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Forum Review

Role of Reactive Oxygen Species in Angiotensin II-Mediated Renal Growth, Differentiation, and Apoptosis

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ABSTRACT

Angiotensin II (ANG II) induces cell-cycle arrest of cultured proximal tubular cells, resulting in cellular hypertrophy. This ANG II-mediated hypertrophy is associated with the induction of $p27^{Kip1}$, an inhibitor of G_1 phase cyclin-dependent kinase cyclin complexes. We have recently demonstrated that ANG II-mediated expression of $p27^{Kip1}$ and induction of cellular hypertrophy depend on the generation of reactive oxygen species (ROS). The effects of ROS are mediated by stimulation of mitogen-activated protein (MAP) kinases. p44/42 MAP kinase directly phosphorylates $p27^{Kip1}$ at serine-threonine residues and increases thereby its half-life time. AT_2-receptor activation has been implicated in apoptosis and/or cell differentiation. Recent studies, however, revealed a more indirect role of hypoxia in the antiproliferative effects of ANG II transduced through AT_2 receptors. We found that SM-20 is down-regulated in ANG II-stimulated PC12 cells that express only AT_2 receptors. It turned out that SM20 is the rat homologue of a dioxygenase that regulates hypoxia-inducible factor 1 (HIF-1). ANG II induces HIF-1α by a posttranscriptional mechanism suggesting that SM20 down-regulation leads to stabilization of HIF-1. Thus, ANG II-induced ROS generation plays a pivotal role in several pathophysiological situations, leading to renal growth regulation and remodeling after injury. *Antioxid. Redox Signal*. 7, 1337–1345.

INTRODUCTION

ANGIOTENSIN II (ANG II) was originally identified as a vasoconstrictor and potent stimulus of aldosterone release from the suprarenal glands. More recently, it has been discovered that this peptide plays an important role in the regulation of glomerular filtration and tubular transport. Research in the last decade provided convincing evidence that ANG II modulates cell growth (39, 52, 55). ANG II could either stimulate growth (proliferation, hypertrophy) or act as a growth suppressor (apoptosis, antiproliferative with induction of differentiation). The type of effect depends on cell type, subtype of ANG II receptors, and presence of other cytokines (52). In the kidney, ANG II is important for organogenesis during development (53). ANG II-mediated differentiation of cells, mainly transmitted through the ANG II type 2 (AT_2) receptor, is pivotal in this setting (53). Under pathophysiological conditions, ANG II is involved in compensatory renal hypertrophy, as well as proliferation of renal cells such as mesangial cells and renal fibroblasts (52). Furthermore, ANG II may also mediate apoptosis of renal cells under certain conditions (39).

We have studied for 10 years the molecular mechanisms of how ANG II induces hypertrophy in proximal tubular cells (55). Reactive oxygen species (ROS) have a pivotal function in this response (17). Moreover, a pertinent relationship between ANG II, ROS, hypertension, and vascular injury has been described (52). ROS, as signal intermediates, may also modulate some of ANG II's effect on renal hemodynamics (50). Finally, a close relationship between ANG II-induced ROS generation, renal infiltration of immune cells, and hypertension has been postulated mainly on experimental grounds (38). In this relationship, ANG II-induced oxidative stress leads to the infiltration of interstitial mononuclear

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cells, which may further locally generate ANG II (38). Interstitial renal inflammation causes local tissue injury resulting in altered sodium handling (38). A basic understanding of the processes of how ANG II stimulates the generation of ROS in the kidney will be reviewed briefly before the molecular mechanisms of ROS-mediated growth modulation will be described in detail. Proinflammatory effects of ANG II will be not reviewed here, and the interested reader is referred to excellent reviews (37, 45).

ANG II-INDUCED ROS FORMATION

More than 10 years ago, it was suggested that ANG II may induce ROS formation (51). Although ROS were not directly measured in these experiments, the assumption stems from acute ANG II-infusion experiments into naive rats in the presence or absence of different free radical scavengers, and these scavengers partly inhibited vascular hyperpermeability and cellular damage (51). *In vitro* studies demonstrated that treatment of cultured vascular smooth muscle cells (VSMC) with ANG II for 4–6 h increased intracellular superoxide anion (O_2^-) concentration (12). This ANG II-stimulated O_2^- production was transduced through the ANG II type 1 (AT_1) receptor and was caused by an activation of membrane-bound NAD(P)H oxidase because the flavoprotein inhibitor diphenyleneiodonium (DPI) and p22phox antisense oligonucleotides attenuated this response (12, 24, 49, 65). AT_1 receptor-transduced ROS formation, depending on NAD(P)H oxidase, has also been described in the kidney (34). Similar observations have been made in rat aortas when the endogenous renin–angiotensin system was stimulated using the two kidneys–one clip (2K-1C) hypertension model (18). Pharmacological inhibitor studies of vascular homogenates from 2K-1C animals demonstrated that the major source of O_2^- was a NAD(P)H oxidase that was activated by a protein kinase C-dependent mechanism (18).

The mechanisms of how ANG II activates NAD(P)H oxidase are incompletely understood. ANG II stimulates p22phox transcription in VSMC, rat aortas, and renal proximal tubular cells (48). This increase in p22phox mRNA expression was accompanied by an increase in NAD(P)H oxidase activity (48). Further evidence for an important role of p22phox in ANG II-mediated O_2^- generation emanated from antisense experiments interfering with p22phox expression (49). Binding of p22phox to p47phox is significantly increased by ANG II-mediated serine phosphorylation of p47phox, indicating an additional indirect mechanism of increased NAD(P)H oxidase activity (26). Interestingly, p47phox is necessary for ANG II-induced ROS formation, but suppresses NAD(P)H oxidase in the absence of ANG II (27). ANG II also stimulates the transcription of p67phox in rabbit aortic adventitial fibroblast (35, 48). More recently, a pivotal role of a gp91phox homologue (termed Nox-2) containing NAD(P)H oxidase has been implicated in ROS generation in cardiomyocytes and renal cortex (2, 5). In mesangial cells, Nox-4, another gp91phox homologue, has been identified and is increased after ANG II challenge (11). In addition, ANG II causes rapid activation of Rac1, an upstream protein involved in the assembly of the NAD(P)H oxidase system (11). Superoxide dismutase is also up-regulated in the kidneys of ANG II-infused rats (5). All these effects are mediated through AT_1 receptors, and ANG II fails to generate ROS in AT_{1a}-receptor knockout mice (11). In contrast, activation of AT_2 receptors has been implicated to inhibit ROS formation by down-regulation of p22phox, Nox-1, and p67phox (5). However, the situation could be much more complex under pathophysiological conditions. It has been reported that the AT_2-receptor subtype mediates renal production of nitric oxide (NO) by stimulation of various NO synthases (41). In certain circumstances, NO synthases could generate ROS in addition to NO (43). This is particularly the case if the substrate L-arginine or the cofactor tetrahydrobiopterin is low. In such situations, NO synthases become uncoupled and generate significant amounts of ROS (43). Whether AT_2-receptor activation plays indeed a major role in this process is currently unclear.

However, stimulation of NAD(P)H oxidase expression may not necessarily be the only mechanism of how ANG II contributes to ROS generation. ANG II markedly increases urinary excretion of proteins, including metal-bearing proteins such as transferrin (1). These transition metals are potent catalysts for oxidative stress, indicating a more indirect mechanism of how ANG II could increase renal ROS generation.

ANG II-MEDIATED GROWTH RESPONSES AND ROS IN RENAL CELLS TRANSDUCED THROUGH AT_1 RECEPTORS

Renal growth processes are part of an adaptive process of surviving nephrons during chronic renal injury (52). Although initially helping to maintain renal function, these compensatory renal growth processes are detrimental in the long-term, leading to glomerulosclerosis, interstitial fibrosis, and tubular atrophy. There is accumulating evidence that ANG II is an important cytokine involved in such compensatory growth processes (52). Particularly, a role of ANG II has been implicated in hypertrophy. This growth response could be defined as an increase in cell size, RNA content, and protein synthesis, without concomitant changes in cellular DNA content. Hypertrophy is an active process in which glomerular cells reenter the cell cycle from G_0. Entry into G_1 is associated with an increase in protein and RNA synthesis, in anticipation of DNA replication in S phase. Cells undergoing hypertrophy, however, do not progress into S phase, but are arrested in G_1. In addition to cell cycle-dependent hypertrophy, there is also evidence that hypertrophy can be induced by cell cycle-independent mechanisms, due to an inhibition of protein degradation.

More than 10 years ago, we discovered that ANG II as a single factor induces hypertrophy of cultured mouse proximal tubular cells (54). This effect was also observed in porcine LLC-PK_1 cells (58) and was subsequently confirmed by other groups in additional species (6, 16). The hypertrophic growth response was specific for proximal tubular cells because ANG II turned out to be a mitogen for mouse cells isolated from the ascending limb of Henle's loop (59). The hypertrophic action of ANG II in proximal tubular cells is mediated through high-

affinity AT_1 receptors, involves activation of G proteins, and is independent of Na^+/H^+ exchanger stimulation (54). ANG II, as well as mitogenic factors such as epidermal growth factor (EGF), activates a genetic pattern typical of G_0–G_1-phase transition (54). In contrast to EGF, which leads to successful progress through the cell cycle resulting in proliferation, ANG II-induced hypertrophy is associated with arrest in the G_1 phase (57). We investigated how ANG II mediates this growth arrest and found that the peptide stimulates expression of $p27^{Kip1}$, an inhibitor of G_1 phase cyclin-dependent kinase (CDK)/cyclin complexes (56). CDK/cyclin complexes are necessary for driving cells through the different phases of the cell cycle. Specific G_1 cyclins have been identified (cyclin D1–3, which associate with CDKs 4 and 6, and cyclin E, which associates with CDK 2). Therefore, activation of CDK 4,6/cyclin D and CDK 2/cyclin E complexes are necessary for transit from G_1 into the S phase, where DNA synthesis occurs. $p27^{Kip1}$ inhibits kinase activity of G_1-phase CDK/cyclin heterodimers. We tested a functional role of ANG II-induced $p27^{Kip1}$ expression in tubular hypertrophy using antisense technology (56). Transfection of tubular cells with $p27^{Kip1}$ antisense, but not missense, oligonucleotides abolished the ANG II-mediated hypertrophy and facilitated entry into the S phase of the cell cycle (13, 56). To overcome intrinsic specificity problems associated with antisense oligonucleotides and to test beyond any doubt the role of $p27^{Kip1}$ in ANG II-induced hypertrophy of proximal tubules, we established proximal tubular cells in culture from $p27^{Kip1}$ −/− mice and tested the effects of ANG II on hypertrophy (62). ANG II induced hypertrophy and cell-cycle arrest of $p27^{Kip1}$ +/+ proximal tubular cells, but facilitated cell-cycle progression of two $p27^{Kip1}$ −/− proximal tubular cell lines without inducing hypertrophy (62). ANG II activated CDK 4/cyclin D kinase activity in $p27^{Kip1}$ +/+ and −/− tubular cells, but stimulated CDK 2/cyclin E activity only in wild-type cells (62). However, in the presence of ANG II, reconstituting $p27^{Kip1}$ expression in $p27^{Kip1}$ −/− tubular cells using an inducible expression system restored G_1-phase arrest and the hypertrophic phenotype (62). These findings are clear evidence that $p27^{Kip1}$ is required for ANG II-induced hypertrophy of proximal tubular cells.

There was no increase in $p27^{Kip1}$ mRNA expression, indicating a posttranscriptional mechanism of up-regulated $p27^{Kip1}$ protein (56). As it has been demonstrated in VSMC that ANG II induces ROS (45), and that the concomitant hypertrophy may also depend on this mechanism (38), we further investigated whether similar mechanisms are operative in tubular cells. ANG II stimulates the accumulation of O_2^- in tubular cells (13). Addition of two different antioxidants (*N*-acetylcysteine, Tiron) completely abolished measurable O_2^- concentrations (13). ANG II up-regulates p22phox mRNA expression in proximal tubular cells. Induction of O_2^- generation was transduced by AT_1 receptors and was inhibited by a DPI or p22phox antisense oligonucleotide, indicating involvement of membrane NAD(P)H oxidase (13). ANG II-stimulated hypertrophy was attenuated by DPI, antioxidants, and p22phox antisense oligonucleotides (13). The ANG II-induced expression of $p27^{Kip1}$ protein and cellular hypertrophy were reduced by similar treatments. Generation of O_2^- by xanthine supplementation also stimulated $p27^{Kip1}$ expression and induced hypertrophy in LLC-PK_1 cells.

What is the link between O_2^- generation and $p27^{Kip1}$ induction? We investigated whether mitogen-activated protein (MAP) kinases may serve as a signal intermediate between ANG II-induced oxidative stress and induction of $p27^{Kip1}$ (14). ANG II induces a biphasic phosphorylation pattern of p44/42 MAP kinase with an early phosphorylation already after 2 min and a later second phosphorylation peak after prolonged incubation (12 h) in cultured proximal tubular cells (14). This phosphorylation of p44/42 MAP kinase causes activation of the enzyme. Exogenous hydrogen peroxide (H_2O_2) also stimulates a biphasic phosphorylation of p44/42 MAP kinase. DPI as well as *N*-acetylcysteine prevents ANG II-induced p44/42 MAP kinase phosphorylation (14). The MAP kinase kinase inhibitor PD98059 completely inhibits ANG II-induced $p27^{Kip1}$ expression and cell hypertrophy. However, PD98059 did not attenuate ANG II-stimulated intracellular synthesis of oxygen radicals. Transient transfection with p44/42 MAP kinase antisense, but not sense, phosphorothioate-modified oligonucleotides also attenuates ANG II-induced MAP kinase phosphorylation, $p27^{Kip1}$ expression, and hypertrophy. Furthermore, induction of $p27^{Kip1}$ by H_2O_2 was also abolished in the presence of PD98059. Although ANG II induces phosphorylation of the stress-activated p38 MAP kinase, inhibition of this enzyme with SB203580 failed to attenuate induced $p27^{Kip1}$ expression and hypertrophy. These data provide evidence that ANG II-mediated oxygen stress leads to the phosphorylation of p44/42 MAP kinase in proximal tubular cells (14). Accordingly, we consequently asked if p44/42 MAP kinase may in turn directly phosphorylate $p27^{Kip1}$. Active p42 MAP kinase directly phosphorylates recombinant $p27^{Kip1}$ *in vitro* (63). In parallel, p44/42 MAP kinase immunoprecipitated from tubular cells phosphorylates recombinant $p27^{Kip1}$. Further proof that p44/42 MAP kinase directly phosphorylates $p27^{Kip1}$ was provided by phospho amino acid mapping (63). We mutated three potential MAP kinase phosphorylation sites of $p27^{Kip1}$, and showed that changing the Ser^{178} to alanine prevented $p27^{Kip1}$ expression (63). Functional studies showed that Ser^{178} mutation, in contrast to wild-type or Ser^{10}, and Thr^{187} mutations failed to promote hypertrophy, showing the role for specific protein phosphorylation in mediating the effect of ANG II on cell hypertrophy.

Atrial natriuretic peptide (ANP) modulates ANG II-induced tubular hypertrophy by specifically interfering with this signal transduction pathway (15). ANP and its fragments 3–28 and 4–27 prevent ANG II-induced cell-cycle arrest. ANP inhibits >80% of ANG II-induced $p27^{Kip1}$ protein expression in cultured proximal tubular cells. ANP stimulates expression of MKP-1, a phosphatase involved in dephosphorylation of p44/42 MAP kinase, up to 12 h (15). It also prevents the ANG II-mediated second phosphorylation peak of MAP kinase after 12 h of stimulation. 8-Bromo-cyclic GMP mimicked all the effects of ANP (15). The effect of ANP on ANG II-mediated hypertrophy of LLC-PK_1 cells is regulated on the level of MAP kinase phosphorylation, a key step in the induction of $p27^{Kip1}$. ANP does not attenuate ANG II-stimulated ROS generation.

An outline of the signal transduction pathway is given in Fig. 1. Although primarily derived from cell-culture studies, similar mechanisms are operative *in vivo* (60). Infusion of

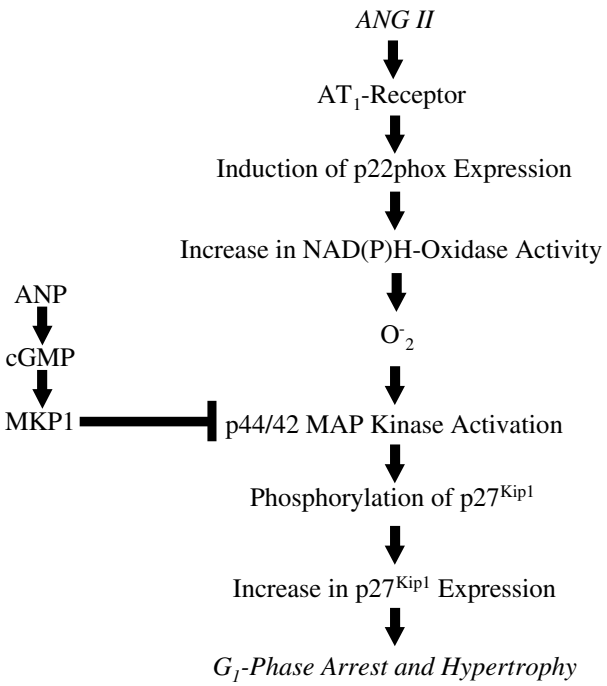

FIG. 1. Overview of ANG II-induced tubular hypertrophy. After binding to AT_1 receptors, ANG II induces up-regulation of the NAD(P)H oxidase subunit p22phox. The increase in O_2^- generation leads to activation of p44/42 MAP kinase. This kinase directly phosphorylates $p27^{Kip1}$, increasing its half-life time. Cells are then arrested in the G_1 phase and undergo hypertrophy. ANP, through increases in cyclic GMP (cGMP), up-regulates the phosphatase MKP1, which in turn dephosphorylates p44/42 MAP kinase. As a result, $p27^{Kip1}$ expression is not increased and ANG II-induced hypertrophy is prevented.

ANG II into naive rats [rate of 250 ng/min into male Wistar rats (body weight, 200 g)] for 7 days increases formation of O_2^- in tubular cells. Furthermore, ANG II infusion stimulates protein expression of $p27^{Kip1}$ (Fig. 2). Infusion of ANG II concomitantly reduces tubular proliferation, indicating G_1-phase arrest. Immunoprecipitation experiments revealed that the increased $p27^{Kip1}$ protein associates with CDK 2 (60). Coadministration of the radical scavenger dimethylthiourea abolished this ANG II-mediated $p27^{Kip1}$ expression without reducing systemic blood pressure. Dimethylthiourea infusion attenuates the ANG II-mediated G_1-phase arrest of tubular cells (60). However, infusion of norepinephrine did not induce O_2^- or $p27^{Kip1}$ expression, despite a significant increase in blood pressure (60). Thus, similar to observations in cell-culture systems, ANG II induces $p27^{Kip1}$ expression in renal tubular cells *in vivo*. This effect is mediated by O_2^-. As tubular hypertrophy depends on G_1-phase arrest and may promote subsequent development of interstitial fibrosis, administering oxygen radical scavenger may be a therapeutic tool to counteract ANG II-dependent remodeling of renal tubular cells.

Another signal transduction pathway between ANG II and ROS has been described in the regulation of the protein kinase Akt in mesangial cells (10). In these cells, ANG II induces activation of Akt. Antioxidants (*N*-acetylcysteine, DPI) inhibited ANG II-induced Akt activation and hypertrophy of mesangial cells (10). However, Akt phosphorylates $p27^{Kip1}$ and impairs thereby the nuclear import of $p27^{Kip1}$, opposes $p27^{Kip1}$-mediated G_1-phase arrest, and would facilitate proliferation and not hypertrophy (28). Therefore, ANG II-induced Akt phosphorylation through ROS as signal intermediates and subsequent hypertrophy could not be mediated by increased $p27^{Kip1}$ expression. Yet an alternative explanation of how this pathway contributes to hypertrophy has been recently discovered by the observation that ANG II-induced ROS formation influences translation initiation processes in VSMC (36). The limiting step for protein synthesis that must be stimulated in hypertrophy is translation initiation. This step is critically dependent on phosphorylation of the PHAS-I (insulin-responsive protein, heat- and acid-stable)–eIF4E (eukaryotic initiation factor 4E) complex in which phosphorylation of PHAS-I releases eIF4E, which forms an active complex with eIF4G (36). ANG II-induced phosphorylation of Ser^{65} of PHAS-I is ROS-dependent and is mediated by Akt (36). Thus, ANG II-stimulated ROS contribute through Akt to cellular hypertrophy by increasing the initial steps of protein synthesis.

ANG II-MEDIATED INHIBITION OF GROWTH, INDUCTION OF DIFFERENTIATION, AND APOPTOSIS: ROLE OF THE AT_2 RECEPTOR

Activation of the AT_2 receptor has different effects on growth depending on tissue type and presumably other cofactors (Fig. 3). The majority of studies, as described above, demonstrate that growth stimulatory effects (proliferation or hypertrophy) of ANG II are transmitted through the AT_1 receptor. Some interesting recent data suggest that AT_2-receptor engagement may play a role in cardiac hypertrophy (23, 40). Targeted deletion of the mouse AT_2 receptor prevented left ventricular hypertrophy resulting from pressure overload induced by aortic constriction (40). In addition, ventricular hypertrophy and cardiac fibrosis induced by chronic ANG II infusion were also abolished in these AT_2-receptor knockout mice (23). However, in many other studies, ANG II-dependent cardiac hypertrophy was transduced by AT_1 receptors (3, 25), and it is not entirely clear whether the data obtained from AT_2-receptor knockout mice may result from deletion of other growth-modifying genes.

ANG II could induce apoptosis of renal cells under certain conditions (64, 66). Tubular apoptosis is a prominent feature of diabetic Ren-2 rats that overexpressed renin and ANG II (4). ANG II induces apoptosis in cultured tubular cells, as well as podocytes in some studies (66), but this is not a general finding (62). Some data suggest that ROS are an important mediator of ANG II-induced apoptosis (47). For example, Tanaka and co-workers have shown that hypoxia and reoxygenation induce apoptosis in cultured glomerular endothelial cells (47). This response was associated with a decrease in Bcl2 expression, but an increase in Bax mRNA transcripts accompanied by translocation of Bax protein from the cytosol to mitochondria. Such a change in the Bcl2/Bax ratio is an important prerequisite for apoptosis (33). Moreover,

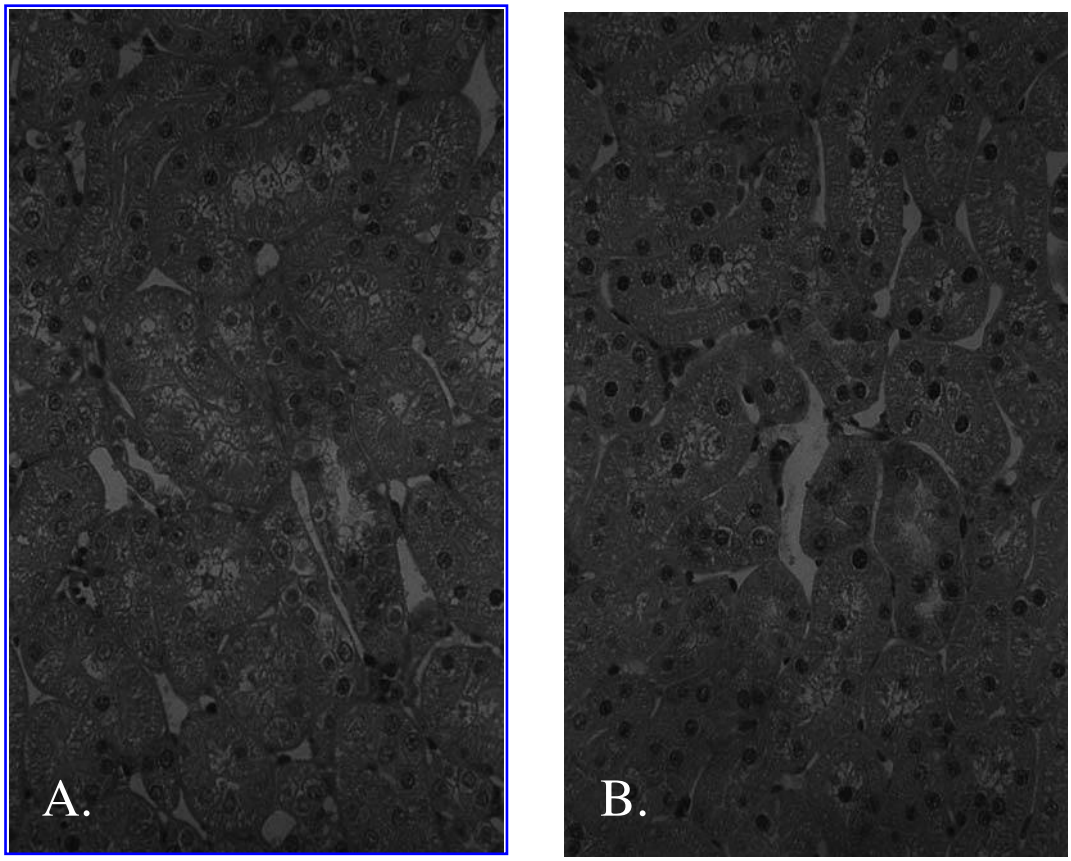

FIG. 2. ANG II stimulates tubular $p27^{Kip1}$ expression *in vivo*. Rats were infused with either solvent (phosphate-buffered saline; **A**) or ANG II (**B**) for 7 days using osmotic minipumps. ANG II infusion increased immunohistochemical staining for $p27^{Kip1}$. This effect was independent of hypertension because norepinephrine infusion failed to increase $p27^{Kip1}$ despite a similar increase in blood pressure (not shown).

treatment of endothelial cells with H_2O_2 up-regulated Bax and induced apoptosis, indicating that ROS, presumably generated in mitochondria, are important mediators (47). Overexpression of dominant-negative hypoxia-inducible factor-1α (HIF-1α) abrogated Bax expression and apoptosis, suggesting that HIF-1α is an upstream regulator of Bax (46).

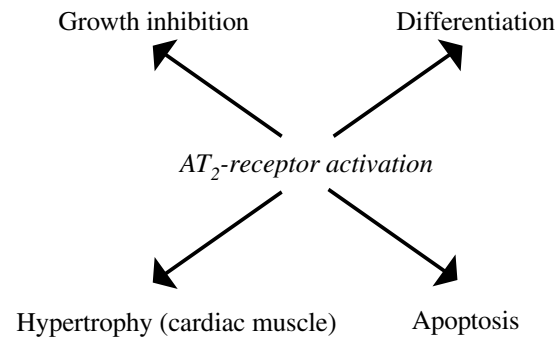

FIG. 3. Overview of the different growth modulatory effects transduced through AT_2 receptor. Why activation of AT_2 receptors causes in one tissue apoptosis and in another hypertrophy is not well understood, but the presence of other growth factors is presumably important.

Accumulating evidence suggests that cell-cycle proteins have also a critical role in apoptosis. Studies by Shankland's group revealed that there was a marked increase in glomerular cell apoptosis in nephritic $p27^{Kip1}$ −/− mice compared with nephritic $p27^{Kip1}$ +/+ controls (32). In addition, apoptosis was also increased in $p27^{Kip1}$ −/− mesangial cells in culture when apoptosis was induced by survival factor deprivation or by exposing cells to cycloheximide, and reconstituting $p27^{Kip1}$ levels by transfection of rescued $p27^{Kip1}$ −/− cells from apoptosis (19). Apoptosis was also increased in tubulointerstitial cells following obstruction in $p27^{Kip1}$ −/− mice compared with control $p27^{Kip1}$ +/+ mice (20). In apoptotic $p27^{Kip1}$ −/− mesangial cells, cyclin A-CDK 2 was also activated without a preceding increase in CDK 2/cyclin E activity, suggesting that uncoupling of CDK 2 activity from the scheduled sequence of cell-cycle protein expression may lead to an inappropriate and premature initiation of G_1/S-phase transition, causing the cell to respond by apoptosis, rather than proliferation (20).

It has been suggested that activation of AT_2 receptors is responsible for proapoptotic effects of ANG II (64), but this issue remains controversial because AT_1-receptor antagonists also attenuated apoptosis in certain models (7). A direct formation of ROS after activation of the AT_2 receptor has so far not been demonstrated, making AT_1-receptor stimulation nec-

essary if one assumes a key role of ROS in apoptosis. Moreover, overexpression of AT_2 receptors in myocardium did not increase ANG II-mediated apoptosis (44). A potential indirect link between AT_2 receptors and apoptosis has been found in humans with congenital anomalies of the kidney and urinary tract, called CAKUT (22, 31). There was a close positive association between patients with CAKUT and mutations of the AT_2-receptor gene on the X chromosome (22, 31). As delayed apoptosis of the mesenchyme during renal organogenesis presumably hinders the normal development of the kidneys and urinary tract (21), impaired function of the AT_2 receptor may explain these congenital abnormalities.

However, it appears that ANG II-induced apoptosis is not a general finding. For example, we found no evidence of apoptosis in primary cultures of tubular cells exposed to ANG II (9). AT_1-receptor blocker treatment of rats with unilateral ureteral obstruction also failed to modulate apoptosis in the obstructed kidney (9). Why ANG II induces apoptosis only in certain circumstances is not known, but the presence of other growth or surviving factors is presumably important for this response.

ANG II exhibited antiproliferative effects without causing apoptosis in PC12 cells, a pheochromocytoma cell line, as well as in neuronal cells that exclusively express AT_2 receptors (29, 30, 61). Antiproliferative effects of ANG II in PC12 cells are not associated with apoptosis, but rather represent a differentiation process (30). To better identify genes involved in the antimitogenic actions of ANG II, we performed differential display analysis of PC12 cells after challenge with ANG II (61). One identified gene selected for further study that was down-regulated by ANG II in PC12 cells was SM-20 (61). ANG II suppresses mRNA expression of SM-20 in PC12 cells already after 30 min as detected by northern blotting. This effect was antagonized by an AT_2-receptor blocker, but not by losartan. SM-20 transcripts were also reduced by ANG II acting on AT_2 receptors in rat glomerular endothelial cells that express both AT_1 and AT_2 receptors (61). SM-20 antisense, but not sense, phosphothioate-modified oligonucleotides reduced baseline proliferation of PC12 cells. In contrast, inducible overexpression of SM-20 prevents the antiproliferative effects of ANG II in PC12 cells. Recent findings show a homology of SM-20 with enzymes involved in the regulation of HIF-1. SM-20 is a mammalian homologue to the EGL-9 gene of *C. elegans* (8). EGL-9 is a dioxygenase that regulates HIF-1α by prolyl hydroxylation (8). EGL-9 is an oxygen sensor, and induction of this enzyme leads to prolyl hydroxylation of HIF with subsequent degradation of HIF-1 by a multiprotein complex with the von Hipple–Lindau tumor-suppressor protein as an important component (Fig. 4). HIF-1 is a transcription factor that activates a wide variety of target genes involved in growth and differentiation (21). It has been shown that HIF-1 is expressed in normoxic conditions in many tissues, suggesting an important role in normal cell homeostasis (21). Thus, one could speculate that ANG II may induce HIF-1 through down-regulation of SM-20. Indeed, we have obtained preliminary evidence that ANG II upregulates HIF-1 in PC12 cells (Fig. 5). Moreover, other proteins such as zinc finger homoeodomain enhancer have been identified that play a role in ANG II-induced differentiation through AT_2-receptor activation (42).

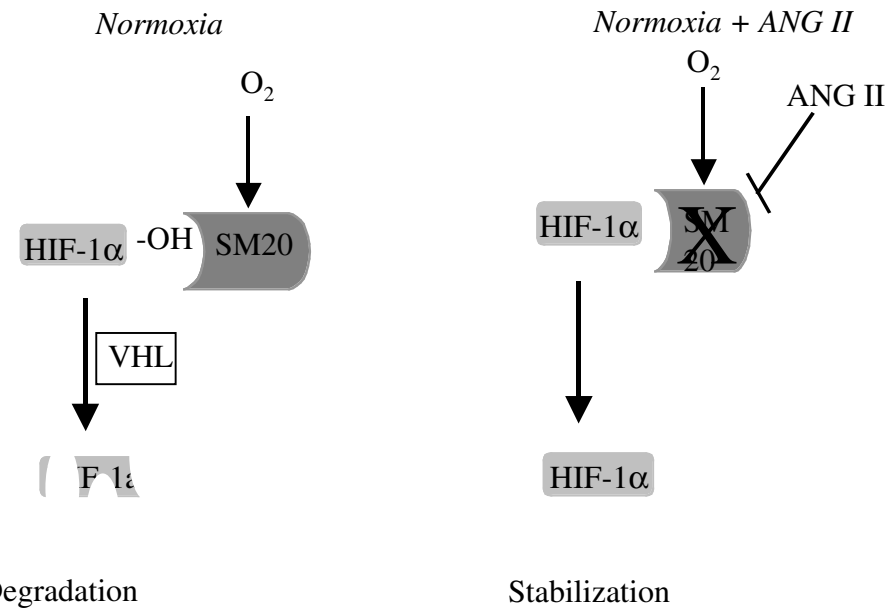

FIG. 4. Regulation of HIF-1α expression. HIF-1α steady-state levels are regulated by prolyl hydroxylation that controls interaction with the von Hipple–Lindau tumor-suppressor protein (VHL) and ultimately HIF-1α degradation by the proteasome. Under normoxic conditions, dioxygenases including SM-20 are activated. HIF-1α is hydroxylated and destroyed. In contrast, under hypoxic conditions, these dioxygenases are down-regulated, leading to the absence of hydroxylation and stabilization. We have shown that ANG II leads to a suppression of SM-20, an important dioxygenase, resulting in stabilization of HIF-1α even under normoxia.

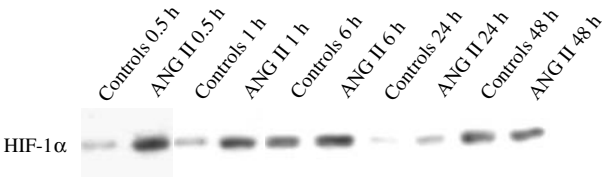

FIG. 5. Western blot for HIF1-α expression. PC12 cells, a rat pheochromocytoma cell line that exclusively expresses AT_2 receptor, were treated for different time periods with a single dose of 10^{-7} *M* ANG II. ANG II challenge up-regulates HIF-1α expression already after 0.5 h, suggesting a posttranscriptional mechanism. Induction of HIF-1α protein by ANG II was detected up to 24 h.

PERSPECTIVES

Depending on the target cell and presence of other growth factors, ANG II may either mediate growth stimulatory effects (*e.g.,* hypertrophy), induce apoptosis, or induce differentiation. Why ANG II has such different effects is incompletely understood and could be only partially explained by activation of different receptor subtypes and distinct signal transduction systems. Nevertheless, ROS are important signal intermediates in transducing ANG II-induced hypertrophy. In addition, the relationship between HIF-1 expression that is induced in hypoxia and ANG II-mediated down-regulation of HIF-1-regulating enzymes suggests an even more complex network between the vasoactive peptide and oxygen metabolism.

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ABBREVIATIONS

ANG II, angiotensin II; ANP, atrial natriuretic peptide; AT_1 and AT_2 receptors, angiotensin II type 1 and 2 receptors, respectively; CAKUT, congenital anomalies of the kidney and urinary tract; CDK, cyclin-dependent kinase; DPI, diphenyleneiodonium; EGF, epidermal growth factor; eIF4E, eukaryotic initiation factor 4E; HIF, hypoxia-inducible factor; H_2O_2, hydrogen peroxide; 2K-1C, two kidneys–one clip; MAP kinase, mitogen-activated protein kinase; NO, nitric oxide; O_2^-, superoxide anion; PHAS-I, insulin-responsive protein, heat- and acid-stable; ROS, reactive oxygen species; VSMC, vascular smooth muscle cells.

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