Podocyte Injury Augments Intrarenal Angiotensin II Generation and Sodium Retention in a Megalin-Dependent Manner

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Abstract—We have previously shown that podocyte injury increases the glomerular filtration of liver-derived Agt (angiotensinogen) and the generation of intrarenal Ang II (angiotensin II) and that the filtered Agt is reabsorbed by proximal tubules in a manner dependent on megalin. In the present study, we aimed to study the role of megalin in the generation of renal Ang II and sodium handling during nephrotic syndrome. We generated proximal tubule-specific megalin KO (knockout) mice and crossed these animals with NEP25 mice, in which podocyte-specific injury can be induced by injection of the immunotoxin LMB2. Without podocyte injury, renal Agt staining was markedly diminished and urinary Agt increased in KO mice. However, renal Ang II was similar between KO and control mice on average: 117 (95% CI, 101–134) versus 101 (95% CI, 68–133) fmol/g tissue. We next tested the effect of megalin KO on intrarenal Ang II generation with podocyte injury. Control NEP25 mice showed markedly increased renal Ang II staining and renal Ang II levels: 450 (336–565) fmol/g tissue. Megalin KO/NEP25 mice showed markedly diminished Agt reabsorption and attenuated renal Ang II: 199 (156–242) fmol/g tissue (P<0.001). Compared with control NEP25 mice, megalin KO/NEP25 mice excreted 5-fold more sodium in the urine. Western blot analysis showed that megalin KO decreased NHE3 and the cleaved α and γ forms of Epithelial Na Channel. These data indicate that Agt reabsorbed by proximal tubules via megalin in nephrotic syndrome is converted to Ang II, which may contribute to sodium retention and edema formation by activating NHE3 and Epithelial Na Channel. (Hypertension. 2019;74:509-517. DOI: 10.1161/HYPERTENSIONAHA.118.12352.) • Online Data Supplement

Key Words: angiotensin II ■ angiotensinogen ■ kidney diseases ■ low density lipoprotein receptor-related protein-2 ■ nephrotic syndrome ■ podocyte

Using kidney- and liver-specific Agt (angiotensinogen) KO (knockout) mice, we previously demonstrated that the liver is the major source of Agt protein and Ang II (angiotensin II) in the kidney. On the contrary, proximal tubular cells transcribe a large amount of Agt mRNA, but this does not significantly contribute to renal Ang II. Furthermore, we demonstrated that podocyte injury increases glomerular filtration of liver-derived Agt, which was accompanied by increased renal Ang II generation independently of renal Agt mRNA. Filtered Agt is reabsorbed by proximal tubular cells in a manner dependent on megalin. Megalin is a multiligand receptor that is intensely expressed on the apical membrane of proximal tubules. Agt protein is one of the ligands of megalin and is taken up by proximal tubules via megalin-dependent endocytosis. These observations led us to speculate that the Agt reabsorbed via megalin may contribute to the production of intrarenal Ang II.

Severe podocyte injury causes nephrotic syndrome, in which the urinary excretion of sodium is suppressed. Intrarenal Ang II may work on transporters and channels in the regulation of blood pressure and urinary concentration through AT1 (Ang II type 1) receptor expressed in renal tubules. We hypothesized that intrarenal Ang II contributes to sodium retention in podocyte injury.

In the present study, we tested whether megalin is involved in intrarenal Ang II generation with or without podocyte injury using proximal tubular cell-specific megalin KO mice. We also studied the effect of megalin KO on urinary sodium excretion.
Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animal Experiments

The protocols for animal experiments were approved by the Animal Experimentation Committee of Tokai University School of Medicine, in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

In the present study, mice carrying megalin-loxP, KAP-Cre, Ndrg1-CreERT2, and NEP25 were used. Detailed information about methods for the animal experiments was shown in the online-only Data Supplement.

Assays for Renal Ang II Content and Renal and Urinary Agt Protein

The renal Ang II content was determined by radioimmunoassay as described previously. Western blot analysis for Agt was performed with whole kidney samples. Detailed information about its methods was shown in the online-only Data Supplement.

Urinary Agt was determined by ELISA (IBL, Fujioka, Japan) as described previously.

Assays for Sodium Channels and Transporters

Western blot analysis for sodium channels and transporters was performed with membrane protein samples. Detailed information about its methods was shown in the online-only Data Supplement.

We confirmed that the amount loaded was in the linear range of detection for each protein (Figure S1 in the online-only Data Supplement).

The following antibodies were used at the indicated dilutions: rabbit polyclonal antibodies against α (1:5000), β (1:40000), and γ Epithelial Na Channel (EhNAC) (1:60000; gifts from Johannes Lofling at the University of Zurich); rabbit polyclonal antibodies against phosphorylated Na-K-Cl cotransporter (NKCC2) at threonine 96 and 101 (1:1000); and phosphorylated Na-Cl cotransporter (NCC) at threonine 53 (1:5000; gifts from David H. Ellison at Oregon Health & Science University); rabbit polyclonal anti-Na-H Exchanger-3 (NHE3) (1:1000; 21790-1-AP; Proteintech, Rosemont, IL); mouse monoclonal antibody against pNHE3 at serine 552 (1:800; 14D5; Novus, Centennial, CO); rabbit polyclonal anti-β-actin (1:1000; No. 4967; Cell Signaling Technology, Danvers, MA); and HRP-conjugated mouse anti-rabbit IgG antibody (1:5000; NA9340V; GE Healthcare, Chicago, IL). There are 2 cleavage sites in αEnaC that give rise to 2 cleavage products.

Histology and Immunostaining

Periodic acid-Shiff staining and immunostaining were performed on 2-μm serial paraffin sections. Rabbit polyclonal anti-mouse/rat Agt antibody (1:50; IBL, Fujisoka, Japan) and rabbit polyclonal anti-rat megalin antibody (1:1000; generated by A.S.) were used at the indicated dilution. Detailed information about its methods was shown in the online-only Data Supplement.

Statistical Analysis

Detailed information about methods for the statistical analyses was shown in the online-only Data Supplement.

Results

Generation of Proximal Tubule-Specific Megalin KO Mice

To delete the megalin gene in the proximal tubule, we first generated MegalinloxP/loxP; Ndrg1-CreERT2TG/WT mice. Initial trials revealed that the megalin gene was most effectively deleted when male mice were treated with tamoxifen (100 μg/g body weight) 5× starting at 3 weeks of age. However, megalin staining remained in the S3 segment, which showed a compensatory increase in the reabsorption of Agt protein (data not shown). To delete the megalin gene in the S3 segment, we further mated mice with KAP-Cre transgenic mice. Later, we found that 2 cycles of tamoxifen treatment deleted the megalin gene in MegalinloxP/loxP; KAP-CreWT/WT; Ndrg1-CreERT2TG/WT mice as efficiently as in MegalinloxP/loxP; KAP-CreTG/WT; Ndrg1-CreERT2TG/WT mice. In addition, these 2 types of mice showed similar phenotypes. We, therefore, combined these animals, designated them as megalin KO mice, and compared these mice with MegalinloxP/loxP; KAP-CreWT/WT; Ndrg1-CreERT2WT/WT mice (megalin control).

Megalin Has No Impact on Ang II in the Kidney Without Podocyte Injury

Renal megalin mRNA and protein were markedly decreased in megalin KO mice (Figure 1A and 1B). Agt protein was stained in a granular pattern in the proximal tubule of S1 and S2 segments of megalin control mice. In contrast, Agt staining was undetectable in the proximal tubule of megalin KO mice (Figure 1B). Western blot analysis showed that the kidneys of megalin KO mice contained less Agt protein than those of megalin control mice (Figure 2).

The urinary albumin/creatinine ratio in megalin KO mice was, on average, 0.56 (95% CI, 0.50–0.63), which was greater than that in megalin control mice at 0.079 (95% CI, 0.069–0.090; P<0.0001). In addition, megalin KO mice showed a markedly higher urinary Agt/creatinine ratio than in megalin control mice: 21.6 (95% CI, 19.5–23.7) versus 0.0998 (95% CI, 0.0850–0.115) μg/mg (P<0.0001; Figure 1C). These data indicate that the majority of renal Agt protein detected by immunostaining was reabsorbed by proximal tubules via megalin.

We next measured Ang II in the renal homogenate. Renal Ang II content in megalin KO mice was, on average, 117 (101–134) fmol/g tissue, which was comparable to that in megalin control mice at 101 (68–133) fmol/g tissue (Figure 1D). This result indicates that the Agt protein reabsorbed via megalin does not contribute to renal Ang II when podocytes are intact.

Megalin control and KO mice showed similar systolic blood pressure (113 [108–118] versus 104 [99–109] mmHg; Figure S2), urine volume (1380 [1130–1620] versus 1590 [1370–1820] mL/d), and urinary sodium (0.58 [0.52–0.64] versus 0.57 [0.53–0.61] mEq/mg) and chloride (0.74 [0.65–0.83] versus 0.73 [0.65–0.80] mEq/mg) excretion. Both types of mice showed normal renal histology (Figure 1B). Renal renin, Ang converting enzyme (Ace), and Agt mRNAs were similar between the 2 groups (data not shown).

Megalin KO Decreases Ang II in Kidneys With Podocyte Injury

We next studied the effect of megalin KO on Ang II in kidneys with podocyte injury. Podocyte injury was induced in NEP25/megalin KO mice by injecting LMB2 (Figure 3). At 7 days after the induction of podocyte injury, both types of mice showed similar massive proteinuria,
with urinary albumin/creatinine ratios of 155.4 (84.9–226.0) and 125.8 (94.9–156.6), respectively (Figure S3).

Immunostaining revealed that the Agt protein reabsorbed in proximal tubular cells was markedly increased in NEP25/megalin control mice, whereas no Agt staining was observed within the proximal tubular cells of NEP25/megalin KO mice (Figure 3B). Notably, NEP25/megalin KO mice showed massive proteinaceous casts, which were intensely stained for

Figure 1. Effect of megalin KO (knockout) on Agt (angiotensinogen) and Ang II (angiotensin II) in the kidney without podocyte injury. Inactivation of the megalin gene was confirmed by Reverse transcription polymerase chain reaction (A) and immunohistochemistry (B, top). Both megalin control (Cont) and megalin KO mice showed normal renal histology (B, middle, PAS [Periodic acid-Shiff] staining). While Agt protein was stained in a granular pattern in the S1 and S2 segments of the proximal tubule of megalin Cont mice, Agt staining was undetectable in megalin KO mice (B, bottom). Urinary Agt excretion was markedly increased in megalin KO mice (C). There was no significant difference in renal Ang II between megalin Cont and KO mice (D). Horizontal bars represent geometric means in A and C and arithmetic means in D. Cr indicates creatinine; and NS, nonsignificant.

Figure 2. Western blot analysis of renal Agt (angiotensinogen) protein. When podocytes were intact, the renal Agt content in megalin KO (knockout) mice was lower than that in megalin control (Cont) mice. With podocyte injury, the renal Agt content was increased in NEP25/megalin Cont mice and further increased in NEP25/megalin KO mice. Error bars represent means±95% CIs. Note that the sample in the third lane from the left in the megalin KO group (arrowhead) was obtained from a mouse with abnormally high albuminuria and proteinaceous casts. This mouse was, therefore, excluded from all analyses.
Agt. Western blot analysis confirmed that renal Agt content was markedly increased in NEP25/megalin control mice compared with megalin control mice without injury (Figure 2). NEP25/megalin KO mice showed a larger amount of Agt protein in Western blot analysis than NEP25/megalin control mice, indicating that a large amount of Agt protein was contained in the proteinaceous casts of NEP25/megalin KO mice. The urinary Agt/creatinine ratio was dramatically increased in both NEP25/megalin control mice (56.6 [42.0–71.2] μg/mg) and NEP25/megalin KO mice (72.4 [56.2–88.6] μg/mg; Figure 3C).

Renal Ang II content was markedly increased in NEP25/megalin control mice, as reported previously. The increase in renal Ang II was significantly attenuated in NEP25/megalin KO mice (450 [336–565] versus 199 [156–242] fmol/g tissue; P<0.001; Figure 3D). Agt and renin mRNA levels were increased in both NEP25/megalin control mice and NEP25/megalin KO mice. Agt and Ace mRNAs were slightly higher in NEP25/megalin KO mice than in NEP25/megalin control mice (Figure S4). None of these changes in mRNA explained the attenuation of renal Ang II in NEP25/megalin KO mice.

Systolic blood pressure was comparable between NEP25/megalin control and NEP25/megalin KO mice (Figure S5A). Urine volume was decreased in only NEP25/megalin control mice (Figure S6A). Urinary Na/creatinine and Cl/creatinine ratios were markedly decreased in NEP25/megalin control mice, but this decrease was attenuated in NEP25/megalin KO mice (Figure S7).

Figure 3. Effect of megalin KO (knockout) on Agt (angiotensigen) and Ang II (angiotensin II) in the kidney with podocyte injury. Inactivation of the megalin gene was confirmed by Reverse transcription polymerase chain reaction (A) and immunohistochemistry (B, top). Agt staining in proximal tubular cells was markedly increased in NEP25/megalin control (Cont) mice, whereas no Agt staining was observed within proximal tubular cells of NEP25/megalin KO mice (B, bottom). NEP25/megalin KO mice showed massive proteinaceous casts, which were intensely stained for Agt (B, middle, PAS [Periodic acid-Schiff] staining and bottom). NEP25/megalin Cont and KO mice showed similarly high urinary Agt/creatinine (Cr) ratios (C). Renal Ang II was markedly increased in NEP25/megalin Cont mice and significantly decreased in NEP25/megalin KO mice (D). Horizontal bars represent geometric means in A and C and arithmetic means in D. NS indicates nonsignificant.

Figure 4. Effect of megalin KO (knockout) on urinary sodium and chloride excretion. When podocytes were intact, there was no significant difference between megalin control (Cont) and KO mice. Podocyte injury markedly suppressed urinary sodium (A) and chloride (B) excretion. Compared with NEP25/megalin Cont mice, this suppression was attenuated in NEP25/megalin KO mice at 4 d after podocyte injury. Horizontal bars represent geometric means. Cr indicates creatinine; and NS, nonsignificant.
In contrast to the mild morphological changes in tubules of NEP25/megalin control mice, NEP25/megalin KO mice developed severe and extensive cast nephropathy with tubular dilatation and the flattening of tubular cells (Figure 3B). The observed attenuation of sodium retention in NEP25/megalin KO mice on day 7 can potentially be attributed to tubular injury. To rule out this possibility, we analyzed sodium retention at an earlier time point on day 4.

**Megalin KO Attenuated the Suppression of Urinary Excretion of NaCl in Podocyte Injury**

Four days after the induction of podocyte injury, NEP25/megalin KO mice showed only mild morphological changes in the tubules. Megalin KO mice excreted more urinary Agt on day 4; this result was similar to that obtained before LMB2 injection but different from that observed on day 7 (Figure S8A). Importantly, similar to the results on day 7, the renal Ang II content in NEP25/megalin KO mice on day 4 was less than that in NEP25/megalin control mice (Figure S8B). Furthermore, as was the case with the mice on day 7, the urinary excretion of sodium (0.057 [0.042–0.074] versus 0.21 [0.15–0.26] mEq/mg; P<0.0001) and chloride (0.59 [0.52–0.66] versus 0.83 [0.67–0.99] mEq/mg; P<0.05) was markedly suppressed in NEP25/megalin control mice, and this suppression was attenuated in NEP25/megalin KO mice (Figure 4). Systolic blood pressure was comparable between the 2 groups (Figure S5B). Urine volume was suppressed in NEP25/megalin control mice compared with that in NEP25/megalin KO mice (Figure S6B).

To elucidate the molecular mechanisms of sodium retention induced by podocyte injury, we analyzed ENaC, NHE3, phosphorylated NKCC2, and phosphorylated NCC by Western blot analysis. Without podocyte injury, no difference was observed in the amount of cleaved forms of the α- or γ-subunit (35 or 70 kD, respectively) or the β-subunit of ENaC in the membrane fraction between megalin control and megalin KO mice (data not shown). After the induction of podocyte injury, the amounts of the cleaved forms of α- and γ-subunits were markedly increased in NEP25/megalin control mice, whereas the noncleaved forms of the α- and γ-subunits (90 and 85 kD, respectively) and the β-subunit were not changed (data not shown). The increases in the cleaved forms of α- and γ-subunits were significantly attenuated in NEP25/megalin KO mice treated with LMB2 (both P<0.001; Figure 5A through 5C). The administration of amiloride—an ENaC inhibitor—to NEP25 mice at 3 days after LMB2 injection significantly increased urinary sodium excretion during the subsequent 15 hours, confirming that ENaC plays an important role in sodium retention in nephrotic syndrome in NEP25 mice (Figure S9). Next, we measured plasma aldosterone and found no difference between NEP25/megalin control and NEP25/megalin KO mice.
(Figure S10). We also measured urinary aprotinin-dependent plasmin-like activity and observed no significant difference between NEP25/megalin control and NEP25/megalin KO mice on day 4 (Figure S11A). Additionally, the change in activity from day 0 to day 4 was not different between the 2 types of mice (Figure S11B).

After the induction of podocyte injury, the amount of NHE3 was significantly increased in NEP25/megalin control mice and was significantly attenuated in NEP25/megalin KO mice treated with LMB2 (Figure 5D). In contrast, there was no difference in pNHE3—the inactive form of NHE3 (Figure 5E).

Quantitative analysis of phosphorylated NKCC2 and phosphorylated NCC showed high variability among the samples, and no significant difference was detected (Figure S12).

Discussion

In the present study, we demonstrated that intrarenal Ang II was increased by podocyte injury and that this increase was attenuated by the genetic inactivation of megalin in the proximal tubules. This result suggests that a portion of plasma Agt filtered through glomeruli and reabsorbed by proximal tubules is converted to Ang II in the nephritic state. In contrast, when podocytes were intact, megalin KO showed no impact on renal Ang II. Because our previous study indicated that the disruption of the Agt gene in the liver markedly decreases renal Ang II,1 baseline renal Ang II is probably generated from liver-derived Agt in the capillary lumen or the interstitium, which was not detected by our immunostaining.

Podocyte injury increases glomerular leakage of renin, as well as Agt, and the leaked renin is also reabsorbed by proximal tubular cells via megalin.3 In addition, a large amount of ACE is expressed in the brush border and basolateral membrane of proximal tubular cells.2,11 Giani et al4,15 reported that tubular ACE contributes to intrarenal Ang II production and sodium retention using a hypertensive mouse model induced by nitro-L-arginine methyl ester. These data collectively suggest that Ang II is likely formed in proximal tubular cells via megalin. These mice also showed no impact on renal Ang II. Because our previous study indicated that the disruption of the Agt gene in the liver markedly decreases renal Ang II,1 baseline renal Ang II is probably generated from liver-derived Agt in the capillary lumen or the interstitium, which was not detected by our immunostaining.

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Studies using collecting duct-specific renin KO mice revealed that high Ang II increases renin production in the collecting duct and that the increased renin augments local Ang II activity.16,17 This mechanism may also function in NEP25 mice, which have high renal Ang II levels. This possibility is supported by the results of the present study, in which the activation of ENaC but not NKCC2 or NCC was detected. Theoretically, more Agt protein is delivered to the collecting duct is not the rate-limiting factor for Ang II generation.

Sodium retention is involved in the development of systemic edema in nephrotic syndrome. Two opposing mechanisms are proposed for its pathogenesis. The underfill mechanism puts forth that sodium retention is an adaptive response to volume depletion. The overfill mechanism explains that sodium retention is primarily caused by an intrinsic inability of the nephrotic kidney to normally excrete sodium. Supporting the latter hypothesis, a previous micropuncture study using unilateral nephritics rats showed that sodium retention occurred in the collecting duct of Puromycin aminonucleoside-perfused kidneys.18 The 2 mechanisms likely contribute to pathogenesis to a variable degree in individual patients. As observed in human patients with nephrotic syndrome, NEP25 mice showed sodium retention and edema formation after induction of podocyte injury. NEP25/megalin KO mice showed attenuated sodium retention compared with NEP25/megalin control mice. Urinary sodium excretion was greater in NEP25/megalin KO mice at 4 days after the induction of podocyte injury, before the kidney showed severe tubular injury. Our study suggests that renal Ang II induced by podocyte injury may be involved in sodium retention in nephrotic syndrome, accounting for the overfill mechanism.

Consistent with urinary sodium excretion, NEP25/megalin KO also showed less activated NHE3 and cENaCs and γENaCs. Because the proximal tubule reabsorbs 60% to 75% of the filtered sodium, mainly via NHE3,19–21 and Ang II, increased NHE3 protein and activity in renal proximal tubular cells,22–27 this result may suggest that increased NHE3 is involved in sodium retention in nephrotic syndrome. However, the interpretation of the NHE3 data is not simple. First, NHE3 is more intensely expressed in the thick ascending limb than the proximal tubule in mice. Second, not all NHE3 proteins exert the function of sodium reabsorption. A significant portion of NHE3 is complexed with megalin in the nonendosomal dense granules of proximal tubular cells and has no Na+/H+ exchange activity.28,29 Other studies indicated that inactive and active forms of NHE3 are located in different parts of proximal tubule microvilli and that high blood pressure induces movement from inactive dense to active light membrane segments.30,31 Kocinsky et al32 reported that NHE3 phosphorylated at serine 552 is localized to the coated pit of the brush border membrane and reflects the inactive form of NHE3. In the present study, there was no difference in pNHE3 between NEP25/megalin control and KO mice. We, therefore, speculate that NHE3 contributes to sodium retention activated by renal Ang II.

In a previous study, the activation of ENaC was reported to contribute to sodium retention in nephrotic rats induced by puromycin aminonucleoside33 or by HgCl2.34 We also confirmed that amiloride markedly augmented urinary sodium excretion in NEP25 mice. Several lines of evidence have indicated that Ang II regulates ENaC activity independently of aldosterone. First, ENaC expression was observed in the absence of the adrenal gland35 or mineralocorticoid receptor.36 Second, the α-subunit of ENaC was decreased in AT1a (Ang II type 1a) receptor KO mice, in spite of the higher plasma aldosterone.37 Third, a functional study using isolated perfused cortical collecting duct showed that Ang II increased apical sodium transport, which was completely inhibited by an ENaC blocker.38 Fourth, Mamenko et al39 demonstrated that Ang II directly increased the open probability of the ENaC channel via the AT1 receptor and induced
apical translocation of the α-subunit of ENaC in an additive manner with aldosterone.39 These findings collectively support the possibility that renal Ang II induced by podocyte injury is involved in sodium retention. In this study, there was no difference in plasma aldosterone between NEP25/megalin control and KO mice. This finding indicates that the difference in sodium reabsorption cannot be ascribed to plasma aldosterone and suggests that increased renal Ang II does not act on the adrenal gland.

Svenningsen et al40 proposed that proteolytic processing by urinary serine protease plays an important role in the activation of ENaC in the nephrotic state. Thus, plasminogen filtered aberrantly through glomeruli is converted to plasmin by the plasminogen activator in the tubular lumen. This serine protease cleaves an inhibitory peptide segment from the γ-subunit, which increases the open channel probability. Aprotinin—a serine protease inhibitor—can abolish γENaC cleavage and sodium retention,41 suggesting that ENaC activation by serine protease is the major molecular mechanism explaining the overfill phenomenon. However, this mechanism cannot explain the increased cleavage of the γ-subunit of ENaC in NEP25/megalin control mice compared with NEP25/megalin KO mice because both mice showed heavy proteinuria and, therefore, likely show similar high activity of urinary serine protease. In addition, we could not detect differences in urinary plasmin activity between NEP25/megalin control and NEP25/megalin KO mice.

The proposed mechanism of renal Ang II generation and its potential function on sodium homeostasis in nephrotic syndrome is illustrated in Figure 6. Our study indicates that Ang II is generated from Agt reabsorbed by proximal tubular cells rather than from Agt in the tubular lumen. However, it is not known why reabsorption via megalin is necessary to generate renal Ang II and why NKCC2 and NCC were not affected by megalin KO. The detailed mechanism and precise location of Ang II generation and transport need to be clarified to solve this conundrum.

Perspectives

The present study revealed the novel concept that in podocyte injury, a large amount of Agt protein absorbed by proximal tubular cells via megalin after filtration through the glomeruli is converted to intrarenal Ang II, which may activate NHE3 and ENaC. This notion may explain the pathogenesis of edema formation in nephrotic syndrome. To understand the precise pathogenesis in more detail, further elucidation of the location in which Agt is converted to intrarenal Ang II and the pathway through which intrarenal Ang II acts on NHE3 and ENaC would be necessary.

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Disclosures

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**Novelty and Significance**

**What Is New?**
- Our study demonstrated for the first time that glomerular filtered plasma angiotensinogen can be a source of renal angiotensin II.

**What Is Relevant?**
- Our findings explain the overfill mechanism of sodium retention in nephrotic syndrome.

**Summary**
This study shows that in nephrotic syndrome, plasma angiotensinogen leaked into the renal tubular lumen is reabsorbed by proximal tubules via megalin and converted to angiotensin II, which may contribute to sodium retention and edema formation by activating NHE3 (Na-H Exchanger-3) and ENaC (Epithelial Na Channel).