Long-Term Regulation of ENaC Expression in Kidney by Angiotensin II


Abstract—We carried out semiquantitative immunoblotting of kidney to identify apical sodium transporter proteins whose abundances are regulated by angiotensin II. In NaCl-restricted rats (0.5 mEq Na/200 g BW/d), the type 1 angiotensin II receptor (AT₁ receptor) antagonist, candesartan, (1 mg/kg of body weight per day SC for 2 days) markedly decreased the abundance of the α subunit of the epithelial sodium channel (ENaC). This subunit has been shown to be rate-limiting for assembly of mature ENaC complexes. In addition, systemic infusion of angiotensin II increased αENaC protein abundance in rat kidney cortex. The decrease in αENaC protein abundance in response to AT₁ receptor blockade was associated with a fall in αENaC mRNA abundance (real-time RT-PCR), consistent with transcriptionally mediated regulation. The effect of AT₁ receptor blockade on αENaC expression was not blocked by spironolactone, suggesting a direct role of the AT₁ receptor in regulation of αENaC gene expression. Candesartan administration was also found to increase the abundances of the β and γ subunits. The increase in β and γENaC protein abundance was not associated with a significant increase in the renal abundances of the corresponding mRNAs, suggesting a posttranscriptional mechanism. Immunocytochemistry confirmed the increase in β and γENaC protein abundance and demonstrated candesartan-induced ENaC internalization in collecting duct cells. The results support the view that the angiotensin II receptor regulates ENaC abundance, consistent with a role for angiotensin II in regulation of collecting duct function. (Hypertension. 2003;41:1143-1150.)

Key Words: receptors, angiotensin II ▪ angiotensin antagonist ▪ sodium channels ▪ aldosterone

Long-term control of blood pressure is closely tied to sodium balance and extracellular fluid volume regulation, both of which are controlled in part by the renin-angiotensin-aldosterone system (RAAS).¹ Angiotensin II has important nonrenal effects that are instrumental in the control of blood pressure as both a vasoconstrictor and a regulator of aldosterone secretion. In addition, angiotensin II has direct effects on the renal tubule in regulating NaCl reabsorption.² The direct antinatriuretic effects of angiotensin II appear to be particularly important in conditions of dietary sodium restriction or contraction of extracellular fluid volume.³

Regulation of renal tubule sodium transport by angiotensin II has been investigated chiefly in relatively short-term experiments with observations within a few minutes of angiotensin II addition.⁴⁻⁷ However, there is growing evidence that a variety of mediators of transport regulation in the kidney, such as vasopressin⁸ and aldosterone,⁹ work by both short-term and long-term actions. The long-term actions are associated with adaptive increases in abundance of transporter proteins, whereas short-term actions are generally associated with regulated trafficking or posttranslational modifications of the transporter proteins.

The antinatriuretic effects of angiotensin II on sodium transport are mediated by binding of angiotensin II to the type 1 angiotensin II receptor (AT₁ receptor).¹⁰ The major objective of the present study was to determine whether there are adaptive changes in sodium transporter protein abundances in the kidney in response to long-term blockade of the AT₁ receptor in rats consuming sodium-restricted diets. The results demonstrate a long-term action of angiotensin II in regulating the abundances of the subunits of the amiloride-sensitive epithelial sodium channel, ENaC, in a pattern similar to that seen with dietary NaCl restriction. Specifically, angiotensin II upregulates expression of αENaC at both the protein and mRNA level while posttranscriptionally decreasing the abundances of both β and γENaC.

Methods

Animal Models
Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were kept in metabolism cages (approved animal protocol 9-KE-5). In each experiment, all rats were ration-fed using a gelled diet¹¹ to control the intake of nutrients and water. The basic gelled diet was prepared by combining commercially available synthetic rat chow containing no added NaCl (formula code 53140000, Zeigler Broth-
ers with deionized water (25 mL/15 g rat chow) and agar (0.5%) for gelation. For most rats, sufficient NaCl was added to the base diet to give them a daily sodium intake of 0.5 mMg/200 g of body weight per day (g BW/d; Low-NaCl Diet). For some rats, sufficient NaCl was added to give the rats 2.0 mMg/200 g BW/d of sodium (NaCl-Replete Diet). All rats were fed enough gelled diet to give them 15 g/200 g BW/d of the synthetic chow and 25 mL/200 g BW/d of water. Thus, water intake and caloric intake were maintained at equal levels in all rats.

Several different experiments compared vehicle-infused and candesartan-infused rats. After a 4-day equilibration period in the metabolic cages, rats were anesthetized with methoxyflurane (Metaphane, Schering-Plough) for subcutaneous implantation of osmotic minipumps (Alzet) containing the AT1 receptor blocker candesartan (CV 11974, Astra Pharmaceuticals, Sodertalje, Sweden; a gift of Dr Peter Morsing). Candesartan was solubilized in 0.02 mol/L Na2CO3 in physiological saline. Experimental rats were given candesartan at a dose of 1 mg/kg per day for 2 days, whereas control rats received only saline/Na2CO3 vehicle. This dose (when given in drinking water) has been shown to be sufficient to block the rise in blood pressure resulting from long-term infusion of angiotensin II.12

One group of rats was treated as above but also received the mineralocorticoid receptor antagonist, spironolactone, for 6 days at a high dose (400 mg/kg BW/d; Sigma). The spironolactone was dissolved in olive oil and added to the gelled food. The dose of spironolactone used was in excess of that needed for virtually complete mineralocorticoid receptor blockade in vivo.3,14 Additional groups of rats were infused with angiotensin II (24.4 ng/min SC) or vehicle by osmotic minipump for 3 days. These rats were maintained on a NaCl intake of 2.0 mMg/200 g BW/d as described above. To minimize baseline levels of angiotensin II, all rats were given the angiotensin-converting enzyme inhibitor, lisinopril, at 3 mg/kg per day by osmotic minipump contemporaneously with angiotensin II infusion.

Urine and Serum Chemistry
Urine and serum were assayed using an autoanalyzer (Monarch 2000 autoanalyzer, Instrumentation Laboratories). Serum aldosterone and vasopressin concentrations were measured by radioimmunoassay (Coat-A-Count Aldosterone, Diagnostic Products Corp; Vasopressin RIA, Alpc).

Semiquantitative Immunoblotting
Immunoblotting procedures for comparing 2 sets of samples of kidney homogenates with regard to relative abundances of specific proteins were described in detail previously.13,14 Preliminary gels were run for the entire set of samples in a given experiment on 12% polyacrylamide/SDS gels, which were stained with Coomassie blue dye to assess equality of loading as described.15,16

Immunocytochemistry
Rat kidneys were perfusion-fixed with a paraformaldehyde-based fixative, and 2 μm paraffin sections were prepared as described previously.14 Sections were labeled following the immunoperoxidase method described by Hager et al.17

Antibodies and Terminology for Apical Sodium Transporters
Affinity-purified primary antibodies recognizing each of the major apical sodium transporter proteins in kidney were prepared: NHE3 (the apical Na-H exchanger of proximal tubule)19, NKCC2 (the bumetanide-sensitive Na-K-2Cl cotransporter of the thick ascending limb),13 NCC (the thiazide-sensitive Na-Cl cotransporter of the distal convoluted tubule),11 and the α, β, and γ subunits of ENaC (the amiloride-sensitive sodium channel of the connecting tubule and collecting duct).19 The specificity of each antibody has been demonstrated by a combination of immunoblotting showing appropriate peptide-ablatable bands and immunocytochemistry showing localization in appropriate membrane domains.

Real-Time RT-PCR
Quantitative, real-time reverse transcription–polymerase chain reaction (RT-PCR, ABI Prism 7900HT) was used to measure relative mRNA abundances in kidneys of vehicle-treated and candesartan-treated rats as previously described.20 Primer sequences are given as supplementary materials in Brooks et al.20 Relative quantitation of gene expression was determined using the comparative C0 method, with validation experiments performed to determine that amplification efficiencies were equal between control and experimental groups.21 Specificity of the amplified products was determined using melting curve analysis and by sequencing of products.

Statistical Analysis
Quantification of the band densities from immunoblots was carried out by laser densitometry (Molecular Dynamics). Values for candesartan-treated rats were compared with controls using an unpaired t test when standard deviations were the same, or by Welch t test when standard deviations were significantly different (INSTAT, Graphpad Software). To facilitate comparisons, we normalized the densitometry values such that the mean for the control group is defined as 100%. P<0.05 was considered statistically significant.

Results
Renal Sodium Transporter Abundance Profiling for Presumptive Determination of Targets for Long-Term Regulation by Angiotensin II
To identify potential renal targets for long-term regulation by angiotensin II, we assessed abundance changes for each of the major apical sodium transporters in kidney by semiquantitative immunoblotting of renal homogenates from NaCl-restricted rats (0.5 mMg Na/200 g BW/d) after subcutaneous infusion of candesartan for 2 days (Figure 1). Densitometric analysis of these immunoblots is summarized in Table I. These preliminary experiments identified αENaC (expressed in the connecting tubule and collecting duct) as a possible target for regulation by angiotensin II, based on apparent downregulation with AT1 receptor blockade. In addition, there were apparent increases in the abundances of the β and γ subunits of ENaC.

Effect of Candesartan Infusion on ENaC Subunit Expression
Additional salt-restricted rats were studied to test explicitly the hypothesis that AT1 receptor blockade via a 2-day infusion of candesartan results in altered ENaC subunit abundances (Figure 2). Candesartan infusion resulted in a large decrease in αENaC abundance (normalized band densities: vehicle-infused control 102±18, candesartan-infused 48±17, P<0.05). As suggested by the preliminary screen, there were also large increases in the abundances of βENaC (normalized band densities: vehicle-infused control 100±3, candesartan-infused 315±50, P<0.01) and γENaC (normalized band densities: vehicle-infused control 100±9, candesartan-infused 332±17, P<0.01). Thus, we conclude that long-term infusion of candesartan alters the abundances of all 3 ENaC subunits. This pattern of changes is similar to what is observed in rats on a sodium-replete diet compared with rats on a sodium-restricted diet.22
Effect of Long-Term Candesartan Infusion on Urine and Serum Composition

Table 2 summarizes urine and serum data for control versus candesartan-treated rats. There were no differences in urine volume or creatinine clearance in response to candesartan infusion. The only significant changes in urinary excretion rates were decreases in $K^+$ and ammonium excretion in response to candesartan. In addition, serum bicarbonate was decreased from 26 ± 0.6 mmol/L in vehicle-infused animals to 20 ± 0.5 mmol/L in candesartan-treated rats, indicating a significant degree of metabolic acidosis.

A separate group of rats was studied by the same experimental protocol to measure aldosterone and vasopressin concentrations. For aldosterone, the values were as follows:

**TABLE 1. Densitometric Analysis of Semiquantitative Immunoblots From Rats Receiving 0.5 mEq Sodium Daily***

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Region</th>
<th>Condition</th>
<th>Control (Vehicle)</th>
<th>Candesartan</th>
<th>$P$ (t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHE3</td>
<td>Cortex</td>
<td>100±20</td>
<td>106±16</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>NHE3</td>
<td>OM</td>
<td>100±6</td>
<td>103±7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>NKCC2</td>
<td>OM</td>
<td>100±4</td>
<td>92±5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>NKCC2</td>
<td>Cortex</td>
<td>100±5</td>
<td>96±5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>NCC</td>
<td>Cortex</td>
<td>100±8</td>
<td>79±13</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>αENaC</td>
<td>Cortex</td>
<td>100±28</td>
<td>65±4*</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>βENaC</td>
<td>Cortex</td>
<td>100±11</td>
<td>164±24*</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>γENaC</td>
<td>Cortex</td>
<td>100±9</td>
<td>147±16*</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SE. All values are normalized by the mean for the control group (n=6 for both control and candesartan-treated groups).

*Apical sodium transporter proteins.

**TABLE 2. Urinary Excretion and Serum Composition of Rats Receiving 0.5 mEq Sodium Daily**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=6)</th>
<th>Candesartan (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>262±10</td>
<td>249±12</td>
</tr>
<tr>
<td>Urine flow rate, mL/day</td>
<td>13.5±1.3</td>
<td>12.4±0.7</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>1.50±0.14</td>
<td>1.22±0.11</td>
</tr>
<tr>
<td>Urinary excretion rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium, mmol/d</td>
<td>0.31±0.05</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>Potassium, mmol/d</td>
<td>1.02±0.08</td>
<td>0.79±0.05*</td>
</tr>
<tr>
<td>Phosphorus, mmol/d</td>
<td>0.22±0.02</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Calcium, mmol/d</td>
<td>1.63±0.13</td>
<td>1.89±0.21</td>
</tr>
<tr>
<td>Creatinine, μmol/d</td>
<td>8.7±0.4</td>
<td>8.4±0.7</td>
</tr>
<tr>
<td>Ammonium, mmol/d</td>
<td>0.97±0.06</td>
<td>0.73±0.07*</td>
</tr>
<tr>
<td>Serum concentrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mEq/L</td>
<td>5.9±0.1</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>Chloride, mEq/L</td>
<td>100±1</td>
<td>106±2</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>30±6</td>
<td>34±7</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>26±0.6</td>
<td>20±0.5*</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*P<0.05.
Subunit mRNA Levels

To address the mechanism of ENaC subunit protein abundance changes following candesartan administration, we repeated the 2-day candesartan-infusion experiment described above to assess the effects of AT1 receptor blockade on ENaC subunit mRNA levels (Figure 3). As can be seen, candesartan infusion was associated with a significant fall in the abundance of αENaC mRNA in whole kidney samples, which was roughly proportional to the demonstrated fall in αENaC protein abundance (compare Figure 2). Because there is no evidence for mRNA stability regulatory sequences in the 3’-UTRs of the αENaC transcript,24 it is likely that the changes in αENaC mRNA are due to altered transcription. In contrast, there were no significant changes in β and γENaC mRNA levels (Figure 3). γENaC mRNA abundance showed a tendency to increase, although any increase that occurred is likely to be far less than the demonstrated increase in γENaC protein abundance (compare Figure 2). Thus, increases in β and γENaC protein levels appear to be largely posttranscriptionally mediated.

Effect of Candesartan on Cellular Localization of ENaC

To examine further the effect of long-term candesartan infusion on ENaC subunit proteins in collecting duct cells, we carried out immunoperoxidase labeling in perfusion-fixed kidneys from NaCl-restricted rats (0.5 mEq Na/200 g BW/d). As illustrated in Figure 4 (βENaC) and Figure 5 (γENaC), candesartan infusion markedly increased β and γENaC labeling in collecting duct principal cells from NaCl-restricted rats, consistent with immunoblotting results. Without candesartan infusion, both subunits were restricted to the apical regions of the collecting duct principal cells as previously seen after dietary NaCl restriction.19,25 In contrast, after candesartan infusion, both subunit proteins were distributed throughout the cells.

Effect of Mineralocorticoid Receptor Blockade on ENaC Response to Candesartan

Because candesartan administration reduces circulating aldosterone levels, it could be argued that the effect of candesartan could have been due solely to a diminished effect of aldosterone on the collecting duct. To test this, we repeated the 2-day candesartan infusion experiment with superimposed administration of a high-dose of the mineralocorticoid blocker spironolactone to both the vehicle-infused and candesartan-infused rats (Figure 6). The spironolactone dose used (400 mg/kg BW/d) was well in excess of that needed for virtually complete mineralocorticoid receptor blockade in vivo.13,14 As seen in Figure 6, candesartan decreased renal αENaC abundance even in the presence of spironolactone administration, supporting the view that the effect of candesartan on αENaC abundance was largely independent of the mineralocorticoid receptor. However, candesartan failed to alter β and γENaC abundances in the presence of high-dose spironolactone (Figure 6). Indeed, in contrast to the increases in β and γENaC abundances seen in response to candesartan (Figures 1 and 2), the abundances of these 2 subunits did not increase. Thus, we conclude that the increases in β and γENaC seen in response to candesartan infusion are likely to be due to the associated decrease in circulating aldosterone level. These results support the view from previous studies that αENaC is regulated independently of the other 2 subunits in kidney.22,26,27

![Figure 3: Effect of long-term AT1 receptor blockade on ENaC subunit mRNA expression levels in whole kidney homogenates in rats. Both vehicle-infused control (n=6) and candesartan-treated rats (n=6) ate a low sodium diet (0.5 mEq Na/200 g BW/d). Messenger RNA levels were determined by real-time RT-PCR. Small vertical bars represent standard error of the mean. Asterisks indicate significant differences in mRNA levels for candesartan-treated rats versus control rats (P<0.05).](image)

![Figure 4: Immunoperoxidase labeling of βENaC in outer medulla from control and candesartan-treated rats. Candesartan treatment was associated with a marked increase in immunoperoxidase labeling in principal cells of the collecting duct (representative of 3 control rats vs 3 candesartan-treated rats).](image)
Effect of Candesartan on the Abundance of the Thiazide-Sensitive Cotransporter of the Distal Convoluted Tubule

The initial screen of transporters responding to candesartan infusion was equivocal with regard to the response of the thiazide-sensitive Na-Cl cotransporter (a target for regulation by aldosterone) with a mean band density after candesartan infusion reported to be 79% of control (NS, Table 1). To readdress this response, we carried out immunoblotting for this cotransporter in whole kidney homogenates from additional candesartan-infused versus vehicle-infused rats. As seen in Figure 7, there was no change in the abundance of this cotransporter, whereas the previously demonstrated changes in the abundances of the 3 ENaC subunits were confirmed.

Does Candesartan Have Effects on ENaC Abundance in the Absence of Dietary NaCl Restriction?

Figure 8 shows the effects of candesartan-infusion on whole kidney sodium transporter protein abundances in rats on a NaCl-replete diet, giving a sodium intake similar to that achieved by ad libitum feeding with standard rodent chows (2.0 mEq Na/200 g BW/d). Experimental rats were treated with 1 mg/kg per day candesartan for 2 days; control rats received vehicle over the same period of time. Increases in β and γENaC seen in response to candesartan administration with low NaCl diet (Figures 1, 2, and 7) were not seen with candesartan infusion to rats on a NaCl-replete diet (Figure 8). Interestingly, the decrease in αENaC abundance in response to candesartan infusion persisted despite the higher level of NaCl intake, supporting the view from previous observations in AT1 receptor knockout mice that a sodium-replete diet does not ablate the dependence of αENaC expression on AT1 receptors.

Effect of Angiotensin II Infusion on Renal Sodium Transporter Protein Abundances

Potentially, candesartan could have produced its effects on αENaC expression via mechanisms not directly related to AT1 receptor blockade. If the effects of candesartan-infusion on αENaC expression are due to AT1 receptor blockade and...
Discussion

We carried out an immunoblotting survey of renal homogenates to determine which of the major renal tubule sodium transporters undergo changes in abundance in response to long-term administration of the AT1 receptor blocker, candesartan. This screen pointed to the 3 subunits of ENaC as potential targets for long-term regulation by angiotensin II (Figure 1). An independent experiment to test this possibility (Figure 2) revealed that the abundance of αENaC was strongly downregulated in response to AT1 receptor blockade with candesartan, whereas the abundances of β and γENaC were increased in response to candesartan infusion. In a previous study, a similar pattern of ENaC subunit abundance changes was seen in response to ablation of the AT1 receptor gene in mice.28 In additional experiments, long-term angiotensin II infusion by osmotic minipump was seen to increase αENaC abundance. These effects of candesartan and angiotensin II infusions considered together support the view that the long-term actions of angiotensin II to regulate sodium absorption by the kidney are mediated in part by regulation of αENaC abundance via the AT1 receptor.

ENaC, the amiloride-sensitive sodium channel of the collecting duct and connecting tubule, plays a critical role in the renal regulation of sodium excretion and blood pressure.29 It is a hetero-oligomer consisting of α, β, and γENaC subunits. Among the 3 subunits, the production of the α subunit is rate limiting for assembly of the mature ENaC complexes.30 Under NaCl-replete conditions, αENaC is present in collecting duct cells at very low levels, whereas β and γENaC appear to be sequestered intracellularly.19,25 With dietary NaCl restriction, αENaC is strongly induced in the kidney and all 3 subunits are readily detectable in the apical region of the cells, presumably in the apical plasma membrane.19,25 Aldosterone has been seen to strongly upregulate the abundance of αENaC in kidney, both at the protein28 and mRNA26 levels and, until now, has been believed to be the main factor responsible of the induction of αENaC in the setting of dietary NaCl restriction. In the present study, AT1 receptor blockade with candesartan in NaCl-restricted rats had a consistent effect of decreasing the abundance of αENaC (demonstrated in multiple experiments), and this effect was not blocked by spironolactone at a dose that has been found to be sufficient to fully block the mineralocorticoid receptor.13 The view that αENaC abundance is regulated via AT1 receptor occupation has derived additional support from a previous study in which ablation of the AT1 receptor gene in mice resulted in a marked decrease in αENaC protein abundance despite an increase in circulating aldosterone levels.28

These data add to evidence from micropuncture studies6,31,32 and patch clamp studies of isolated collecting ducts33 that angiotensin II has the direct effect of regulating ion transport in the connecting tubule and collecting duct. Additionally, immunohistochemical studies34 and RT-PCR studies35 have provided evidence for expression of AT1 receptors in collecting duct.
Interestingly, increases in NaCl intake to a “normal” level failed to block the ability of candesartan administration to decrease αENaC abundance (Figure 8). In our previous studies, increases in NaCl intake also did not block the ability of ablation of the AT1 receptor gene to decrease αENaC abundance. Such increases in dietary NaCl intake are generally sufficient to suppress the peripheral renin-angiotensin-aldosterone system, but their effects on the intrarenal renin-angiotensin system are less clear. The continued action of candesartan or AT1 receptor ablation of suppressing αENaC expression supports the view that the AT1 receptor is normally at least partially occupied, even in the absence of dietary NaCl restriction. Indeed, immunolocalization of AT1 receptors in the kidney revealed that the AT1 receptor is expressed in the apical, but not the basolateral, plasma membrane of collecting duct principal cells, supporting the view that angiotensin II regulation in the collecting duct may be mediated by intrarenally produced angiotensin II.

Although αENaC abundance decreases in response to AT1 receptor blockade, it is interesting that the abundances of β and γENaC increase in response to candesartan administration, as illustrated both by immunoblotting results (Figures 1, 2, and 7) and immunocytochemistry (Figures 4 and 5). The fact that these subunit abundances are changed in opposite directions implies that the stoichiometry of ENaC complexes in collecting duct cells is not invariant within the cell, confirming previous studies, which have demonstrated non-coordinate regulation of α versus β/γENaC abundances. The upregulation of β and γENaC protein abundance was not associated with significant changes in the corresponding mRNA levels (Figure 3) and was blocked by the mineralocorticoid receptor blocker, spironolactone (Figure 6), whereas the downregulation of αENaC abundance was associated with a corresponding change in mRNA level (Figure 3) and was not blocked by spironolactone (Figure 6). Thus, the regulation of αENaC versus the regulation of β/γENaC protein abundances appear to be independent processes. In our previous studies, we have identified 2 factors that increase β and γENaC abundance independent of αENaC, namely, vasopressin and metabolic alkalosis. Both factors appear to be ruled out in the present studies as factors in the β/γENaC protein abundance increases in response to candesartan because vasopressin levels fell in response to candesartan administration and the candesartan-treated rats manifested metabolic acidosis rather than alkalosis. Hypothetically, the acidosis could have been, in part, due to an increase in amiloride-sensitive sodium absorption in the collecting duct, which has been associated with a voltage-mediated decrease in proton secretion in this segment. One potential explanation for the increase in β and γENaC protein abundance following candesartan is a decrease in trafficking of these subunits to the apical plasma membrane. Recent pulse-chase studies in cultured cells have demonstrated that trafficking of ENaC to the cell surface results in a decrease in the half-life of β and γENaC protein, presumably because retrieval of ENaC from the plasma membrane is associated with degradation of ENaC protein.

**Perspective**

This study has identified the α subunit of ENaC as a target for long-term regulation by angiotensin II in the kidney. The regulation of αENaC abundance by angiotensin II appears to be mediated by AT1 receptors and is associated with concomitant changes in αENaC mRNA. Because αENaC is normally rate limiting for assembly of mature ENaC complexes, this effect of angiotensin II is likely to be involved in the overall sodium-retaining action of angiotensin II.

**References**


Long-Term Regulation of ENaC Expression in Kidney by Angiotensin II
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