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Nuclear factor- κ B as a hormonal intracellular signaling molecule: focus on angiotensin II-induced cardiovascular and renal injury

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Abstract

Purpose of review—Nuclear factor- κ B (NF- κ B) has recently emerged as a novel intracellular signaling molecule for hormones, cytokines, chemokines, and growth factors. The purpose of this article is to highlight the role of NF- κ B as an intracellular signaling for angiotensin II and clinical perspectives of targeting NF- κ B signaling in treating hypertensive and renal diseases.

Recent findings—A selective review of recently published work provides strong evidence that activation of NF- κ B signaling by angiotensin II mediates the detrimental effects of angiotensin II on the transcription of cytokines, chemokines and growth factors. Angiotensin II stimulates AT₁ receptors to activate NF- κ B signaling via both canonical (classical) and noncanonical (alternative) pathways. Intracellular angiotensin II may also induce NF- κ B activation and transactivation of target genes. Nearly 800 NF- κ B inhibitors have been described, but none has advanced to clinical trials. However, angiotensin converting enzyme inhibitors and AT₁ blockers are beneficial in treating angiotensin II-induced hypertensive and renal injury in part by inhibiting NF- κ B activation.

Summary—Angiotensin II induces the transcription of cytokines, chemokines and growth factors, leading to target organ injury. These responses to angiotensin II are caused primarily by AT₁ receptor-activated NF- κ B signaling. Targeting NF- κ B signaling with angiotensin converting enzyme inhibitors, AT₁ blockers, and specific NF- κ B inhibitors may represent a novel approach in treating angiotensin II-induced hypertensive and renal diseases.

Keywords

angiotensin II; cytokines; G protein-coupled receptor; intracellular signaling; nuclear factor- κ B

Introduction

Nuclear transcription factor- κ B (NF- κ B), which was first described in the 1980s [1–3], is one of the most important transcription factors playing critical roles in the regulation of innate immunity, inflammatory responses, and cell growth and apoptosis. NF- κ B belongs to the super NF- κ B transcription factor family, including two subfamily NF- κ B proteins (e.g., p100, p105, and Relish) and Rel proteins (e.g., c-Rel, RelA, RelB, Dorsal and Dif) [4•,5•]. These proteins show a Rel homology domain (RHD), which is necessary for DNA binding and dimerization, and interactions with inhibitory κ B proteins (I κ Bs). NF- κ B and Rel proteins form different homodimers and heterodimers in response to different stimuli and bind to DNA regulatory κ B sites. The p50/RelA dimers and the p52/RelB dimers are the most common and important

elements in the NF- κ B family [5•,6–8]. 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 diverse target genes [6,7]. The structures, biology and potential roles of NF- κ B in cancer development, innate immunity responses, inflammatory diseases, and cell growth and apoptosis have been comprehensively reviewed [4•,5•,9,10,11•]. This article only focuses on the roles of NF- κ B as an intracellular signal for the vasoactive hormone angiotensin II (Ang II), its upstream signaling pathways and downstream effectors, and the perspectives of targeting NF- κ B signaling in Ang II-dependent and hypertension-induced organ injury.

NF- κ B: an important intracellular signaling for angiotensin II-induced effects

NF- κ B is widely expressed in different mammalian tissues including humans and can be activated by a variety of stimuli. The proinflammatory cytokine and chemokine families, including tumor necrosis factor (TNF) α , IL-1 and IL-6, microbial lipopolysaccharide (LPS), and reactive oxygen species (ROS) are the most commonly described factors that induce NF- κ B activation [4•,5•,9,10,11•]. There is accumulating evidence from the last decade, however, that the vasoactive peptide Ang II also activates NF- κ B, which may serve as an intracellular signaling for Ang II-induced effects [12–15]. Ang II is known to induce target organ damage in cardiovascular, hypertensive and renal diseases by activating a number of pro-inflammatory cytokines, chemokines and growth factors [16,17,18•,19]. Early studies showed that infusion of Ang II in rats increased renal and vascular smooth muscle cell (VSMC) NF- κ B binding activity, and activation of NF- κ B was associated with increases in inflammatory cell infiltration and tubulo-interstitial inflammatory responses [16,17,18•,19]. In rats harboring human renin and angiotensinogen genes and thereby producing high circulating and tissue Ang II, NF- κ B binding activity was substantially increased and inhibition of NF- κ B was found to ameliorate Ang II-induced inflammatory target organ injury in the heart and kidney [15]. Ang II also appears to induce inflammatory responses in rats with unilateral ureteral obstruction [20], mice with atherosclerosis and aneurysm [21], and in experimental diabetic nephropathy [22], via activation of NF- κ B.

Most recent studies in cultured cells or in animal models of Ang II-induced tissue injury further support an important role of NF- κ B in mediating the detrimental effects of Ang II. *In vitro*, Ang II stimulates the expression and transcription of the inflammatory cytokine IL-6 in VSMCs and the effect is mediated by NF- κ B [23,24,25•,26]. Ang II also induces the expression and transcription of the chemokine family member monocyte chemoattractant protein 1 (MCP-1) or insulin-like growth factor I receptors (IGF-IR) via a NF- κ B-dependent pathway in rat preadipocytes [27], and IL-6 transcription in rat VSMCs [26,28,29]. We recently demonstrated that Ang II stimulated NF- κ B activation and proliferation in immortalized rabbit proximal tubule cells [17,30]. The effects of Ang II on activation of NF- κ B signaling *in vitro* were replicated in a variety of animal studies with Ang II-induced tissue injury or inflammatory diseases, suggesting the physiological and pathophysiological relevance of NF- κ B activation by Ang II. Although not being a purely Ang II-dependent model of renal disease, 5/6 nephrectomy in rats significantly increased the expression of NF- κ B in the renal cortex, which could be inhibited by the angiotensin converting enzyme (ACE) inhibitor enalapril [31]. In spontaneously hypertensive rats, the development of hypertension-induced vascular inflammatory responses was associated with activation of NF- κ B and other inflammatory markers, such as IL-1 β and IL-6, whereas AT $_1$ receptor blockade with candesartan attenuated these responses [32]. This suggests that endogenous Ang II plays a role in inducing NF- κ B activation. Using a more direct approach, Ozawa *et al.* [18•,19] chronically infused Ang II in rats to develop Ang II-induced renal tubulo-interstitial injury or fibrosis. In addition to severe

systemic hypertension, Ang II significantly increased NF- κ B binding activity, as assessed by electromobility shift assay, expression of MCP-1 and TGF- β 1 mRNA levels, renal cortical tubulo-interstitial macrophage infiltration and collagen deposition and proteinuria [18,19]. Because the NF- κ B inhibitor parthenolide blocked these effects of Ang II, these results clearly implicate an important role of NF- κ B in the development of Ang II-induced renal injury.

NF- κ B activation by angiotensin II: role of upstream signaling molecules and downstream effectors

What intracellular signaling mediates NF- κ B activation by cytokines, chemokines, or growth factors has been extensively investigated for two decades since its discovery. Although not fully understood, the upstream signaling pathways that regulate NF- κ B activation have been well characterized. It is clear that in the absence of stimulation by cytokines, chemokines, or hormones, NF- κ B remains inactive in the cytoplasm, being bound to the I κ Bs. Now, there is a consensus in the NF- κ B research community that two major upstream signaling pathways participate in the activation of NF- κ B; the canonical (or classical) and noncanonical (or alternative) pathways [5,9,11,33]. The activation of the upstream I κ B kinase (IKK) complex that includes the catalytic kinase subunits (IKK α and IKK β) and a regulatory subunit (IKK γ), or NF- κ B essential modulator (NEMO), is required for the canonical pathway [5,9,10]. Cytokines, chemokines or peptide hormones bind to their cell surface receptors with various adaptor proteins at their cytoplasmic domains, leading to recruitment of intracellular IKK α , IKK β , and NEMO and activation of the IKK complex. Activated IKK then induces phosphorylation of I κ B at its serine residues, followed by ubiquitination and degradation of the inhibitory I κ B by proteasomes [5,10]. The released NF- κ B dimer (p50/RelA) is translocated to the nucleus and acts as the transcription factor to regulate target genes encoding inflammatory cytokines, chemokines, cell adhesion molecules, growth factors or hormonal signaling proteins [10]. This classical NF- κ B signaling plays a dominant role in controlling NF- κ B activation and upregulation of inflammatory cytokines, chemokines and growth factors, and is mainly responsible for the well documented effects of NF- κ B in cells and tissues [10,11,34]. By contrast, the alternative pathway is less well understood, but its activation does not involve direct I κ B degradation [10,11]. The differences between the classical and alternative pathways leading to NF- κ B activation are that the latter pathway responds largely to B-cell-related signaling and recruits two IKK α subunits but not NEMO [5,10,11]. It is the upstream NF- κ B-inducing kinase (NIK) that phosphorylates the IKK complex, leading to selective release of the other NF- κ B dimer (p52/RelB) [10,11]. The alternative pathway appears to play an important role in premature B cell survival and lymphoid organ development [5,10,11]. Although a third pathway of NF- κ B activation directly via p50 or p52 homodimers has been suggested, the manner by which it activates NF- κ B and how this pathway regulates NF- κ B signaling remain poorly understood [5].

While the signaling pathways leading to NF- κ B activation by classical cytokines or chemokines, such as TNF α , LPS or IL-1 β , have been well characterized, the upstream intracellular signaling that mediates NF- κ B activation by Ang II is not fully understood. It is generally accepted that AT $_1$ receptor-activation of its cell surface G protein-coupled receptors (GPCRs) is required for NF- κ B activation, though how activation of AT $_1$ receptors by Ang II would lead to phosphorylation of the IKK complex and release of NF- κ B dimers, (p50/RelA) or p52/RelB, is unclear [13,14,20,22,24,27,30]. Although AT $_2$ and AT $_4$ receptors or other Ang II fragments, such as angiotensin III or IV, have been reported by some groups to activate NF- κ B independent of AT $_1$ receptors *in vitro* and *in vivo* [13,14,35,36], the precise roles of these non-AT $_1$ receptor-mediated mechanisms remain to be confirmed [37]. Indeed, Wu *et al.* [38] have shown that rather than activating NF- κ B signaling, stimulation of AT $_2$ receptors by Ang II in fetal VSMCs (which express AT $_1$ and AT $_2$) actually inhibited AT $_1$ receptor-mediated NF- κ B DNA binding, I κ B degradation and production of MCP-1. In experimental diabetic

nephropathy, Lee *et al.* [22] showed that valsartan, an AT₁ receptor blocker, was much more effective than PD 123319, an AT₂ receptor blocker, in blocking NF-κB activation and attenuating glomerulosclerosis and tubulointerstitial inflammatory cell infiltration. In a recent study, we also noted that losartan, but not PD 123319, blocked Ang II-induced activation of NF-κB in rabbit proximal tubule cells [30]. The absence of effects of AT₂ receptor blockade on NF-κB activation is probably expected, because it is well documented that the AT₂ receptor differs substantially from the AT₁ receptor in their genome structures, the patterns of their expression and cellular distribution, biological actions and signaling transduction mechanisms [39,40]. AT₂ receptors are expressed only sparsely in major cardiovascular and renal cells or tissues in adults and most of biological actions of AT₂ receptor activation by Ang II appear to oppose those of AT₁ receptor activation [41]. By contrast, Ang III and Ang IV are the biological active fragments of Ang II and both peptides retain relatively high binding affinity for AT₁ receptors (albeit less potent than Ang II) [40,42]. Ang III and Ang IV have been shown to activate AT₁ receptors to induce cardiovascular and renal effects, which can be blocked by AT₁ receptor antagonists [43–45]. Since most of these studies used pharmacological blockers, which require special preparations, more specific approaches, such as use of small interfering RNA (siRNAs) *in vitro* or mice with total AT₁, AT₂ or AT₄ receptor knockout, may be warranted [37].

Ang II appears to induce NF-κB activation via a series of complex cellular mechanisms. The growth effects of Ang II are mainly mediated by activation of cell surface AT₁ receptors, which leads to activation of phospholipase C (PLC)/IP₃/Ca²⁺/protein kinase C (PKC) signaling, transactivation of epidermal growth factor receptors and activation of receptor tyrosine kinase (RTK), and MAP kinases ERK 1/2 or p38 MAP kinase [40,46–48]. Activation of these classical AT₁ receptor signaling may in turn help recruit adaptor proteins and activate the IKK complex and phosphorylation of IκB, leading to degradation of IκB [13,23,25•,38]. Recent evidence suggests that both canonical (IκB-dependent) and noncanonical (IκB-independent) signaling pathways may induce activation of NF-κB by Ang II. Zhang *et al.* [23,49] recently reported dual signaling pathways for NF-κB activation by Ang II in VSMCs. In these studies, deletion of IKK or knocking down IKK using a dominant negative IKK adenovirus or siRNA to IKKβ decreased NF-κB activation by approximately 70%, suggesting that Ang II induces NF-κB activation via IKK [49]. Zhang *et al.*, however, found that activation of IKK by Ang II also led to phosphorylation of p65 subunit rather than degradation of IκB. This alternative pathway in part activates the Ras/MEK1/ERK1/2 pathway and its downstream effector, ribosomal S6 kinase (RSK) [49]. Interestingly, both pathways can act independently, but also act cooperatively to induce maximal NF-κB activation. If both IKK and RSK pathways were inhibited, Ang II-induced phosphorylation of p65 subunit and NF-κB activation and translocation to the nucleus would be completely blocked [49,23]. In VSMCs, activation of NF-κB by Ang II also appears to involve ROS [49] or RhoA signaling pathways [25•]. The RhoA signaling plays an important role in GPCR signaling-mediated cardiovascular hypertrophic responses. In the latter study, Ang II induced rapid phosphorylation of RelA at serine residue 536 and its nuclear translocation, whereas inhibition of RhoA by C3 exotoxin or a dominant negative RhoA blocked Ang II-induced RelA Ser536 phosphorylation and IL-6 expression [25•]. These studies provide further evidence that at least in VSMCs, Ang II appears to induce NF-κB signaling primarily via the noncanonical, IκB-independent pathway, involving RhoA/Rac and NIK [50,51•].

Whether Ang II activates NF-κB in cells other than VSMCs through similar mechanisms is not well understood. A novel pathway leading to NF-κB activation by Ang II has recently been described in hepatocytes, involving three proteins: CARMA3, Bcl10 and MALT1 [52•]. CARMA3 (caspase recruitment domain10/Bimp1) is a member of the CARMA family proteins associated with integrating upstream signaling PKC with its downstream signaling proteins [53]; Bcl10 is a bridging protein [54]; and MALT1 is an effector protein associated with the

IKK activation [52•]. McAllister-Lucas *et al.* [52•] demonstrated that Ang II appears to use the classical (canonical) signaling pathway to activate NF- κ B in hepatocytes via phosphorylation and degradation of I κ B α . Dominant negative mutants, siRNAs, or gene targeting against these proteins all abolished AT₁ receptor-dependent activation of NF- κ B. The upstream signaling pathways that mediate Ang II-induced NF- κ B activation in kidney cells have not been well characterized. In rat mesangial cells, Lorenzo *et al.* [36] found that PKC was not involved in Ang II or angiotensin III-induced NF- κ B activation, although AT₁ and AT₂ receptors were responsible for the effects of Ang II whereas AT₂ receptors mediated those caused by Ang III. In kidney epithelial cells, constitutive expression of RhoA blocked NF- κ B activation induced by LPS while the RhoA kinase inhibitor Y27632 or a dominant negative RhoA mutant had opposite effects, suggesting that the RhoA signaling may also play an important role in mediating Ang II-activated NF- κ B in renal epithelial cells [55]. Finally, Ozawa and Kobori [18•] recently reported that the Rho–NF- κ B axis plays an important role in the development of Ang II-induced renal injury. Taken together, Rho (or RhoA) kinase appears also to mediate Ang II-induced activation of NF- κ B signaling and tissue injury in the kidney.

Targeting NF- κ B signaling pathways in angiotensin II-induced cardiovascular, hypertensive and renal injury

Ang II is widely implicated in the pathogenesis of many progressive cardiovascular and renal diseases, including diabetic nephropathy and Ang II-induced hypertensive tubulo-interstitial fibrosis. In addition to increasing blood pressure and inducing salt and fluid retention, Ang II acts as a potent pro-inflammatory cytokine, chemokine and growth factor [18•,56,57]. As reviewed above, Ang II can affect the transcription of multiple genes, which regulate sodium transport, cell growth and proliferation, and inflammatory responses. The upstream signaling pathways mediating these effects of Ang II appear to converge on the activation of NF- κ B, acting as a common intracellular signaling for Ang II to induce target organ injury. Since NF- κ B transcription factors regulate vital physiological and pathological responses, including immune responses, inflammation development, cell growth or apoptosis, it is not surprising that targeting NF- κ B activation is an attractive approach for therapeutic intervention [4•,10,34]. Indeed, Gilmore and Herscovitch [4•] recently categorized nearly 800 inhibitors that target the NF- κ B signaling pathways ranging from upstream signaling to downstream effectors. There are three key steps for NF- κ B activation leading to target organ injury: extracellular cytokines, chemokines or hormones binding or activating their respective cell surface receptors that triggers the canonical or noncanonical NF- κ B signaling pathways [5•,9]; cytoplasmic activation of the NEMO/IKK complex and degradation of I κ B (canonical), or the NIK-mediated activation of NF- κ B (noncanonical) [5•,10,11•]; and nuclear translocation of NF- κ B and its effects on target gene transcription [5•,9,10,11•]. Although these NF- κ B inhibitors show certain potencies and specificities for blocking a particular step of NF- κ B signaling in experimental cells or animals, the clinical relevance of these compounds in treating cardiovascular and renal diseases remains uncertain [58]. None of these inhibitors has been advanced to clinical trials. Moreover, caution has been raised regarding whether inhibition of NF- κ B signaling is beneficial or detrimental, because NF- κ B not only induces tissue injury, but also plays important roles in different physiological processes [58–60]. Recent studies show that total inhibition of the NF- κ B pathway in macrophages leads to more severe atherosclerosis in mice, possibly by affecting the pro-inflammatory and anti-inflammatory balance that controls the development of atherosclerosis [58,60]. Kisseleva *et al.* [61•] showed that mice with endothelial cell-specific inhibition of I κ B α with Tie2 promoter/enhancer-I κ B α (S32A/S36A) mutation had enhanced sensitivity to LPS-induced increase in vascular permeability and a marked loss in tight junction formation, suggesting that NF- κ B plays an important role in the maintenance of vascular integrity and response to proinflammatory cytokines. By

contrast, Henke *et al.* [62••] showed that vascular endothelial cell-specific NF- κ B suppression markedly attenuated L-NAME- or Ang II-induced hypertensive renal injury in mice.

It should be mentioned that none of these inhibitors of NF- κ B signaling is specific for blocking Ang II-induced NF- κ B activation and subsequent expression and transcription of genes for proinflammatory cytokines, chemokines, growth factors or peptide hormones. Although not classified as gene transcription-modulating or anti-NF- κ B signaling drugs, ACE inhibitors and AT₁ receptor antagonists may in part be used to inhibit NF- κ B activation specifically induced by Ang II in addition to their antihypertensive effects [63]. Many cardiovascular, hypertensive and renal diseases are commonly associated with increased formation of circulating and tissue Ang II. Ang II binds and activates its cell surface AT₁ receptors to induce NF- κ B activation either via the canonical NEMO/IKK/I κ B pathway [13,29,49] or via the noncanonical NIK-dependent, Ras/MEK1/ERK1/RSK-dependent, or RhoA small GTP binding protein-dependent pathways [18•,25•,51•]. The growth-promoting and proliferative effects of Ang II may be partly mediated by activation of NF- κ B signaling. Conversely, clinical beneficial effects of ACE inhibitors and AT₁ receptor blockers may also in part due to their effects on inhibition of NF- κ B signaling [18•,20,22]. Finally, the potential role of intracellular or intracrine Ang II in activating NF- κ B signaling should also be considered especially for chronic inflammatory cardiovascular and renal diseases [64–67]. Intracellular Ang II may stimulate cytoplasmic and nuclear AT₁ receptors to induce phosphorylation of cytoplasmic and nuclear proteins. Intracellular kinases activated by Ang II include PKC, MAP kinases (ERKs), p38 kinases, JAK-STAT signaling, and calcineurin phosphatases [40,46–48,63]. Some of these kinases are directly or indirectly associated with either upstream or downstream NF- κ B signaling pathways [23,28,51•]. Furthermore, Ang II and AT₁ receptors are known to translocate to the nucleus [64,68], where Ang II may activate transcription of its precursor angiotensinogen via NF- κ B signaling [69,70]. These interactions between Ang II and NF- κ B signaling and their underlying mechanisms require further investigation.

Conclusion

In summary, we have witnessed tremendous effort and progress in studying the NF- κ B signaling within two decades of its discovery. The structures and biology of upstream and downstream NF- κ B signaling proteins, the canonical and noncanonical pathways leading to its cytoplasmic activation and nuclear translocation and transactivation of target gene expression, have been characterized. The roles of NF- κ B signaling in the regulation of innate immunity and inflammatory responses, cancer development, cell growth and apoptosis, and Ang II-induced target tissue injury are increasingly recognized. More than 25 000 articles have been published in the field and close to 800 inhibitors have been described to target NF- κ B signaling. Specific NF- κ B inhibitors, however, have not yet been used in humans to treat diseases associated with NF- κ B activation. From a clinical perspective in nephrology and hypertension, translational research should become the increasing focus of future studies to target NF- κ B signaling in preventing and treating Ang II-induced cardiovascular, hypertensive and renal injury.

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- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 109).

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