Ang II Accumulation in Rat Renal Endosomes During Ang II-Induced Hypertension
Role of AT₁ Receptor

Jia L. Zhuo, John D. Imig, Timothy G. Hammond, Sheyla Orengo, Edmund Benes, L. Gabriel Navar

Abstract—Hypertension induced by long-term infusion of angiotensin II (Ang II) is associated with augmented intrarenal Ang II levels to a greater extent than can be explained on the basis of the circulating Ang II levels. Although part of this augmentation is due to AT₁ receptor–dependent internalization, the intracellular compartments involved in this Ang II accumulation remain unknown. In the present study, we sought to determine whether Ang II trafficking into renal cortical endosomes is increased during Ang II hypertension, and if so, whether the AT₁ receptor antagonist, candesartan, prevents this accumulation. Compared with controls (n=12; 114±2 mm Hg), Ang II-infused rats (n=12; 80 ng/kg/min, SC, for 13 days) developed hypertension with systolic blood pressure rising to 185±4 mm Hg by Day 12. In Ang II hypertensive rats, plasma renin activity was suppressed, whereas plasma and kidney Ang II levels were increased by 3-fold (348±58 versus 119±16 fmol/mL) and 2-fold (399±39 versus 186±26 fmol/g). Intracellular endosomal Ang II levels were increased by more than 10-fold (1100±283 versus 71±12 fmol/mg protein), whereas intermicrovillar cleft Ang II levels were increased by more than 2-fold (88±22 versus 37±7 fmol/mg protein). Flow cytometric analysis detected significant increases in AT₁A receptor antibody binding in endosomal and intermicrovillar clefts of Ang II–infused rats. The hypertension induced by Ang II was prevented in rats treated concurrently with candesartan (2 mg/kg/d, 114±2 mm Hg). Candesartan treatment (n=8) also prevented increases in kidney (215±19 fmol/g), endosomal (96±29 fmol/mg protein), and intermicrovillar cleft Ang II levels (11±2 fmol/mg protein). These results indicate that there is substantial intracellular accumulation of angiotensin peptides in renal cortical endosomes during Ang II–dependent hypertension via an AT₁ receptor–mediated process. (Hypertension. 2002;39:116-121.)

Key Words: kidney • endosomes • angiotensin II • AT₁ receptor • hypertension, experimental

The importance of angiotensin II (Ang II) in the development and maintenance of hypertension is well documented in several animal models of experimental hypertension, such as two-kidney, one-clip renal hypertension (2K1C), the Ren-2 gene transgenic rats, and the Ang II–infused model. The Ang II–dependent increases in arterial blood pressure are commonly associated with higher levels of circulating and intrarenal Ang II levels, structural abnormalities, and functional derangements in the kidney. One important feature in the renin-angiotensin system profile observed in these rats is that renal Ang II levels are greater than can be explained on the basis of circulating Ang II and suppressed renin expression. This suggests that angiotensin peptides continue to be generated intrarenally via a renin-independent pathway, or that circulating Ang II mulates in one or more compartments within the kidney.

Previous studies have shown that intrarenal Ang II levels are increased in the contralateral nonclip kidney of 2K1C hypertensive rats and in kidneys of Ang II–infused rats and Ren-2 transgenic rats. Because blockade of the AT₁ receptor with losartan normalizes blood pressure and prevents augmentation of intrarenal Ang II levels, this enhanced uptake of Ang II within the kidney appears to be mediated by the AT₁ receptor. These results suggest that Ang II levels are augmented in intracellular compartments by an AT₁ receptor–mediated internalization mechanism and are protected from degradation to some extent. Recent studies have localized angiotensin peptides in renal endosomes and intermicrovillar clefts, thus implicating them as potential sites for intracellular accumulation in the kidney.
ney. Renal endosomes and intermicrovillar clefts from rat renal cortex also contain angiotensin converting enzyme (ACE) and AT1 receptors. In the present study, we first sought to determine whether intracellular accumulation of circulating or intrarenally formed Ang II into renal endosomes is increased during Ang II–dependent hypertension. Second, studies were performed to examine whether AT1 receptor blockade with candesartan would prevent Ang II accumulation in whole kidney, renal endosomes, and intermicrovillar clefts. Third, flow cytometric analysis was used to measure AT1 receptor antibody binding in renal intracellular endosomes isolated from normotensive and Ang II hypertensive rats. The present study demonstrates that during Ang II–induced hypertension, Ang II levels are markedly increased in parallel in the kidney, intermicrovillar clefts, and endosomes via an AT1 receptor–mediated process.

Methods

Animal Preparation
Ang II–induced hypertensive rats were prepared as described previously. Adult male Sprague-Dawley rats (Charles River Laboratories, Montreal, Quebec, Canada) were divided into three groups: Group 1 rats served as controls and did not receive any treatment (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12); Group 2 rats received Ang II infusions at 80 ng/min via osmotic minipumps (n=12). Rats receiving chronic Ang II infusions developed hypertension, Ang II levels are markedly increased by almost 3-fold in Ang II–infused rats compared with controls. Concurrent administration of candesartan elevated Ang II hypertensive rats, and conversely, it was elevated more than 6-fold in Ang II–infused rats treated concurrently with candesartan (Table). Long-term Ang II infusion also significantly decreased plasma Ang I levels compared with controls (38±9 versus 70±9 fmol/mL). In contrast, plasma Ang II concentrations increased by almost 3-fold in Ang II–infused rats compared with controls. Concurrent administration of candesartan elevated PRA markedly and increased plasma Ang II levels further above the levels seen in rats receiving Ang II infusion alone (Table). The increase in Ang II as a consequence of stimulation of PRA is presumably due to the blockade of the feedback inhibition of renin release by Ang II in the juxtaglomerular apparatus (JGA).
Effects of Long-Term Ang II and/or Candesartan on Kidney and Endosomal Ang II Levels

Kidney Ang II levels in control rats averaged 186 ± 26 fmol/g kidney weight and were increased to 399 ± 39 fmol/g in Ang II–infused rats (P < 0.05) (Figure 1). Unlike plasma Ang II levels, which increased further above the levels of Ang II–infused rats (Table), kidney Ang II levels in candesartan-treated Ang II–infused animals did not increase significantly from the levels observed in control rats (215 ± 19 fmol/g kidney weight). Endosomal Ang II levels increased from 71 ± 12 fmol/mg in control animals to 1100 ± 283 fmol/mg endosomal protein in the Ang II–infused rats, whereas intermicrovillar cleft Ang II levels increased from 37 ± 7 fmol/mg to 88 ± 22 fmol/mg endosomal protein. Candesartan prevented the Ang II–induced increases in both endosomal and intermicrovillar Ang II levels (Figure 1).

Effects of Long-Term Ang II Infusion on AT1 Receptor Antibody Binding in Renal Endosomes and Intermicrovillar Clefs

To determine whether the increases in kidney, endosomal, and intermicrovillar Ang II levels in Ang II–infused rats are associated with alterations of AT1 receptor expression, flow cytometry was employed to measure AT1 receptor antibody binding in renal cortical endosomes. Classic log concentration antibody binding curves were first determined to select the optimal concentration of AT1 receptor antibody used for measurement of AT1 receptor protein expression in renal endosomes and intermicrovillar clefs (not shown). As shown in Figure 2, compared with control animals, AT1 receptor antibody binding was significantly higher in both intracellular endosomes (control 115 ± 5.5 fluorescence intensity units versus Ang II–infused 161 ± 17.6 fluorescence intensity units, P < 0.05) and intermicrovillar clefs (control 26.4 ± 4.8 fluorescence intensity units versus Ang II–infused 44.7 ± 8.6 fluorescence intensity units, P < 0.05) in Ang II–infused rats at 1:1000 antibody dilution.

Colocalization of Entrapped Endosomal Marker Fluorescein Dextran and AT1 Receptor Antibody Binding

Colocalization of entrapped fluorescein dextran as an endosomal marker and AT1 receptor antibody binding in renal intracellular endosomes by flow cytometry is shown in Figure 3. Panel A shows the background level of autofluorescence without entrapped fluorescein and minimal level of AT1 receptor antibody binding. Colocalization of AT1 receptor antibody binding with entrapped fluorescein dextran in a representative control endosomal sample is shown in Panel B. A marked shift to the right of the 3D frequency histogram (colocalization) was observed in renal endosomes of Ang II–infused rats, indicating an increase in intracellular endosomal AT1 receptor antibody binding (Panel C). Concurrent administration of candesartan prevented the shift to the right of the 3D frequency histogram in renal endosomes of Ang II–infused rats (Panel D). Only a single population of renal endosomes was observed in all 3 groups of rats.

Discussion

Internalization of the Ang II–AT1 receptor complex following binding of the agonist to its membrane-bound receptors has been suggested to play an important role in mediating biological actions of Ang II in kidney cells. Although fluorescence-labeled Ang II and AT1 receptors have been localized in endosomes and/or lysosomes, the actual presence of internalized Ang II and AT1 receptors in intracellular compartments has been reported only recently. We demonstrated that rat renal cortical endosomes and intermicrovillar...
Figure 3. Colocalization of entrapped endosomal fluorescein dextran and AT1a receptor antibody binding using flow cytometry in endosomes prepared from the control rats, Ang II-infused rats, and rats treated concurrently with Ang II and candesartan. Each panel displays data on 2000 individual endosomes with axes on log scale. For the entrapped fluorescence, the origin is at the top left corner. A. Autofluorescence without fluorescein entrapped and with minimal AT1a receptor antibody binding. B, Colocalization of entrapped endosomal fluorescein dextran and AT1a receptor antibody binding in a normotensive control rat (representative of n=4). C, Colocalization of entrapped endosomal fluorescein dextran and AT1a receptor antibody binding in an Ang II-infused rat (representative of n=4). D, Colocalization of entrapped endosomal fluorescein dextran and AT1a receptor antibody binding in a rat treated concurrently with Ang II and candesartan (representative of n=4). Note a marked right shift in 3D frequency histogram in Ang II–infused rats, which was prevented by candesartan, indicating an increase in AT1a receptor antibody binding during long-term Ang II infusion and a restoration back to control with concurrent candesartan treatment.
increased expression of AT₁ receptors is responsible, at least in part, for enhanced Ang II accumulation in intracellular endosomal compartments during Ang II–dependent hypertension. The physiological consequence of increased endosomal AT₁ receptor expression and enhanced intracellular accumulation of Ang II in renal endosomes during Ang II–dependent hypertension is yet to be determined. Accumulating evidence suggests that internalization of AT₁ receptors following binding of the agonist is not solely for the purpose of Ang II trafficking to the lysosomes for degradation and recycling of the receptors back to cell membranes. Rather, this process may be important for full expression of biological actions in different cells. A recent study showed that microinjection of Ang II directly into vascular smooth muscle cells induces an increase in intracellular calcium via an AT₁ receptor–mediated process. In the kidney, endocytosis of the Ang II–AT₁ receptor complex increases phospholipase C and sodium flux and decreases cAMP in cultured proximal tubule epithelial cells following exposure to Ang II in vitro. Likewise, AT₁ receptor–mediated endocytosis of Ang II and increased recycling of AT₁ receptors have been linked to increased phospholipase A₂ activity and increased sodium flux in a tubule cell line expressing rabbit AT₁ receptors (LLC-PK₁). Thus, increased internalization and/or accumulation of Ang II in renal endosomes may enhance proximal tubular sodium reabsorption and, therefore, contribute to the blunted pressure natriuresis relationship in Ang II–dependent hypertension. Chen et al demonstrated Ang II–driven internalization of an AT₁ receptor–green fluorescent protein (AT₁-R-GFP) receptor complex and increased colocalization of the GFP fluorescence in nuclear regions. Long-term Ang II infusions have been shown to lead to increased angiotensinogen mRNA levels causing enhanced intrarenal production of angiotensinogen. Because of the evidence suggesting that the Ang II–AT₁ receptor complex may migrate to the nucleus, it is possible that Ang II–dependent stimulation of angiotensinogen mRNA requires Ang II internalization and direct genomic action as proposed by Re. Furthermore, increased accumulation of endosomal Ang II levels and AT₁ receptors may also play important roles in causing structural injury or derangements during Ang II hypertension. Intracellular Ang II may induce transforming growth factor-β or nuclear transcription factor-κB expression to promote Ang II–induced tubulointerstitial inflammation. Glomerulosclerosis, and renal microvascular hypertrophy. Overall, internalization of Ang II and AT₁ receptors into renal endosomes and intermicrovillar clefts may play important paracrine and intracrine roles in the hypertensinogenic and pathologic actions of Ang II on the kidney. In summary, the present study demonstrates that during Ang II hypertension, Ang II levels are markedly increased in the kidney and in renal cortical endosomes and intermicrovillar clefts. These increases in endosomal and intermicrovillar cleft Ang II levels are associated with enhanced expression of AT₁ receptors in these intracellular compartments and are prevented by concurrent administration of AT₁ receptor blockers. These findings support the hypothesis that intrarenal trafficking/accumulation of angiotensin peptides into renal cortical tubular endosomes is enhanced during Ang II hypertension, and this process is mediated by AT₁ receptors. Thus, increased AT₁ receptor expression and internalization of Ang II–AT₁ receptor complex may be important for full expression of hypertensinogenic, paracrine, and intracrine actions of Ang II in the kidney during Ang II–dependent hypertension.

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References


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