Role of Bradykinin in Renoprotective Effects by Angiotensin II Type 1 Receptor Antagonist in Salt-Sensitive Hypertension

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To elucidate whether bradykinin is involved in the renoprotective effect produced by angiotensin II type 1 receptor antagonist (AT1A) in chronic salt-sensitive hypertension, Dahl salt-sensitive rats receiving a high-salt (8%) diet were treated either with an AT1A (candesartan, 1 mg/kg/day), a bradykinin B2 receptor antagonist (BKB2A; FR172357, 30 mg/kg/day) or a combination of AT1A and BKB2A for 7 weeks. None of the treatments changed the markedly increased systolic blood pressure induced by a high-salt diet. However, chronic treatment with AT1A significantly improved the histological hallmarks of renal damage—i.e., glomerular sclerosis and cell proliferation—despite the presence of severe hypertension. This beneficial action of AT1A was abolished by the concomitant administration of BKB2A. In agreement with these histologically based findings, increases in levels of creatinine clearance induced by AT1A were also reversed back to the basal levels when BKB2A was administered in conjunction with AT1A. Furthermore, urinary excretions of nitrate plus nitrite and prostaglandin E2 increased moderately in response to the administration of AT1A alone, but not in combination with BKB2A. Thus, the blockade of bradykinin signaling abrogates the renoprotective actions of the angiotensin II type 1 (AT1) receptor antagonism. Collectively, these data show that when AT1 action is chronically blocked, endogenous bradykinin plays a pivotal role in preventing the progression of glomerular injury in salt-sensitive hypertension. (Hypertens Res 2003; 26: 265–272)

Key Words: angiotensin II type 1 and type 2 receptors, glomerulus, bradykinin, nitric oxide, prostaglandins

Introduction

Angiotensin II (Ang II) is one of the most important endocrine regulators of systemic blood pressure homeostasis, and works by controlling vascular tone and circulating blood volume (1, 2). In addition to these hemodynamic actions, Ang II promotes the growth of a variety of cell types in vivo as well as in vitro in a blood-pressure-independent or non-hemodynamic manner (1–5). Both the hemodynamic and the non-hemodynamic effects of Ang II are elicited primarily through the Ang II type 1 (AT1) receptor (1, 2, 4, 5). Therefore, a main therapeutic strategy for treating hypertension and its related organ damage is attenuation of the activation of AT1 receptors by angiotensin converting enzyme inhibitors (ACEI) or AT1 receptor antagonists (AT1A) (1, 6).

Bradykinin, a product from a kininogen substrate by kallikrein action, causes vasodilatation and natriuresis through the release of nitric oxide (NO) and prostaglandins (PGs) (7). Thus far, two types of kinin G protein-coupled receptors, B1 and B2, have been characterized (8). The B2 receptor mediates the majority of the cardiovascular and renal actions of bradykinin (7). Acute blockade of the B2 receptor substantially decreases renal blood flow and natriuretic response (9), and chronic blockade of the B2 receptor increases susceptibility to Ang II-induced hypertension (10).
The interaction between Ang II type 2 (AT2) activity and the bradykinin system has been shown to be of importance in the regulation of systemic blood pressure, natriuresis and cardiovascular damage (11, 12), but the role played by the bradykinin system in the glomerular injury caused by chronic hypertension remains unclear. We have previously shown that the administration of AT1A ameliorates the glomerular damage caused by chronic salt-sensitive hypertension without lowering systemic blood pressure. This effect was attributed to AT1A-induced suppression of the expression of transforming growth factor-β1 in glomeruli (13), which in turn was induced by inhibition of activator protein-1 activity (14). Given that AT1A are capable of enhancing the AT2-bradykinin system in vivo (15, 16), the underlying mechanism whereby AT1A prevents glomerular damage in salt-sensitive hypertension most likely involves the activation of endogenous AT2 receptor signaling, which, in turn, counteracts the effects of AT1 activation.

To fill this gap in our knowledge, we here investigated the chronic effect of bradykinin B2 receptor antagonists (BKB2A) on the glomerular damage induced by high-salt intake in the presence or absence of AT1A. The present study provides a new understanding of the mechanism by which AT1A exert renoprotective effects on salt-sensitive hypertension.

**Methods**

**Materials**

Male Dahl-Iwai salt-sensitive rats (n = 28) at the age of 5 weeks were purchased from Japan SLC (Shizuoka, Japan). All rats were housed in climate-controlled metabolic cages with a 12:12-h light-dark cycle. The rats received either a low (0.3%) or a high (8%) salt (MF, Oriental Yeast, Tokyo, Japan) diet, with distilled water provided ad libitum. Candesartan cilexetil: an AT1A, and FR172357: a BKB2A, were generously provided by Takeda Chemical Industries (Osaka, Japan) and Fujisawa Pharmaceutical Co. (Osaka, Japan), respectively. The experimental protocol was approved by the Animal Ethics Review Committee of Okayama University Graduate School of Medicine and Dentistry.

**Experimental Protocol**

All rats were fed a 0.3% salt diet during the 1-week control period, followed by an 8% salt diet from 6 weeks of age for the duration of the experimental period. The 28 rats were then divided randomly into four groups and treated with drugs dissolved in 0.3 ml of 2% gum Arabic solution for 7 weeks using gavage as follows: a control group treated with vehicle alone; an AT1A group treated with candesartan cilexetil (1 mg/kg/day); a BKB2A group treated with FR172357 (30 mg/kg/day); and an AT1A + BKB2A group treated with a combination of candesartan cilexetil and FR172357 (1 mg/kg/day and 30 mg/kg/day, respectively). FR172357 was administered every 12 h based on its half-life and a previous study on the effect of HOE-140 and FR172357 on allergic inflammation (17). Systolic blood pressure (SBP) and heart rate (HR) were measured weekly at 9 AM in conscious, restrained, and warmed condition using tail cuff plethysmography (BP-98A; Softron, Tokyo, Japan). Urine samples were collected over a 24-h period and stored at -80°C until assay. Urinary protein excretion and N-acetyl-β-glicosaminidase (NAG) activity were determined using a Bio-Rad protein assay kit (Bio-Rad, Richmond, USA) and NAG test pack (Shionogi Pharmacoeutical, Osaka, Japan), respectively. Urinary hormone levels, i.e., aldosterone, nitrate plus nitrite (NOx), cyclic guanosine monophosphate (cGMP) and prostaglandin E2 (PGE2) excretion, were determined using an SPAC-S-Aldosterone RIA kit (Daichi Radioisotope, Tokyo, Japan), an NO^-2/NO^-3 assay kit (Dojindo, Kumamoto, Japan), a cGMP immunoassay (R&D Systems Inc., Minneapolis, USA) and a Prostaglandin E2 assay system (Amersham Pharmacia Biotech AB, Uppsala, Sweden), respectively.

Rats were killed by decapitation after the 7-week treatment and the kidneys were removed quickly for histological assessment. Trunk blood samples were collected and stored at -80°C until assay. Serum concentrations of electrolytes, creatinine, total protein and urinary creatinine were measured by an autoanalyzer system. Creatinine clearance was determined based on body weight correction. Plasma renin activity (PRA) was determined by a radioimmunoassay for angiotensin I (Dinabot Radioisotope, Tokyo, Japan).

**Histological Examination**

For observation by light microscopy, a portion of each kidney was fixed in 10% buffered paraformaldehyde, embedded in paraffin, sectioned into 4 μm slices, and stained with periodic acid-Schiff (PAS) reagent. The severity of glomerular sclerosis was estimated according to the mesangial injury score (MIS) system established by Raij et al. (18). In brief, the severity was graded from 0 to +4 based on the area of glomerular involvement out of more than 100 glomeruli examined from each group. A +1 lesion represents an involvement of 25% area of glomerulus while a +4 lesion indicates 100% involvement of a glomerulus. In addition, glomerular cellularity was determined by counting the total number of nuclear cells in each glomerulus.

**Statistical Analysis**

All values were expressed as the means ± SEM and statistically analyzed by analysis of variance. Values of p < 0.05 were considered to indicate statistical significance.
Results

To assess the hemodynamic effects of AT1A or BKB2A on Dahl salt-sensitive hypertensive rats, SBP, HR and urine volume were examined weekly throughout the observation period. In this study, 30 mg/kg/day of FR172357 was orally administered to exert a saturated effect on bradykinin B2 receptor antagonism. HOE-140 has been widely used to block B2 actions in vivo studies, in which 500 µg/kg/day of HOE-140 was shown to abolish the hemodynamic actions induced by ACEI (19–21). The concentration of FR172357 used here was sufficient to neutralize B2 activation efficiently, and was chosen based on a dose-effects study on the concentrations of HOE-140 and FR172357 needed to inhibit inflammatory effects specifically evoked by bradykinin stimulation (17).

As shown in Fig. 1A, SBP levels were markedly increased by chronic intake of 8% high salt for 7 weeks (Control group). Notably, however, none of the three treatments—AT1A (AT1A group), BKB2A (BKB2A group) or a combination of both (AT1A & BKB2A group)—resulted in significant differences in SBP levels (Fig. 1A). In addition, HR levels were not altered either by high-salt intake (Control group) or treatments with AT1A, BKB2A or AT1A & BKB2A treatment.

Fig. 1. Effect of AT1A and BKB2A on changes in SBP and HR. Male Dahl salt-sensitive rats were fed a high-salt diet (8%) from the age of 6 weeks for 7 weeks. The rats were concomitantly treated with the following drugs: the Control group, treated with vehicle alone; the AT1A group, treated with candesartan cilexetil (1 mg/kg/day); the BKB2A group, treated with FR172357 (30 mg/kg/day); and the AT1A + BKB2A group, treated with a combination of candesartan cilexetil and FR172357 (1 mg/kg/day and 30 mg/kg/day, respectively). Levels of SBP (A) and HR (B) were measured weekly using tail cuff plethysmography. Data are shown as the means ± SEM (n = 7). AT1A, angiotensin II type 1 receptor antagonist; BKB2A, bradykinin B2 receptor antagonist; SBP, systolic blood pressure; HR, heart rate.

Fig. 2. Effect of AT1A and BKB2A on urine volume. Male Dahl salt-sensitive rats were fed a high-salt diet (8%) from the age of 6 weeks for 7 weeks. Rats were treated with the drugs described in the legend of Fig. 1. Urine volume collected over a 24 h-period was measured weekly. Data are shown as the means ± SEM (n = 5). Abbreviations are the same as Fig. 1.
titatively verified by assessment using the MIS grading system established by Raij et al. (18), as well as by measurement of glomerular cellularity (Fig. 4). Although BKB2A therapy alone did not affect the glomerular lesions induced by high-salt intake, AT1A treatment significantly reduced the extent of glomerular damage induced by salt-sensitive hypertension. Importantly, this histological improvement induced by AT1A was totally abolished by co-treatment with BKB2A. In agreement with the pathohistological changes, a functional marker of glomerular filtration rate, creatinine clearance level, was significantly improved by AT1A treatment but not by BKB2A (Fig. 5). Moreover, co-treatment with BKB2A clearly abolished the increment of creatinine clearance produced by AT1A (Fig. 5). Thus, the renoprotective effect of chronic AT1A treatment in the glomeruli of salt-sensitive hypertensive rats seems likely to be bradykinin-dependent.

After 7 weeks of treatment, the urinary protein excretion levels were 367 ± 74, 247 ± 6, 382 ± 81, and 253 ± 35 mg/day in the Control, AT1A, BKB2A, and AT1A + BKB2A groups, respectively. Excretions of urinary NAG were 0.917 ± 0.04, 0.711 ± 0.12, 0.772 ± 0.05, and 0.735 ± 0.03 U/day in the Control, AT1A, BKB2A and AT1A + BKB2A groups, respectively. Urinary levels of both protein

Table 1. Effect of AT1A and BKB2A on Organ Weight and the Body Weight Ratio

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AT1A</th>
<th>BKB2A</th>
<th>AT1A + BKB2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>363.6 ± 7.8</td>
<td>371.4 ± 3.4</td>
<td>350.1 ± 4.6</td>
<td>363.6 ± 9.2</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.75 ± 0.03</td>
<td>1.72 ± 0.02</td>
<td>1.66 ± 0.02</td>
<td>1.73 ± 0.05</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.43 ± 0.02</td>
<td>1.36 ± 0.02</td>
<td>1.39 ± 0.03</td>
<td>1.34 ± 0.03</td>
</tr>
<tr>
<td>Kidney/body weight (%)</td>
<td>0.452 ± 0.012</td>
<td>0.444 ± 0.005</td>
<td>0.462 ± 0.010</td>
<td>0.453 ± 0.005</td>
</tr>
<tr>
<td>Heart/body weight (%)</td>
<td>0.371 ± 0.011</td>
<td>0.351 ± 0.004</td>
<td>0.384 ± 0.015</td>
<td>0.368 ± 0.004</td>
</tr>
</tbody>
</table>

AT1A, angiotensin II type1 receptor antagonist; BKB2A, bradykinin B2 receptor antagonist. Control group: vehicle alone; AT1A group: candesartan cilexetil (1 mg/kg/day); BKB2A group: FR172357 (30 mg/kg/day); AT1A + BKB2A group: a combination of candesartan cilexetil and FR172357 (1 mg/kg/day and 30 mg/kg/day, respectively). Data are shown as the means ± SEM (n = 7).
and NAG were reduced by AT1A but not by BKB2A. However, the changes induced by AT1A were not significantly abolished by co-treatment with BKB2A. To assess the discrepancy between the histological changes in glomeruli and the urinary levels of protein and NAG, tubulo-interstitial regions were also microscopically evaluated; however, there were no distinguishable changes produced by any of the treatments in the interstitium.

To determine the factors involved in regulation of the histological remodeling in the glomeruli, we examined several humoral indicators in blood or urinary samples collected at the end of the experimental period. Among the four experimental groups, no significant changes were found in serum concentrations of sodium and chloride, total protein, PRA level (Table 2) or urinary excretion of aldosterone (data not shown). The levels of urinary NO and PGE2 but not cGMP excretions were moderately higher in the AT1A group than in the other groups, but this difference was not statistically significant (Table 3).

**Discussion**

Our present study demonstrates that bradykinin plays a pivotal role in the renoprotective effects induced by chronic AT1 antagonism in salt-sensitive hypertension. To exclude the possible influence of secondary alteration caused by blood pressure change in the glomerular injury, SBP levels in the experimental groups were equilibrated throughout the experimental period. The physiological and histological results revealed that bradykinin B2 blockade abolishes the glomerulo-protective effects produced by chronic AT1A therapy in salt-sensitive hypertension, suggesting that the beneficial effect of AT1A on glomerular protection is, at least in part, mediated through the activation of bradykinin B2.
Table 2. Effect of AT1A and BKB2A on Serum Electrolytes, Total Protein, and Plasma Renin Activity Levels

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AT1A</th>
<th>BKB2A</th>
<th>AT1A + BKB2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mmol/l)</td>
<td>142.1 ± 0.3</td>
<td>142.4 ± 0.5</td>
<td>143.9 ± 0.5</td>
<td>143.9 ± 0.4</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>102.0 ± 0.2</td>
<td>101.7 ± 0.8</td>
<td>102.3 ± 1.0</td>
<td>101.9 ± 0.7</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.53 ± 0.13</td>
<td>5.61 ± 0.13</td>
<td>5.33 ± 0.13</td>
<td>5.43 ± 0.18</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>1.16 ± 0.38</td>
<td>1.76 ± 0.32</td>
<td>3.05 ± 0.87</td>
<td>1.90 ± 0.53</td>
</tr>
</tbody>
</table>

Data are shown as the means ± SEM (n = 7). For abbreviations and a description of the experimental groups, see Table 1.

Table 3. Effect of AT1A and BKB2A on Urinary Levels of Nitrate Plus Nitrite (NO$_2$-NO$_3$), cGMP and PGE2:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AT1A</th>
<th>BKB2A</th>
<th>AT1A + BKB2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary NO$_2$ (µmol/day)</td>
<td>1.45 ± 0.62</td>
<td>2.25 ± 0.47</td>
<td>1.31 ± 0.61</td>
<td>1.89 ± 0.53</td>
</tr>
<tr>
<td>Urinary cGMP (ng/day)</td>
<td>36.4 ± 2.7</td>
<td>32.9 ± 4.4</td>
<td>36.8 ± 9.0</td>
<td>35.3 ± 4.0</td>
</tr>
<tr>
<td>Urinary PGE2 (ng/day)</td>
<td>58.6 ± 8.9</td>
<td>87.8 ± 27.2</td>
<td>54.7 ± 5.0</td>
<td>51.5 ± 4.7</td>
</tr>
</tbody>
</table>

Data are shown as the means ± SEM (n = 5). For a description of the experimental groups, see Table 1. cGMP, cyclic guanosine monophosphate; PGE2, prostaglandin E2; other abbreviations are the same as Table 1.

AT1 receptors are widely distributed throughout the normal adult kidneys, including the mesangial, epithelial, endothelial and vascular smooth muscle cells in glomeruli (2, 6), and they contribute to the progression of renal damage related to hypertension induced by both the systemic and local renin-angiotensin systems. In contrast, a second Ang II receptor, AT2, is expressed at low levels in normal adult kidneys and increases in response to injury (22, 23). This receptor has been shown to exhibit effects that counteract those of AT1, including vasodilatation, growth-inhibition, and apoptosis (1, 11, 24–26). Mice lacking AT2 receptors have been shown to exhibit sustained sodium retention (27, 28) and a hypersensitive pressor response to Ang II (27), suggesting that AT2 receptors counteract the sodium-retaining and hypertensive actions of Ang II. Furthermore, a study using mice overexpressing the AT2 receptor has provided compelling evidence that AT2 stimulation by Ang II causes the enhancement of bradykinin, NO and cGMP production through a mechanism by which AT2 activation facilitates intracellular acidification of vascular smooth muscle cells as well as the activation of kininogenase (29).

The counterbalancing effect of the kinin-kallikrein system to AT1 action was earlier demonstrated in bradykinin B2 receptor-deficient mice, which bear distinct abnormalities in the heart, including left ventricular hypertrophy, myocardial damage and cardiac failure (30). Of interest, this cardiac remodeling was abrogated by chronic treatment with AT1A (31), suggesting that endogenous AT1 actions are responsible for the cardiovascular phenotype caused by the B2 receptor gene deletion. Furthermore, the B2 knockout mice are specifically susceptible to hypertension in response to endogenous Ang II but not to norepinephrine (32). Therefore, it seems likely that development of hypertension and cardiovascular remodeling entails the interaction between endogenous AT1 and bradykinin B2 actions.

Regarding the importance of the bradykinin system in the Dahl hypertension model, Hirawa and colleagues have reported that in vivo infusion of exogenous kallikrein, a kinin activator, functionally and morphologically attenuates glomerular damage in salt-sensitive rats without affecting systemic blood pressure. Furthermore, these changes were completely abolished by additional infusion of HOE-140 (33). Taken together with our present findings, these results suggest that activation of bradykinin is critical to prevent and/or buffer glomerular injury caused by salt-sensitive hypertension.

The precise mechanism by which bradykinin exerts its renoprotective effects without lowering systemic blood pressure remains uncertain, although recent researches have focused on the roles of potent regulators downstream of bradykinin: NO and PGs. Accumulating evidence from in vitro studies has shown that NO exhibits anti-proliferative effects on a variety of cell types either in a cGMP-dependent or -independent manner (34–36). In the kidney, NO plays key roles not only in the suppression of DNA synthesis and proliferation of glomerular mesangial cells through cGMP generation (37), but also in the alteration of glomerular microcirculation (38). Bradykinin also stimulates the release of PGs from a variety of cell types (39–41). The activation of the PGs: system attenuates collagen expression in the cardiac fibroblasts (42) while PGE2 induction inhibits growth factor-induced DNA synthesis (43). We also previously revealed that depletion of NO is critical to the development of both hypertension and glomerular damage in Dahl salt-sensitive rats even under salt-restricted conditions (44), suggesting that the progress of hypertension and glomerular injury shown in the Dahl hypertension model is highly dependent on the synthesis of endogenous NO. Thus, endogenous bradykinin contributes to the preservation of glomerular function via enhancement of the activity of NO and PGs in...
the kidney. In the present study, however, we cannot exclude the possibility that AT1A and BKB2A directly affected hemodynamics in the renal glomeruli, which could modulate endogenous NO and PGs synthesis. A further study assessing the microcirculation of renal glomeruli will be needed to explore the evidence on this issue.

The beneficial effects of AT1A are mediated through a dual mechanism of action. The first mechanism involves the direct antagonism of AT1 receptor signaling, and the second mechanism involves the subsequent interruption of the negative feedback of AT1 signaling, resulting in increases of systemic renin (45) and Ang II (46, 47). When the AT1 receptors are chronically blocked by AT1A, the increased levels of Ang II activate the signaling pathway of the unblocked AT2 receptors, which causes the enhancement of the endogenous bradykinin B2 system (1, 15, 16). Because of the rapid degradation of bradykinin in vivo, the beneficial effects of AT1A, which are mediated through the increase in bradykinin levels, would most likely be augmented by co-administration with ACEI, which blocks the degradation of bradykinin (6). In this regard, an in vivo study using sodium-depleted rats by Siragy and colleagues has demonstrated that renal interstitial bradykinin and cGMP levels are significantly increased when either ACEI or AT1A are administered individually, and when administered together they cause an even higher increase of these factors (15). Furthermore, these effects have been shown to be abolished by blockade of the AT2 receptors, demonstrating the pivotal role of AT2 action in the activation of the intrarenal bradykinin-cGMP system (15). The role of AT2 receptors in the renoprotective mechanisms in salt-sensitive hypertension has yet to be elucidated.

Collectively, these data demonstrate that the bradykinin B2 receptor blockade neutralizes the renoprotective effects of chronic AT1A treatment without changing systemic blood pressure in salt-sensitive hypertension. This finding suggests that the activation of the endogenous bradykinin system and the subsequent augmentation of NO and PGs synthesis is critical for the beneficial effects induced by chronic AT1A treatment in this model, and that these effects presumably occur through the activation of AT2 receptors.

References


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