Proximal tubule cell-specific adenoviral transfer of an intracellular angiotensin II protein elevates blood pressure by activating AT$_1$ receptors in the rat kidney

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Abstract

Whether intracrine angiotensin II (Ang II) plays a physiological role in the regulation of blood pressure is unknown. We tested the hypothesis that proximal tubule (PT) cell-specific expression of an intracellular Ang II fusion protein (ECFP/AII) via intrarenal adenoviral transfer can increase blood pressure by activating AT₁ receptors in the kidney. Adenovirus-mediated transfer of ECFP/AII was induced in the rat renal cortex via microinjection. The sodium and glucose transporter 2 (sGLT2) promoter was used to drive ECFP/AII expression specifically in PTs. Intrarenal transfer of ECFP/AII resulted in a time-dependent, PT cell-specific expression of ECFP/AII throughout the cortex, which was visualized by fluorescent imaging by day 7, peaked by day 14, and persisted for 5 weeks after intrarenal transfer. No significant ectopic expression of ECFP/AII was visualized in the glomeruli, the medulla or in other extrarenal tissues. In ECFP/AII-transferred rats, systolic blood pressure increased from a control level of 122 ± 4 mmHg to 141 ± 8 mmHg by day 7 (p<0.01), and to 148 ± 6 mmHg by day 14 (p<0.01), respectively. At the peak of ECFP/AII expression, urinary sodium excretion was decreased by 23% (p<0.01), while urinary potassium excretion were not affected. Losartan normalized blood pressure and urinary sodium responses to control levels. Ang II levels were increased by 121% in the kidney (p<0.01) and PTs by more than 2-folds (p<0.01), respectively, but remained unaltered in plasma or urine. Our results suggest that intrarenal adenoviral transfer of an intracellular Ang II protein specifically in PTs of the kidney can increase arterial pressure by activating AT₁ receptors in the kidney.
Introduction

- In vivo, endocrine and paracrine angiotensin II (Ang II) increases blood pressure by binding to its cell surface receptors to induce diverse central, cardiovascular, adrenal and renal effects.

- However, binding of Ang II to its cell surface receptors also evokes receptor-mediated endocytosis or uptake of extracellular Ang II via AT$_1$ (AT$_{1a}$) receptors, which may act as an intracellular hormone.

- In vitro, Ang II can induce novel intracellular and/or nuclear actions in the absence of cell surface receptors or in the presence of cell surface AT$_1$ receptor blockade.

- Whether in vivo cell- or tissue-specific expression or transfer of an intracellular Ang II protein may alter arterial blood pressure remains virtually unknown.
Hypotheses

- Intrarenal adenoviral transfer of an intracellular cyan fluorescent Ang II protein (ECFP/AII) selectively in proximal tubules increases arterial blood pressure in rats and mice.

- The blood pressure effect of intrarenal adenoviral transfer of ECFP/AII in proximal tubules is mediated by AT$_1$ (AT$_{1a}$) receptors.
Methods

- Intrarenal adenoviral transfer of ECFP/AII selectively in proximal tubules of the kidney was induced by microinjections in the superficial cortex.

- Adult male Sprague-Dawley rats were microinjected with:
  - saline as control,
  - ECFP/AII (20 μl of 1 in 5 diluted Ad-sglt2-ECFP/AII per injection).
  - ECFP/AII + losartan treatment for 2 weeks (20 mg/kg/day, p.o.).
  - ECFP/AIlc, encoding a scrambled sequence of ECFP/AII.
  - had access to fixed amounts of water and rat chows for 24 h in a metabolic cage before (basal) and 7 day (week 1) and 14 days (week 2) after intrarenal ECFP/AII transfer was induced.

- Two groups of wild-type and AT$_{1a}$ receptor-knockout mice were included to determine the role of AT$_{1a}$ receptors.

- Measurements of weekly systolic pressure, 24 h urine and urinary sodium excretion, lithium clearance, and Ang II levels in plasma, kidney, isolated proximal tubules, and urine.
Construction of proximal tubule-specific adenoviral vector of intracellular Ang II protein (ECFP/AII)

A. Construction of pECFP/AII plasmid by Dr. Julie Cook of Ochsner Clinic.

B. Subclone the gene of interest, ECFP/AII, into a proximal tubule-specific promoter Sglt2 vector, constructed by Drs. Rubera and Tauc of France.

C. Construction of an adenoviral vector encoding recombinant human Ad-sglt2-ECFP/AII by Vector BioLabs (2.5 x 10^{11} PFU/ml).

Figure 1

I: Human Ad5-sequences (wt1-458); includes 5’ L-ITR and packaging signal.
II: transgene Sglt2-ECFP/AII-PolyA.
III: Human Ad5 sequences (wt 3513-35935; E3 region deleted, includes 3’ R-ITR. E3 deletion: nts 28587-30464.
The sodium and glucose transporter 2 (sGLT2) promoter drives ECFP/AII expression selectively in proximal tubules of the kidney.
The sodium and glucose cotransporter 2 (sGLT2) promoter drives ECFP/AII expression selectively in proximal tubules of the kidney.

**Figure 2b.** A representative cyan fluorescent image of an isolated proximal tubule expressing ECFP/AII (A-C). The glomerulus expressed minimal ECFP/AII (D-F). Magnification: 15 X.
Effects of proximal tubule-specific transfer of ECFP/AII on ectopic ECFP/AII expression in extra-renal tissues

Figure 3. The lack of ectopic expression of Ad-sglt2-ECFP/AII in extra-renal tissues 2 weeks after intrarenal adenoviral transfer of ECFP/AII in the rat kidney. Magnification: 15 X.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on systolic blood pressure in rats

Figure 4. ECFP/AII, but not ECFP/AIIc, increased systolic blood pressure in rats and the effect was blocked by concurrent losartan treatment. *p<0.05, *p<0.01 vs its basal control. #p<0.05, ##p<0.01 vs. ECFP/AII. n = 8-10.
Effects of proximal tubule-specific transfer of ECFP/AII on systolic blood pressure in wild type and AT$_{1a}$-KO mice

**Figure 5.** ECFP/AII increased systolic blood pressure in wild-type (WT) but not in AT$_{1a}$-KO mice. **p<0.01 vs. WT basal. #p<0.05 vs. WT basal. ##p<0.01 vs. WT mice transferred with ECFP/AII. n = 8-10 for each group.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on urine excretion in rats

Figure 6. ECFP/AII decreased 24 h urine excretion at day 14 after intrarenal transfer, which was normalized to control levels by concurrent losartan treatment. N = 8-10 for each group.
Effects of proximal tubule-specific transfer of ECFP/All in the kidney on urinary sodium excretion in rats

**Figure 7.** ECFP/All, but not ECFP/Allc, decreased 24 h urinary sodium excretion on day 14 after intrarenal ECFP/All transfer. **p<0.01 vs. control. +p<0.05 vs. ECFP/All alone. N = 8-10 for each group.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on lithium clearance in rats

**Figure 8.** ECFP/AII decreased lithium clearance on day 14 after intrarenal transfer. Lithium clearance was used as an indirect estimation of proximal sodium reabsorption in the entire kidney. **p<0.01 vs. control. ++p<0.01 vs. ECFP/AII alone. N = 6-8 for each.**
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on whole kidney Ang II levels in rats

Figure 9. ECFP/AII, but not ECFP/AIIc, increased whole kidney Ang II levels on day 14 after intrarenal transfer. **p<0.01 vs. control. +p<0.05 or ++p<0.01 vs. ECFP/AII alone. N = 6-9 for each.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on proximal tubule Ang II levels in rats

**Figure 10.** ECFP/AII, but not ECFP/AIIc, increased Ang II levels in freshly isolated proximal tubules on day 14 after intrarenal transfer. Ang II levels are expressed as pg Ang II per mg extracted protein. *p<0.05 or **p<0.01 vs. control. +++p<0.01 vs. ECFP/AII. N = 7-9 for each.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on plasma Ang II levels in rats

Figure 11. ECFP/AII and ECFP/AIIc had no effects on plasma Ang II levels on day 14 after intrarenal transfer. However, losartan increased plasma Ang II by preventing Ang II from binding to its receptors in tissues. **p<0.01 vs control. ***p<0.01 vs ECFP/AII. N = 8-10 for each group.
Effects of proximal tubule-specific transfer of ECFP/AII in the kidney on urine Ang II levels in rats

Figure 12. ECFP/AII and ECFP/AIIc had no significant effects on Ang II levels in urine on day 14 after intrarenal ECFP/AII transfer. Urine Ang II levels are expressed as pg per ml urine samples. N =10 for each group.
Summary

- Proximal tubule-specific transfer of ECFP/All in rats and mice resulted in:
  - proximal tubule-selective expression of ECFP/All.
  - no ectopic expression of ECFP/All in extra-renal tissues.
  - increases in blood pressure, that was blocked by losartan treatment in rats and in AT$_{1a}$-KO mice.
  - increases in Ang II levels in the whole kidney and isolated proximal tubules.
  - unchanged plasma and urine Ang II levels.
  - decreases in 24 h urine output, urinary sodium excretion and lithium clearance, which was blocked by losartan.

- Proximal tubule-specific transfer of control ECFP/Allc in rats had no significant effects on blood pressure, urinary sodium excretion, and Ang II levels in plasma, kidney, urine and proximal tubules.
Conclusion

- Intrarenal adenoviral transfer of an intracellular Ang II protein selectively in proximal tubules of the kidney can increase arterial pressure by activating AT$_1$ receptors in the kidney.
The current results provide in vivo evidence that intracellular or intracrine Ang II may act in proximal tubules of the kidney to regulate blood pressure.

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