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Novel roles of intracrine angiotensin II and signalling mechanisms in kidney cells

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Key words: angiotensin II, AT₁-receptor signalling, endosomes, kidney, proximal tubule cells, receptor endocytosis

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Abstract

Angiotensin II (Ang II) has powerful sodium-retaining, growth-promoting and pro-inflammatory properties in addition to its physiological role in maintaining body salt and fluid balance and blood pressure homeostasis. Increased circulating and local tissue Ang II is one of the most important factors contributing to the development of sodium and fluid retention, hypertension and target organ damage. The importance of Ang II in the pathogenesis of hypertension and target organ injury is best demonstrated by the effectiveness of angiotensin-converting enzyme (ACE) inhibitors and AT₁-receptor antagonists in treating hypertension and progressive renal disease including diabetic nephropathy. The detrimental effects of Ang II are mediated primarily by the AT₁-receptor, while the AT₂-receptor may oppose the AT₁-receptor. The classical view of the AT₁-receptor-mediated effects of Ang II is that the agonist binds its receptors at the cell surface, and following receptor phosphorylation, activates downstream signal transduction pathways and intracellular responses. However, evidence is emerging that binding of Ang II to its cell surface AT₁-receptors also activates endocytotic (or internalisation) processes that promote trafficking of both the effector and the receptor into intracellular compartments. Whether internalised Ang II has important intracrine and signalling actions is not well understood. The purpose of this article is to review recent advances in Ang II research with focus on the mechanisms underlying high levels of intracellular Ang II in proximal tubule cells and the contribution of receptor-mediated endocytosis of extracellular Ang II. Further attention is devoted to the question whether intracellular and/or internalised Ang II plays a physiological role by activating cytoplasmic or nuclear receptors in proximal tubule cells. This information may aid future development of drugs to prevent and treat Ang II-induced target organ injury in cardiovascular and renal diseases by blocking intracellular and/or nuclear actions of Ang II.

Introduction

Renin was discovered by Robert Tigerstedt in kidney extract more than a century ago, but

interest in the renin-angiotensin-aldosterone system (RAAS) remains stronger than ever.¹ Our knowledge of the RAAS has dramatically evolved from Tigerstedt's early discovery of renin as a "pressure-elevating substance from the kidney" to today's widespread recognition of the RAAS as a dual endocrine and local tissue paracrine and autocrine system.^{2–6} For the first half of a century, the effector of the system, angiotensin II (Ang II), was known mainly as a humoral factor that is formed by the action of kidney-derived rate-limiting enzyme renin. Renin cleaves the liver-synthesised substrate angiotensinogen to form Ang I, which is converted to Ang II by the lung endothelium-derived angiotensin-converting enzyme (ACE). Ang II raises systemic blood pressure (BP) by causing vasoconstriction and promotes sodium and fluid retention by stimulating aldosterone synthesis and release from the adrenals. Over the last two decades, the RAAS is no longer considered as a circulating system alone and its roles have been expanded and redefined. For example, prorenin, renin and its substrate angiotensinogen have been shown to be synthesised and taken up in tissues other than the kidney and liver.^{7–9} ACE is widely expressed in tissues beyond the pulmonary endothelium so that Ang II may be produced in any tissues where renin, angiotensinogen and ACE are co-existent. ACE is also no longer the only enzyme to convert Ang I to Ang II because chymase has been shown to produce Ang II in human tissues.¹⁰ Finally, novel biological actions have been uncovered for other components of the system, such as Ang (1–7)¹¹ and Ang IV.^{12,13} It is now well accepted that Ang II and its biologically active metabolites can be produced at local tissues and act both systemically as circulating peptides and locally as paracrine and/or autocrine factors.

There is now increasing evidence that Ang II may also function as an intracrine peptide, which may be synthesised and exert biological effects within the cells.^{14–19} The potential role of intracellular Ang II could be traced back to an early study in which radiolabelled Ang II was found in the nuclei of vascular smooth muscle and cardiac cells following systemic administration.²⁰ However, Re

later defined the concept of intracrine Ang II as the effector peptide that is either synthesised within a cell via actions of renin, angiotensinogen and ACE or internalised from extracellular Ang II.^{14,21} Evidence supporting a functional role of intracellular (or intracrine) Ang II has been reported in various target tissues or cells. For instance, Re and associates have demonstrated in the liver that intracellular Ang II induced important actions in hepatocytes.²²⁻²⁴ De Mello and colleagues have consistently shown that intracellular dialysis of renin, angiotensinogen or Ang II into hamster cardiomyocytes significantly altered cell-to-cell communications, supporting an important role for intracellular Ang II in regulating cardiac function.²⁵⁻²⁷ Haller *et al.* showed that microinjection of Ang II directly into rat vascular smooth muscle cells increased intracellular calcium, which could be blocked by intracellular AT₁-receptor blockers.^{28,29} Sigmund and associates recently provided evidence that an intracellular form of renin plays a functional role in the brain.¹⁹ Moreover, Danser's and Mullins' groups have recently reported that prorenin uptake or internalisation induced intracellular signalling or responses that were either dependent or independent of Ang II.^{30,31} The presence and the potential role(s) of intracrine or intracellular Ang II in tissues other than the kidney have been reviewed elsewhere^{5,7,14,17,18} and further detailed discussion of the topic in those tissues is beyond the scope of this article.

The current review instead focuses on the intracrine renin-Ang II system in the kidney with emphasis on proximal tubule cells, because little is known about the presence and potential role(s) of intracellular Ang II in this tissue. Recent evidence suggests that increased synthesis and/or uptake of circulating and locally produced extracellular Ang II by proximal tubule cells may contribute to sodium retention and promote growth and proliferation, leading to the development of hypertension-induced renal injury.³²⁻³⁶ Although Ang II exerts powerful and diverse effects on proximal tubular function and cell growth by activating cell surface type 1 (AT₁) receptors,³⁷⁻⁴¹ Ang II-mediated activation of these receptors also induces endocytosis (internalisation) of the Ang II-AT₁-receptor complex, which may initiate intracellular effects.⁴²⁻⁴⁴ Whether internalised or intracellular Ang II plays a physiological and/or pathological role in proximal tubule cells remains to be further studied.^{45,46} Since proximal tubule cells reabsorb more than 65–70% of glomerular filtered sodium load, and increased sodium reabsorption in this nephron segment by extracellular and intracellular Ang II will promote sodium and fluid retention and consequently the development of hypertension. Acting as a

powerful intracellular cytokine and growth factor, intracellular Ang II may also play an important role in the pathogenesis of Ang II-induced hypertensive renal injury, especially tubulo-interstitial fibrosis.^{35,47,48} By studying intracellular trafficking and intracrine actions of internalised Ang II, novel mechanisms by which Ang II activates intracellular receptors to elicit biological responses may be uncovered. This information may aid future development of drugs to prevent and treat Ang II-induced target organ damage in cardiovascular and renal diseases by blocking intracellular and/or nuclear actions of Ang II.

Overview of proximal tubule renin-angiotensin system

It is well recognised that proximal tubule function is regulated by both circulating and locally formed Ang II.^{34,49} In the kidney, all major components of the RAS, including angiotensinogen, renin, ACE and Ang II receptors, have been demonstrated in proximal tubule cells (figure 1).⁵⁰⁻⁵⁶ Expression of renin, angiotensinogen and ACE ensures local generation of Ang II independent of the circulating RAS, whereas expression of Ang II receptors is essential for Ang II to induce biological actions. There are at least two major classes of Ang II receptors expressed in proximal tubule cells, AT₁ and AT₂, where high-affinity AT₁-receptors occur in both the brush border and basolateral membranes.^{52,55,57-60} AT₁-receptors are G protein-coupled receptors belonging to the superfamily of seven transmembrane-spanning proteins.^{40,61-63} Two subtypes of the AT₁-receptor, designated AT_{1a} and AT_{1b}, have been identified in rodents, but the former is the predominant isoform in the kidney, equivalent to the human AT₁-receptor.⁶¹ The primary actions of Ang II in proximal tubule cells are to stimulate sodium and bicarbonate reabsorption physiologically^{38,39,64,65} and to promote sodium retention and induce cellular growth and differentiation in diseased states.^{35,66-69} Most of the known proximal tubular actions of Ang II are mediated by the AT₁-receptor, which is coupled to a G-protein-regulating mechanism that activates multiple signalling pathways, including phospholipase C, D, and A₂ signalling, mitogen-activated protein kinase (MAPK), and tyrosine kinase.^{37,52,63,70,71} Activation of AT₁-receptors leads to phosphoinositide hydrolysis, mobilisation of intracellular calcium, and inhibition of adenylate cyclase.^{38,45,46,57,72,73} By contrast, only low levels of AT₂-receptors are expressed in proximal tubules and the role of this receptor is much less clearly defined than that of the AT₁-receptor.^{57,60,74-76}

The classic view of Ang II-mediated actions is that Ang II binds its receptors at the plasma membrane and phosphorylation of the receptor

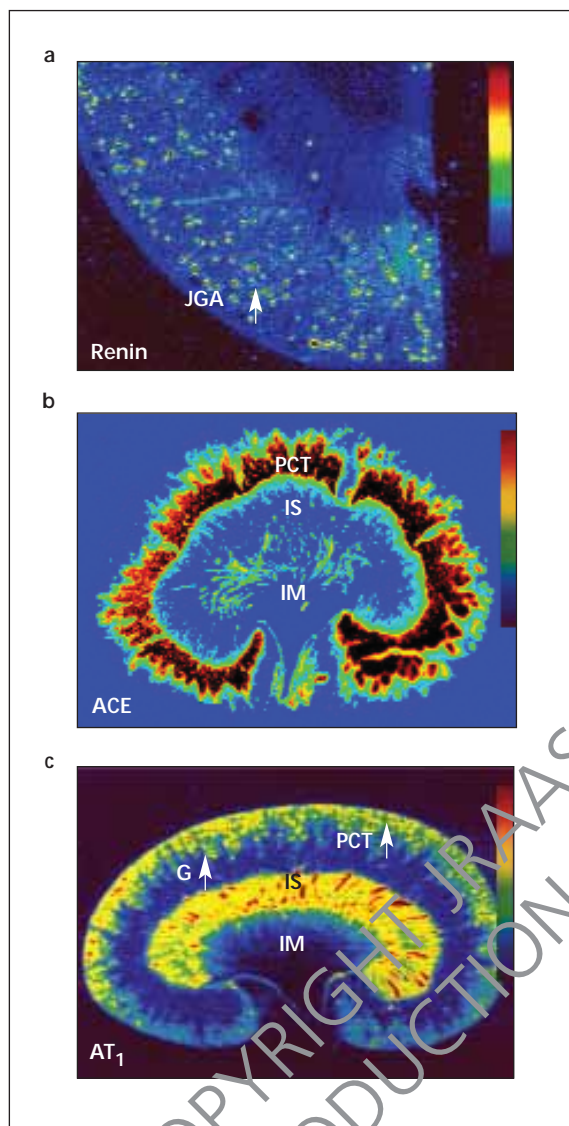


Figure 1 Mapping of active renin; **a**: angiotensin-converting enzyme (ACE); **b**: and Ang II AT₁-receptors; **c**: in the kidney, as visualised by quantitative *in vitro* autoradiography. Active renin in the renal cortex was labelled with [¹²⁵I]-H77, a renin inhibitor; ACE by [¹²⁵I]-351A, a lisinopril derivative; and AT₁-receptors by [¹²⁵I]-[Sar¹,Ile⁸] Ang II. Active renin was localised in the vascular pole of glomerulus corresponding to the juxtaglomerular apparatus (JGA), ACE primarily in proximal tubules (PCT), and AT₁-receptors in the glomerulus (G), proximal tubules and the inner stripe of the outer medulla (IS). IM = inner medulla. Modified from References ^{40,52,58,60,102}

activates downstream signalling and induces intracellular responses.^{37,38,52,57,63,70,71} However, increasing evidence suggests that binding of Ang II to its membrane AT₁-receptors also activates endocytotic (or internalisation) processes that promote trafficking of both the effector and the receptor into intracellular compartments, where interaction of Ang II with its receptors may induce intracellular signalling with consequent biological effects.⁴²⁻⁴⁴ Whether internalised or intracellular Ang II has important intracrine

actions and signalling is not completely understood. For instance, although the concept of intracellular Ang II was introduced many decades ago^{14,20,77,78} a careful MEDLINE search yielded only several dozens of citation on intracellular Ang II and its receptors in all tissues. Thus, 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 internalised Ang II in proximal tubule cells and other tissues.

AT₁-receptor-mediated accumulation of extracellular Ang II in proximal tubule cells

Angiotensin II levels in the kidney are often greater than can be explained by levels of circulating Ang II, but the precise levels and localisation of intrarenal Ang II are not fully understood.^{32,33,36,40,53,79,81} Nanomolar concentrations of Ang II have been reported in the glomerular filtrate,⁸¹ proximal tubular fluid^{79,80,82,83} and cortical interstitial fluid.^{84,85} Conversely, it has recently been suggested that most intrarenal Ang II is cell-associated, though the location of cell-associated Ang II has not been identified to our knowledge.^{34,86} The biological significance of high levels of intrarenal Ang II is not known.

There is considerable evidence that the kidney takes up circulating or extracellular Ang II, and this process may contribute significantly to overall levels of intrarenal Ang II.^{32-34,36,86-88} Navar and associates were among the first to demonstrate that the kidney accumulated circulating Ang II when rats were infused with the exogenous peptide,^{32,33,89} and their findings were later confirmed by many others.^{34,36,88,90} Uptake of circulating or extracellular Ang II by the kidney appears to involve AT₁-receptor-mediated internalisation, because AT₁-receptor antagonists effectively prevent Ang II accumulation in this tissue (figure 2).^{34,36,87-90} While not internalised itself, the AT₂-receptor may play a regulatory role in AT₁-receptor-mediated internalisation of Ang II, since the AT₂-receptor has been shown to antagonise most, if not all, of the known AT₁-receptor mediated actions of Ang II.^{43,61,74,76,91} However, we do not know the cellular localisation of Ang II uptake in the kidney because most previous studies were only concerned with the whole kidney tissue. There is indirect evidence suggesting proximal tubule cells as potential sites of intrarenal Ang II accumulation.⁹² For example, we recently demonstrated increased intracellular uptake of Ang II in renal endosomes of Ang II-infused rats isolated primarily from renal cortical tubules, and this uptake was prevented by the ARB candesartan (figure 2).³⁶ Ang II has also been shown to stimulate AT_{1a}-receptor internalisation

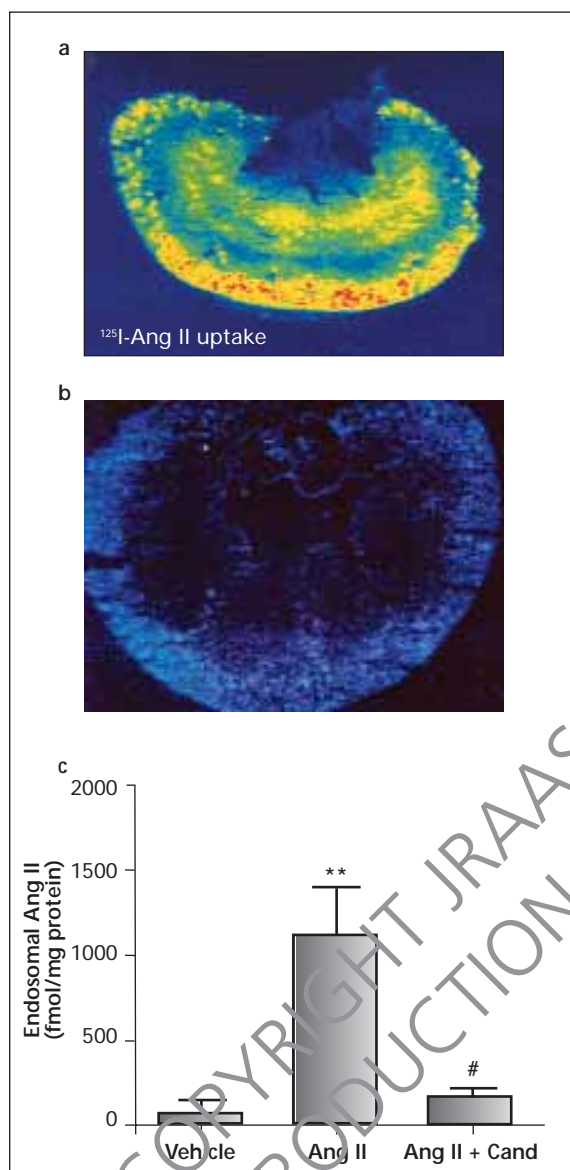


Figure 2
Quantitative *in vitro* autoradiographs showing AT₁-receptor-mediated uptake of circulating [¹²⁵I]-Ang II in the rat kidney. **a**: a control rat kidney; **b**: the kidney of a rat pretreated with losartan to block AT₁-receptors before [¹²⁵I]-Ang II was infused; **c**: renal cortical endosomal Ang II levels in the rats receiving vehicle, Ang II infusion for 2 weeks, or receiving concurrent administration of Ang II and candesartan. ** = *p* < 0.01 vs. vehicle; # = *p* < 0.05 vs. Ang II. Modified from References^{36,40,58,102}

in AT_{1a}-receptor transfected epithelial cell lines, such as opossum kidney (OK) epithelial cells and human embryonic kidney 293 cells (HEK293)^{42,43,91,93,94} but these cells normally do not express the components of the RAS including the AT₁-receptors and thus their physiological relevance remains uncertain. The contribution of receptor-mediated endocytosis to intracellular levels of Ang II has been determined only recently in proximal tubule cells *in vitro*. Using cultured proximal tubule cells which express the RAS and respond to Ang II stimulation,^{95,96} we showed that intracellular Ang II levels were

increased more than two-fold when they were incubated with exogenous Ang II, and this response was inhibited by the ARB losartan and the cytoskeleton microtubule inhibitor colchicine or the tyrosine phosphatase inhibitor phenylarsine oxide (PAO).⁴⁶ These results support the concept that proximal tubule cells take up extracellular Ang II via AT₁-receptor-mediated endocytosis and the process requires interactions with cytoskeleton microtubules and tyrosine phosphatases.

Pathways of intracellular trafficking of internalised Ang II

The specific intracellular compartment(s) where internalised Ang II is trafficked in proximal tubule cells remain to be determined. Likewise, we do not know whether Ang II is translocated to the nucleus following internalisation in these cells, where it may activate nuclear receptors to elicit a nuclear effect. Identification of the intracellular compartment(s) associated with Ang II internalisation would be important in understanding the role(s) of internalised Ang II in these cells. Recent studies suggest that endosomes may be intracellular compartments for internalised Ang II following its binding to cell membrane-bound AT₁-receptors.^{36,86,91,92} In HEK 293 transfected with AT_{1a}-receptors, the Ang II-AT_{1a}-receptor complex was internalised and transported to the endosomes; however, levels of internalised Ang II were not determined.⁹¹ Although Ang II and AT_{1a}-receptors have been reported in endosomes of the rat renal cortex, it was not determined whether they are synthesised intracellularly or accumulated through endocytosis.⁹² In the pig kidney, acute infusion of [¹²⁵I]-Ang II was associated with high levels of relative radioactivity in intracellular fractions, but the precise location of this radioactivity remains unidentified.^{34,86,88} None of these studies identified and/or measured intracellular Ang II levels during chronic Ang II infusion *in vivo* or in cultured proximal tubule cells *in vitro*. Using a different approach, we have recently isolated and purified intermicrovillar clefts (heavy endosomes) containing clathrin-coated vesicles and endosomes and measured intracellular Ang II levels in rats with Ang II-induced hypertension.³⁶ Because we were able to co-localise AT_{1a} receptors with a trapped endosomal marker, fluorescein dextran, the Ang II levels obtained from isolated and purified endosomes most likely represent internalised Ang II in this compartment (figure 2). Nevertheless, since complete elimination of potential contamination between different subcellular and intracellular fractions is impossible, intracellular localisation of Ang II and AT₁-receptors in renal endosomes or other compartment(s) such as the nucleus, still awaits precise morphological confirmation. Although Ang II receptors have been demonstrated in the

nuclei of epithelial cells or isolated from the kidney,⁹⁷⁻¹⁰⁰ there is no study specifically designed to localise internalised Ang II and AT₁-receptors in proximal tubule cells *in vitro* and *in vivo*. It would be interesting to use state-of-the-art electron microscopic autoradiography and immunohistochemistry to localise internalised Ang II and AT₁-receptors, focusing mainly in proximal tubule cells *ex vivo*, and use confocal and fluorescence microscopy for similar studies in cultured proximal tubule cells *in vitro*. The information obtained from the experiments will be important for our understanding of the intracrine role(s) of internalised Ang II in the regulation of proximal tubule sodium transport and BP and in the development of Ang II-induced renal injury.

Internalised Ang II induces intracellular calcium mobilisation by stimulating intracellular AT₁-receptors

The autocrine or intracrine role of internalised Ang II is not well understood to our knowledge. It is generally recognised that in order to exert cellular actions, extracellular Ang II must bind to cell surface receptors and activate intracellular second messenger systems.^{37,61,63,70,101,102} Membrane-bound receptors may be primarily responsible for the acute or immediate effects of Ang II following activation of signal transduction systems.^{42,71} However, binding of Ang II to its membrane AT₁-receptors also activates endocytotic processes that promote trafficking of both the effector and the receptor into intracellular compartments in vascular smooth muscle cells (VSMCs)^{103,104} and AT₁-receptor-expressing epithelial cells.^{94,105,106} We do not know whether internalised Ang II activates cytoplasmic receptors to induce intracellular effects because it is difficult to differentiate intracellular effects from those mediated by the membrane receptors or intracellularly formed peptide.

The fate of internalised AT₁-receptors has been suggested as: a) recycling back to the cell surface after dissociation from internalised Ang II; b) helping dispose of excess Ang II through degradation by intracellular lysosomes; or c) mediating the intracellular actions of Ang II.^{42,43,91,103,106} Recent studies have shown that internalised Ang II and AT_{1a}-receptors are not necessarily sorted to lysosomes for degradation, but may be stored within the endosomal compartments and released into the cytoplasm in response to the acidic endosomal environment.^{104,107,108} Upon release, internalised Ang II may mediate responses within the cells.⁴³ Consistent with the latter pathway, recent evidence suggests that internalisation of AT₁-receptors and Ang II is important for full expression of physiologically relevant actions of

this agonist.^{94,105,106} Schelling *et al.* have previously shown that blockade of AT₁-receptor endocytosis with PAO inhibited activation of protein kinase C and formation of inositol 1,4,5-triphosphate (IP₃) and sodium flux.^{105,106} However, PAO is not only an inhibitor of AT₁-receptor-mediated endocytosis but also a potent tyrosine phosphatase inhibitor,^{104-106,108} which also selectively inhibits the sustained formation of phospholipase C-mediated diacylglycerol (DG) accumulation in VSMCs.¹⁰⁸ Thus, it is difficult to know whether PAO blocks receptor-mediated endocytosis or directly inhibits membrane AT₁-receptor-mediated downstream signalling. Recent studies have demonstrated that microinjection of Ang II directly into the cytosol of VSMCs increases intracellular [Ca²⁺]_i.^{28,29} Since the AT₁-receptor blocker was applied extracellularly to block cell membrane-bound receptors, whereas Ang II was microinjected into the cells, these results raise the possibility that intracellular Ang II acts on cytoplasmic receptors to exert intracrine effects in VSMCs. Indeed, Ang II has been shown to bind to a high-affinity cytoplasmic Ang II binding protein in non-renal tissues, although the consequences of this interaction remain to be determined.^{22,78,109,110}

To determine whether intracellular or internalised Ang II plays any physiological role in proximal tubule cells, microinjection of Ang II directly into the cells may be one of the best approaches available, because it can differentiate the intracellular effects mediated by the microinjected Ang II from those mediated by extracellular Ang II via membrane receptors. Using this novel approach, we recently demonstrated that microinjection of Ang II into proximal tubule cells induced intracellular [Ca²⁺]_i responses and the responses were blocked by co-microinjection with the AT₁-receptor agonist losartan or by pre-treatment of the cells with thapsigargin to deplete intracellular calcium stores or with U-73122 to inhibit phospholipase C.⁴⁵ Intracellular [Ca²⁺]_i response to Ang II stimulation represents a well-described characteristic of actions of Ang II on proximal tubular transport (figure 3).^{37-39,45,49} Our results suggest that intracellular Ang II may play a physiological role in proximal tubule cells by stimulating cytoplasmic AT₁-receptors to mobilise calcium from intracellular stores.

Intracellular Ang II activates nuclear transcription factor NF-κB by stimulating cytoplasmic and nuclear AT₁-receptors

In addition to actively participating in physiological regulation of sodium and fluid reabsorption and BP homeostasis, Ang II has been implicated in the pathogenesis of many

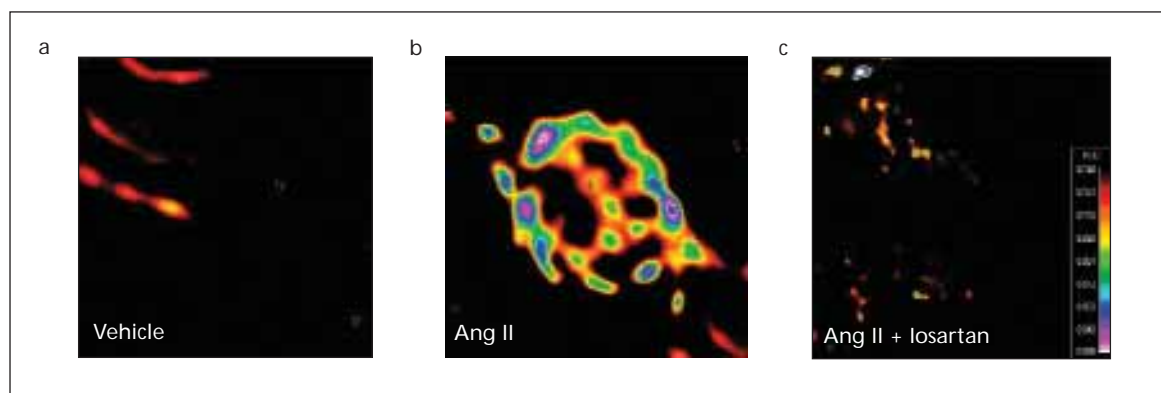


Figure 3

Microinjection of Ang II directly into single proximal tubule cells increased intracellular calcium via activation of intracellular AT_1 -receptors. **a:** control; **b:** Ang II (1 nM); and **c:** co-microinjection of Ang II and losartan (10 μ M). Images were taken 30 seconds after microinjection. The color bar indicates the intensity of calcium signalling with black representing background and red the highest level of signalling.

progressive renal diseases, including Ang II-induced hypertensive tubulo-interstitial fibrosis.^{55,47,48,68} Ang II is not only a powerful vasoactive peptide, but also a potent pro-inflammatory cytokine and growth factor in cardiovascular and renal tissues.¹¹¹⁻¹¹³ There is accumulating evidence that Ang II can affect the transcription of genes related to sodium transport^{114,115} and cell growth and proliferation.¹¹⁴⁻¹¹⁷ Ang II has been shown to induce expression of the epithelial sodium channel (EnaC),¹¹⁵ NHE-3,^{115,118-121} and proto-oncogenes, growth factor genes, extracellular matrix genes and hypertrophic marker genes.^{47,69,111} Although not classified as gene transcription-modulating drugs, ACE inhibitors and AT_1 -receptor antagonists have been shown to inhibit changes in gene expression induced by Ang II.¹¹⁷

The growth-promoting and proliferative effects of Ang II may be partly mediated by internalised Ang II, acting on cytoplasmic and nuclear receptors.^{36,68,111,121} Internalised Ang II may stimulate cytoplasmic receptors to activate a variety of intracellular kinases, leading to phosphorylation of many cytoplasmic and nuclear proteins. Intracellular kinases activated by Ang II include extracellular signal-regulated protein kinase(s) (ERKs)^{63,70,117,122} JAK-STAT signalling^{117,123,124} and calcineurin phosphatase.^{117,125} Activation of calcineurin phosphatase by Ang II may be particularly relevant to increased intracellular $[Ca^{2+}]_i$ by internalised Ang II, because increased intracellular $[Ca^{2+}]_i$ is associated with increased expression of the N^+/H^+ exchanger and hypertrophic and proliferative responses.^{126,127} Internalised Ang II may also be translocated to the nucleus,^{20,97,99,110} where it may activate nuclear calcium^{28,29,45} and stimulate transcription of NHE-3 and pro-inflammatory cytokines and growth factors.^{20,47,69,97,111,128} Binding sites for Ang II with AT_1 -receptor properties have been identified in the nucleus of hepatocytes,¹¹⁰ CHO cells,⁹⁷ HEK

293 cells⁹⁹ and the kidney,^{98,100,109,110} but we do not know whether there are functional nuclear Ang II receptors in proximal tubule cells.

Recent studies indicate that Ang II activates nuclear transcription factor NF- κ B and activator protein-1 (AP-1) in the kidney, leading to long-lasting inflammatory and growth-promoting effects.^{47,48,112,129,130} NF- κ B is an important transcription factor in inflammatory diseases, and activation of NF- κ B by Ang II stimulates transcription of many sodium transporters, cytokines and chemokines, including angiotensinogen, monocyte chemoattractant peptide-1 (MCP-1), TGF β , and RANTES (regulated on activation normal T cell expressed and secreted).^{47,48,112,121} When not activated, NF- κ B exists in an inactive form in the cytoplasm, binding to inhibitory I κ B proteins. Stimulation of cells results in phosphorylation and degradation of I κ B proteins, which releases NF- κ B dimers. These dimers are translocated to the nucleus, where they activate appropriate target genes.^{131,132} After internalisation, Ang II has been localised to the nucleus of VSMCs.²⁰ More recently, Chen demonstrated that Ang II induced internalisation of an AT_{1a} -receptor-green fluorescent protein complex, which is then translocated to the nucleus in transfected CHO cells.⁹⁷ It is likely that internalised Ang II activates nuclear transcription factor NF- κ B by binding to cytoplasmic and nuclear AT_1 -receptors, thereby increasing expression of the Na^+/H^+ exchanger NHE-3, which promotes sodium transport in proximal tubules, and pro-inflammatory cytokines, chemokines and growth factors including angiotensinogen, MCP-1, IL-1 β and TGF- β 1. NF- κ B activation by intracellular Ang II may be particularly relevant because activation of NF- κ B by Ang II is one of the major mechanisms by which Ang II acts as an inflammatory cytokine to induce tubulo-interstitial fibrosis.^{47,48,112,121,129,130,133} In a recent study, we have shown that Ang II

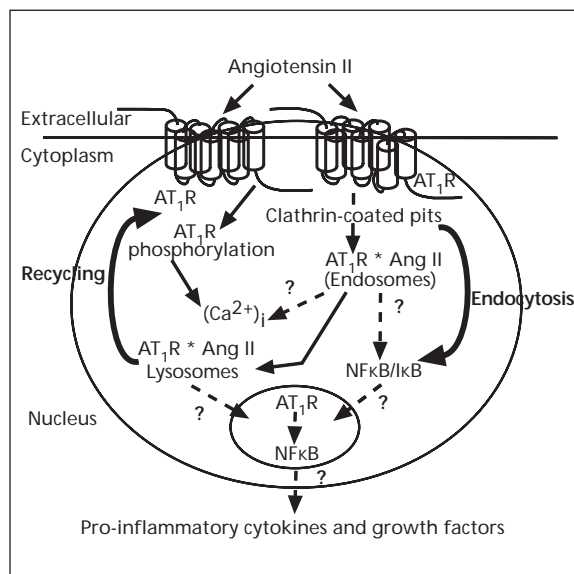


Figure 4

A schematic diagram showing classic and alternative pathways by which extracellular Ang II induces biological actions in proximal tubule cells, namely through activation of cell surface AT₁-receptors under acute physiological settings and/or intracellular AT₁-receptors (cytoplasmic and nuclear) following endocytosis under pathophysiological conditions. While the classic pathway plays an essential role in the physiological regulation of proximal tubular transport, the alternative pathway may play a critical role in the development of hypertension and Ang II-induced tubulo-interstitial injury.

induced growth of proximal tubule cells and activated NF-κB, and these responses were blocked by inhibition of AT₁-receptor-mediated endocytosis of extracellular Ang II with losartan or colchicine.^{20,21} Taken together, these studies suggest that internalised or intracellular Ang II may play an important role in renal cellular growth and fibrotic responses by activating NF-κB signalling.

Perspectives

In summary, significant progress has been made during the last few years on the potential role of intracrine Ang II that is either synthesised intracellularly or internalised via AT₁-receptor-mediated endocytosis in other tissues and kidney cells. Our current understanding of intracellular Ang II and its signalling in proximal tubule cells may be summarised in figure 4. Intra-proximal tubular and renal cortical interstitial fluid compartments contain high levels of extracellular Ang II under physiological conditions, which may be enhanced substantially during Ang II-induced hypertension or other renal diseases associated with activated renin-angiotensin system, such as diabetic nephropathy. The primary effects of extracellular Ang II (circulating and intrarenally formed) are still mediated by cell surface receptors, the peptide is also taken up by proximal tubule cells through AT₁-receptor-

mediated endocytosis. Upon internalisation, Ang II may stimulate cytoplasmic and nuclear AT₁-receptors to increase intracellular [Ca²⁺]_i and activates nuclear transcription factor NFκB, leading to increased expression of the Na⁺/H⁺ exchanger NHE-3 and pro-inflammatory cytokines and growth factors. Increased expression of the Na⁺/H⁺ exchanger NHE-3 and pro-inflammatory cytokines and growth factors by internalised Ang II may contribute to sodium retention and tubulo-interstitial injury in Ang II-dependent hypertension.

Further studies are required to further study the cellular mechanisms by which AT₁-receptor-mediated endocytosis of extracellular Ang II is regulated, the pathways of its intracellular trafficking and signalling, and its potential role(s) in the regulation of proximal tubule function and in the development of target organ injury in hypertensive and progressive renal diseases.

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