of ANG II (7, 32). In addition to its role in the control of blood pressure and fluid and electrolyte regulation, the AT₁ receptor has been implicated in the pathogenesis of various cardiovascular diseases (7, 12, 32). In addition, dietary sodium intake is known to modulate the renin-ANG system. Low-salt diet leads to elevation of plasma renin and aldosterone activity and consequently to decreased AT₁ receptor expression via homologous downregulation. High-salt intake, which causes hypertension in some individuals, induces a decrease in the activity of the circulating renin-ANG system, and this is thought to be involved in the accompanied upregulation of AT₁ receptor expression (1, 3). To define whether a pathophysiological enhancement of AT₁ receptor gene expression in Dahl S rats could explain the phenomenon of salt-sensitive hypertension, we examined the vascular AT₁ receptor expression and the vasoconstrictive effect of ANG II in Dahl R and Dahl S rats fed with either a regular or high-salt diet.

METHODS AND MATERIALS

Materials. ANG II, salts, and other chemicals were purchased from Sigma Chemical (Deisenhofen, Germany). [32P]dCTP, Hybond N-nylon membranes, and 125I-labeled ANG II were obtained from Amersham (Braunschweig, Germany). Antibiotics, serum, and cell culture medium were purchased from GIBCO-BRL (Eggenstein, Germany). RNA-clean was from AGS (Heidelberg, Germany), and losartan was a gift from Merck Sharp & Dohme.

Animals. Dahl S and Dahl R rats of the inbred strain J ⁴  strain were bred and housed at the central animal laboratories of Bayer (n = 20). The rats received a standard rodent chow with 0.4% or 8% NaCl and tap water from the age of 5–6 wk. Blood pressure was measured with the tail-cuff method. After 6 wk, the animals (3 mo, male, 200–300 g) were killed, and the kidney, brain, and aorta were excised and cleaned from connective tissue. Blood was collected via puncture of the right ventricle. The animal experiments were approved by the institutional committee and were in accordance with guidelines for experimental research (Nordrhein-Westfalen, Germany).

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Aortic ring preparations and tension recording. After excision of the descending aorta, the vessel was immersed in Krebs buffer, and adventitial tissue was removed. Rings (2–3 mm) were mounted for recording of isometric tension in organ baths filled with Krebs buffer that was continuously aerated with 95% O2 and 5% CO2. The preparations were attached to a force transducer, and isometric tension was recorded on a polygraph. Tissues were allowed to equilibrate for 90 min. A resting tension of 2 g was maintained throughout the experiment. Drugs were added in increasing concentrations to obtain cumulative concentration-response curves. The drug concentration was increased when vasoconstriction was completed, which took an average of 5–10 min for each step.

mRNA isolation. Vessels were homogenized and lysed in 1 ml of RNA-clean (AGS) and were processed according to the manufacturer’s protocol to obtain total cellular RNA. The RNA was isolated with RNA-clean (AGS) according to the manufacturer’s instructions (21). Two micrograms of the isolated total RNA and 10 pg of the mutAT1 mRNA were reverse transcribed using the Megascript-Kit (Ambion) following the manufacturer’s instructions (21). Twenty-eight cycles were performed under the following conditions: 94°C, 30 s; 55°C, 45 s; 72°C, 45 s. The sequence for the AT1 receptor sense and antisense primers were as follows: 5'-ACCCCTCTACAGCATCTTTGTGGTGGGGA-3' and 5'-GGGAGCGTCTGAGATTCCAGACTCTAATAGA-3', respectively. The samples were used for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA amplification to verify the quantity and quality of the RNA. Quantitative PCR. The aorta, brain, or kidney were isolated, quickly frozen in liquid nitrogen, and homogenized. RNA was isolated with RNA-clean (AGS) according to the manufacturer’s protocol to obtain total cellular RNA. The original AT1 receptor cDNA (15) was digested with MscI and self-ligated. The resulting plasmid lacking the region from base 446 to 734 (mutAT1) was linearized by digestion with Sac I, and a deletion-mutated AT1 receptor mRNA was in vitro transcribed using the Megascript-Kit (Ambion) following the manufacturer’s instructions (21). Two micrograms of the isolated total RNA and 10 pg of the mutAT1 mRNA were mixed and reverse transcribed using random primers. The single-stranded cDNA was amplified by PCR reaction using Taq DNA-polymerase (Boehringer, Mannheim, Germany). Eight-eight cycles were performed under the following conditions: 94°C, 30 s; 55°C, 45 s; 72°C, 45 s. The sequence for the AT1 receptor sense and antisense primers were as follows: 5'-ACCCCTCTACAGCATCTTTGTGGTGGGGA-3' and 5'-GGGAGCGTCTGAGATTCCAGACTCTAATAGA-3', respectively. The same samples were used for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA amplification to confirm that equal amounts of RNA were reverse transcribed. The PCR reactions were performed in triplicate. The maximal density and apparent affinity of binding sites were obtained by nonlinear regression analysis. Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed using the ANOVA test.

RESULTS

Blood pressure. Figure 1 illustrates that systolic blood pressure increased only slightly in Dahl R rats on the 8% NaCl diet, whereas in Dahl S rats on 8% NaCl an increase of systolic blood pressure >200 mmHg developed (P < 0.05).

Functional experiments. To investigate the effect of high-salt diet on the vasoconstrictor effect of ANG II, organ chamber experiments with isolated aortic segments were performed. Developed force of contraction upon incubation with 20 or 80 mmol/l KCl was similar in all groups (data not shown). ANG II caused a profound concentration-dependent effect on aortic contraction in Dahl R and Dahl S rats fed with the low-salt diet. In Dahl R and Dahl S rats, the ANG II caused vasoconstriction was significantly inhibited after the 8% NaCl diet (Fig. 2). Maximal force of contraction decreased in Dahl R rats from 40.7 ± 3.4 to 24.2 ± 2.1% and in Dahl S rats from 30.9 ± 6.3 to 10.1 ± 6.31% of KCl-induced vasoconstriction. ANG II-induced vasoconstriction was significantly higher in Dahl R rats than in Dahl S rats. As control experiments, phenylephrine-induced force of contraction was measured. Figure 2 shows that α-adrenergic-mediated aortic constriction...
was similar in all groups, suggesting that a high-salt diet caused a selective decrease of ANG II-caused vasoconstriction in both Dahl R and Dahl S rats.

**AT1 receptor density.** To examine whether this modulation in ANG II-caused aortic constriction was based on regulation of AT1 receptor expression, radioligand binding assays on cell membranes isolated from thoracic aortas that were excised from Dahl R and Dahl S rats on regular or high-salt diet were conducted. The representative saturation binding assays with 125I-ANG II in Fig. 3 show that the 8% salt diet led, in Dahl S and Dahl R rats, to a decrease of AT1 receptor density without significant changes in receptor affinity (Table 1). These data demonstrate that high-salt intake is associated with a downregulation of vascular AT1 receptor density in vivo in Dahl R and in Dahl S rats.

**Aortic AT1 receptor mRNA.** To assess whether the decrease of aortic AT1 receptor density during high-salt diet was caused by a decrease in mRNA, AT1 receptor mRNA was detected by means of a quantitative PCR. The RT and PCR reaction of the AT1 receptor mRNA was monitored by including an internal standard. This deletion-mutated AT1 receptor mRNA yielding a substantially shorter PCR product (191 bp) enabled distinction of the wild-type and mutated AT1 receptor mRNA (479 bp). Quantity and quality of the included RNA was controlled by an additional PCR reaction from the same RT samples using an external standard (GAPDH). The exponential phase for the used amounts of wild-type and mutated RNA was found to be in a range between 20 and 36 cycles (data not shown). Therefore, 28 cycles were used in our experimental setup. Figure 4 illustrates a representative ethidium bromide-stained agarose gel loaded with PCR reactions generated from aortic RNA of Dahl R rats fed with 0.4 or 8% NaCl diet, indicating that the AT1 receptor mRNA expression was markedly decreased in aortas isolated from rats on a high-salt diet. Figure 5 shows the quantitative analysis indicating that the AT1 receptor mRNA was decreased to 57.8% in Dahl R rats and to 59.0% in Dahl S rats on the high-salt diet [AT1 receptor mRNA-to-internal standard ratio 8.13 ± 1.7 (8% NaCl) vs. 4.7 ± 1.1 (0.4% NaCl) in Dahl R rats and 11.0 ± 3.1 (8% NaCl) vs. 6.5 ± 0.9 (0.4% NaCl) in Dahl S rats]. AT1 receptor mRNA expression was slightly higher in Dahl S than in Dahl R rats irrespective of the dietary intake. However, these differences were not statistically significant. GAPDH mRNA was similar between groups (data not shown).

Kidney and brain AT1 receptor mRNA. In addition, AT1 receptor mRNA expression was assessed in brain Fig. 2. Functional effect of ANG II in Dahl R and Dahl S rats. Force of contraction in response to increasing concentrations of ANG II (A and C) and phenylephrine (B and D) in aortic rings isolated from Dahl R and Dahl S rats on low- or high-salt diet. Each point represents the mean ± SE, n = 10. *P < 0.05.
and kidney of Dahl S and R rats by quantitative PCR. Figure 6 displays that the high-salt diet led to a corresponding downregulation of AT1 receptor mRNA expression in kidney but to an upregulation in brain in both animal models. Basal brain AT1 receptor expression was comparable between Dahl R and Dahl S rats; however, the increase in AT1 receptor expression in response to the high-salt diet was more pronounced in Dahl S than in Dahl R rats. No significant difference of GAPDH mRNA expression was measured between groups (data not shown).

Renin plasma levels. Figure 7 illustrates renin plasma concentrations in Dahl R and Dahl S rats after either a

Table 1. AT1 receptor density and affinity in aortas isolated from Dahl R and Dahl S rats on a low- or high-salt diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Density, fmol/mg protein</th>
<th>Affinity, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4% NaCl diet</td>
<td></td>
<td></td>
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<tr>
<td>Dahl S</td>
<td>11.1 ± 0.6</td>
<td>0.3 (0.18–0.4)</td>
</tr>
<tr>
<td>Dahl R</td>
<td>7.9 ± 0.9</td>
<td>0.3 (0.04–0.56)</td>
</tr>
<tr>
<td>8% NaCl diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dahl S</td>
<td>8.5 ± 0.9</td>
<td>0.2 (0.11–0.24)</td>
</tr>
<tr>
<td>Dahl R</td>
<td>5.6 ± 0.21</td>
<td>0.38 (0.11–0.6)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ranges are in parentheses. AT1, ANG II type 1 receptor; Dahl R, Dahl salt resistant; Dahl S, Dahl salt sensitive.

Fig. 4. Quantitative ANG II type 1 (AT1) receptor mRNA PCR. Representative ethidium bromide-stained agarose gel of an RT-PCR of RNA isolated from aortas excised from Dahl R rats on regular and high-salt diet. The 496-bp DNA fragment corresponds to the AT1 receptor mRNA, and the 191-bp DNA fragment resulted from the mutated AT1 receptor mRNA (mutAT1-R; internal standard); 1 kb DNA marker (GIBCO-BRL) on left.
low- or high-salt diet. As expected, renin was suppressed in Dahl R rats upon high-salt intake. Additionally, renin plasma concentrations were significantly lower in Dahl S rats than in Dahl R (low-salt diet) rats, and a high-salt diet led to a paradoxical increase in renin levels in Dahl S rats.

**DISCUSSION**

The present study demonstrates that increased intake of NaCl decreases vascular AT$_1$ receptor gene expression in Dahl R and Dahl S rats. Because many of the known biological effects of ANG II are mediated by the AT$_1$ receptor, modulation of the responsiveness of this receptor has been a prominent subject of recent research. Conditions of increased renin-ANG system activity cause downregulation of AT$_1$ receptors, whereas a decrease in the activity of the renin-ANG system upregulates the AT$_1$ receptor expression (1, 3, 7, 12, 32).

It is thought that ANG II circulating in the plasma may influence AT$_1$ receptor regulation. Namely, reduced ANG II concentration may lead to upregulation, and increased ANG II plasma levels may cause downregulation of vascular AT$_1$ receptors (1, 3, 13, 18, 22, 23, 28).

It has been shown that a high-salt diet causes suppression of plasma renin levels, whereas a low-salt intake leads to increased renin plasma levels (1, 3). Accordingly, AT$_1$ receptors are regulated in a reciprocal manner in response to dietary changes. AT$_1$ receptors are reportedly upregulated after high-salt intake in the kidney (27) and in the brain (29). More recently, it has been shown that increased dietary salt intake induces an upregulation of vascular AT$_1$ receptors in Sprague-Dawley and Wistar rats (25, 33). Consistently, kidney AT$_1$ receptor expression is enhanced in Sprague-Dawley rats on a high-salt diet (30). Salt-induced AT$_1$ receptor upregulation may potentially be involved in salt sensitivity. We hypothesized that some individuals may react with a more pronounced AT$_1$ receptor overexpression upon high-salt intake leading to salt-sensitive hypertension. To explore this theory, we examined Dahl rats, which represent a well-established animal model for salt-sensitive hypertension. The Dahl S rats develop a low-renin hypertension after a high-salt diet (5). Interventional studies in Dahl rats showed that ACE inhibitors and AT$_1$ receptor antagonists are capable of lowering blood pressure, reducing cardiac hypertrophy, and decreasing mortality (16, 17, 19, 31), suggesting a central role of the renin-ANG system and the AT$_1$ receptor in this animal model. It was therefore reasonable to assume that AT$_1$ receptor regulation may participate in the salt sensitivity of Dahl S rats. Surprisingly, our data show that vascular AT$_1$ receptors are downregulated in Dahl S and Dahl R rats regardless of the development of salt-sensitive hypertension in Dahl S rats, suggesting that AT$_1$ receptor regulation is not
decisively involved in salt-sensitive hypertension in Dahl S rats. Renal cross-transplantation of the kidney of Dahl S and Dahl R rats has illustrated that the kidney of Dahl S rats plays an important role in the development of hypertension (8). It is of note that suppression of plasma renin concentrations to a high-salt diet is blunted in Dahl S rats (6). Moreover, it has been shown that renin activity gradually increased in Dahl S rats after a high-salt diet for 4 wk (29). On the basis of these data, it is thought that the renin-ANG system is inefficiently suppressed during salt loading, which contributes to salt-sensitive hypertension. The present data illustrate downregulation of vascular AT1 receptors and decreased ANG II-induced vasoconstriction after a high-salt diet. This is obviously independent of the circulating renin-ANG system because the suppression of plasma renin concentration in response to a high-salt diet should lead to AT1 receptor upregulation. Our data on renin plasma levels are in agreement with previous findings (6, 26) which demonstrate that renin plasma concentrations are suppressed in Dahl R rats but may be paradoxically increased in Dahl S rats after a high-salt intake. The latter may be due to progressive renal damage and heart failure in Dahl S rats, leading to an activation of the renin-ANG system, which overcomes the initial suppression of renin after salt loading (6, 26). Accordingly, AT1 receptor downregulation may be of compensatory nature in Dahl S rats. This does not apply for Dahl R rats, which display vascular AT1 receptor downregulation after salt loading despite a suppressed renin-ANG system. Therefore, the observed downregulation of vascular AT1 receptors in Dahl rats is probably influenced by additional factors besides the renin-ANG system. The fact that AT1 receptor downregulation occurred not only in aortic but also in kidney tissue supports the idea of a general regulatory phenomenon in these animals. It may be speculated that, e.g., the increased catecholamine plasma levels induce AT1 receptor downregulation (4). This is presumable, since stimulation of β-adrenergic receptors with isoproterenol causes AT1 receptor downregulation in vascular smooth muscle cells (34). In addition, it is well established that the vascular AT1 receptor is subjected to heterologous regulation by, e.g., growth factors and lipoproteins (22, 24). The nitric oxide system may also influence blood pressure regulation in Dahl rats. Namely, it has been reported that renal and aortic constitutive nitric oxide synthase activity is significantly lower in Dahl S rats on a high-salt diet than in Dahl S rats on a low-salt diet or Dahl R rats (15). This could possibly explain the increase in blood pressure in Dahl S rats despite the observed AT1 receptor downregulation.

Interestingly, in Sprague-Dawley rats, salt loading causes an upregulation of vascular AT1 receptor expression (25), suggesting that AT1 receptor regulation in Dahl rats is per se subjected to differential regulatory pathways. On the basis of our data, this does not apply to the brain, since salt loading causes upregulation of brain AT1 receptors, especially in Dahl S rats, suggesting organ-specific regulation of the AT1 receptor. Acting through the AT1 receptor in the brain, ANG has effects on fluid and electrolyte homeostasis, neuroendocrine systems, and autonomic pathways regulating cardiovascular function and behavior. The distribution of ANG receptors in the brain indicates that they play diverse and important physiological roles in the nervous system (2). There is a greater increase of AT1 receptor expression in the brain after a high-salt diet (basal levels are comparable) in Dahl S rats compared with Dahl R rats. It may be speculated that this AT1 receptor upregulation may drive in part the salt-sensitive hypertension in those animals. If so, AT1 receptor downregulation in the aorta and kidney would be considered a compensatory step, although the molecular mechanisms leading to this differential and organ-specific regulatory pathways are not clear.

There is a trend toward higher AT1 receptor expression in the aorta and kidney in Dahl S rats on a low-salt diet compared with Dahl R rats on a low-salt diet. This could be explained by the reduced renin levels in Dahl S rats. On the other hand, the lower renin levels may be induced through AT1 receptor overexpression in Dahl S rats. Nevertheless, the present data cannot clarify this question.

Further investigations concerning the molecular mechanisms underlying this differential AT1 receptor regulation will lead to a better understanding of the role of the AT1 receptor and its regulation in the setting of hypertension. Our results suggest that the renin-ANG system-independent factors, such as the sympathetic nervous system, are involved in this pathophysiologically important modulatory process of AT1 receptor gene.

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REFERENCES


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