Trapping intracellular ANG II to the proximal tubule: powerful in vivo effects on sodium handling and blood pressure

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THE RENIN-ANGIOTENSIN SYSTEM (RAS) acts as a dual endocrine and local tissue paracrine system (1, 6). There is increasing evidence that angiotensin (ANG) II may also function as an autocrine or intracrine peptide which exerts biological effects from within the cell without stimulating plasma membrane bound ANG II type 1 (AT1) receptors (2, 5). The autocrine or intracrine role of ANG II is not well understood, and the mechanisms by which intracellular ANG II exerts its physiological effects are unknown. Since the RAS serves as one of the most powerful regulators of arterial blood pressure by influencing sodium balance, extracellular fluid volume, and renal and systemic vascular resistance, there are multiple cell types in which intracellular ANG II may exert physiological effects. Intracellularly administered ANG II can induce biological effects which are inhibited by intracellular but not extracellular ANG II receptor blockade in vascular smooth muscle cells in vitro (3). However, the ability to distinguish the intracellular actions of ANG II in the physiological responses in an intact animal model had not been determined previously.

In an issue of the American Journal of Physiology-Renal Physiology, the work of Li and colleagues (4) describes a novel approach to establishing the physiological significance of selective in vivo upregulation of intracellular ANG II in proximal tubule epithelial cells. The study was specifically designed to investigate the physiological effects of cytoplasmic ANG II-AT1 receptor mediated effects on proximal tubule epithelial function. The unique contribution of this work is that the authors present convincing evidence for intracrine ANG II effects on sodium reabsorption and blood pressure using in vivo experimental approaches. Li et al. focused attention on the proximal tubule effects of intracellular ANG II since all of the major components of the RAS have been demonstrated in proximal tubule cells. An innovative methodology for targeting expression of ANG II to the proximal tubule was developed by the authors. They provide in vivo evidence that an adenoviral construct encoding a fluorescent fusion of ANG II linked to the enhanced cyan fluorescent protein (ECFP) which is driven by the sodium glucose cotransporter 2 (SGLT2) promoter can effectively deliver an intracellular fluorescent ANG II fusion protein selectively in proximal tubule cells. The adenoviral construct was injected into multiple renal cortical sites of rat and mouse kidneys and resulted in high proximal tubule expression for a period of up to 4 wk without evidence of secretion into the extracellular space. Since ECFP/ANG II is synthesized on free ribosomes and not destined for secretion out of the cells, ECFP/ANG II protein is thus trapped within the cytoplasm of the proximal tubule cell and is unable to act on cell surface AT1 receptors. Confirmation of the expression pattern of ECFP/ANG II was performed by fluorescent immunohistochemical localization using CFP- and ANG II-specific antibodies to cortical proximal tubules with limited expression in glomeruli, cortical connecting tubules, and medullary tissues. Ectopic ECFP/ANG II expression was very low to undetectable in extrarenal tissues. Most interesting was the demonstration of a significantly elevated renal sodium reabsorption and ultimately increased blood pressure in the animals injected with the ECFP/ANG II adenovirus compared with animals injected with the scrambled version of the fusion protein construct. Two weeks following the introduction of the ECFP/ANG II vector, peptide levels of ANG II were significantly increased in the injected kidney and freshly isolated proximal tubules without alterations in the plasma or urinary levels of the peptide, suggesting that the blood pressure effects reflect the intracellular expression of the peptide. These hypertensive effects were observed in both rats and mice injected with the adenovirus and eliminated by AT1 receptor blockade or in AT1a knockout mice. The work of Li et al. provides direct in vivo evidence for intracellular ANG II mediated via the AT1 receptor in the physiological role of proximal tubule reabsorptive function.

It is important to understand the regulatory mechanisms of AT1 receptor-mediated ANG II endocytosis and its contribution to intracellular ANG II levels, intracellular trafficking pathways, and the potential role of internalized ANG II in cellular function. It is not known at the present time whether internalized ANG II is translocated to the nucleus, where it activates nuclear receptors, or whether intracellular ANG II activates cytoplasmic receptors. Future studies are required to determine whether intracellular ANG II-AT1 receptor effects increase Na+/H+ exchanger expression and activity to contribute to sodium retention and hypertension.

The introduction of this unique in vivo approach which limits synthesis of the ANG II peptide that is trapped within the intracellular compartment allowed Li et al. to reveal the physiological responses to overexpression of intracellular ANG II in proximal tubules. This novel approach allowed the authors to convincingly conclude that overexpression of renal proximal tubule intracellular ANG II, which is unable to be secreted, regulates proximal tubular sodium reabsorption and, thereby, sodium excretion and blood pressure in an AT1 receptor-dependent manner. It is expected that this technical approach will be utilized to continue unraveling the mystery of the feedforward mechanism of the RAS to alter renal sodium reabsorptive function and hypertensinogenic effects in physiological and pathophysiological states.
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