The effect of Angioteinsin II receptor type 1 and type 2, in Mediated Overproduction of Reactive Oxygen Specie s, Pro-inflammatory, and Pro-fibrotic actions in cardiovascular cells, Leukocytes, and Monocyte and as a Potential Modulatory in Thrombosis, Cancer and Autoimmunity Treatment and Prevention

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Abstract

Renin Angiotensin system (RAS) regulates multiple physiological, pharmacological and pathological functions throughout the body by interacting with angioteinsinII (AngII) receptor type 1 (AT1R) and Ang II receptor type 2 (AT2R).

However, Ang II is being responsible for damaging several organs like kidney, heart, adrenal cortex, smooth muscles, brain, pancreas, endothelial function and liver. Ang II-induced AT1R activation via Gq/11 stimulates phospholipases A2, C, and D, and activates inositol trisphosphate/Ca2 signaling, protein kinase C isoforms, and MAPKs, as well as several tyrosine kinases (Pyk2, Src, Tyk2, FAK), scaffold proteins (G protein-coupled receptor kinase-interacting protein 1, p130Cas, paxillin, vinculin), receptor tyrosine kinases, and the nuclear factor-kB pathway.

The AT1R also signals via Gi/o and G11/12 and stimulates G protein-independent signaling pathways, such as-arrestin-mediated MAPK activation and the Jak/STAT. Many of the deleterious actions of AT1R activation are initiated by locally generated, rather than circulating, Ang II and are concomitant with the harmful effects of aldosterone in the cardiovascular system.

AT1R-mediated overproduction of reactive oxygen species has potent growth promoting, proinflammatory, and profibrotic actions by exerting positive feedback effects that amplify its signaling in cardiovascular cells, leukocytes, and monocytes.

In contrast, mitogen activated protein kinase (MAPK) signal transduction pathways are normally observed everywhere and extremely regulated process for a eukaryotic cell cycle. Like Ang II, MAPK also contributes several physiological and pathological functions once it is activated. Moreover, MAPK cascade is also considered as a key signaling pathway that strictly controls various stimulated cellular processes, including proliferation, differentiation, inflammation, fibrosis and apoptosis.

Studies demonstrated that how Ang II is responsible for MAPK activation and its further consequences in cytoplasm and nucleus for regulation of several inflammatory and pro-inflammatory cytokines.

In this article, I discuss Angiotensin-(1–12), Ang-(1–7) Metabolism, Angiotensin II and MAPK, Ang II and MAPK in hepatic free radicals generation, Ang II and MAPK in diabetic, Ang II and MAKP in hepatic inflammation, Angiotensin II as a New Potential Therapeutic Target, Angiotensin II and the Renin-angiotensin System

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Key Word: Angiotensin II, MAPK, Metabolism, Free Radicals, Diabetes, Hepatic Inflammation, Cancer, and Autoimmunity

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1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 (AT1) receptor antagonists (ARBs) are widely prescribed antihypertensive agents (1),(2). Contemporary guidelines recommend these drug classes for patients with uncomplicated hypertension, heart failure, previous myocardial infarction, chronic kidney disease, or diabetes (3),(4). However, the results of a recent metaanalysis suggest, largely on the basis of indirect comparisons, that ARBs and ACE inhibitors may not be equivalent in terms of their ability to reduce cardiovascular morbidity and cardiovascular mortality and total mortality (5),(6),(7),(8). Pooled analyses of placebo-controlled and active comparator randomized controlled trials of ACE inhibitors have demonstrated reductions in overall mortality, cardiovascular mortality, and myocardial infarction (5),(6). In contrast, meta-analyses of ARB trials indicate reductions in the risk of stroke but an unchanged or potentially increased risk for myocardial infarction (5),(8),(9). It has been suggested that differences in the pharmacology of ACE inhibitors and ARBs may explain these differences in risk reduction. (6),(10). Contrasting with this body of evidence are the results of the Ongoing Telmisartan Alone and in Combination with Ramipril Global Endpoint Trial (ONTARGET) randomized controlled trial, which enrolled patients at high cardiovascular risk and included a comparison between 8576 patients treated with ramipril and 8542 patients treated with telmisartan.11 This trial reported no difference in terms of the incidence of the primary composite endpoint of cardiovascular death, myocardial infarction, stroke, and heart failure hospitalization between ramipril (16.5%) and telmisartan (16.7%; hazard ratio [HR], 1.01; 95% confidence interval [CI], 0.94–1.09) (11).The objective of this retrospective cohort study was to further explore these discrepant findings in a large clinical registry of patients with diabetes. We focused on patients with diabetes because they constitute a group at high risk for morbidity and mortality. Specifically, we compared mortality and hospitalization outcomes between incident ACE inhibitor users and ARB users in this patient population. In this article I discuss the recognition of Ang II's pathogenic actions is leading to novel clinical applications of angiotensin-converting enzyme inhibitors and AT1R antagonists, in addition to their established therapeutic actions in essential hypertension, diabetics, hepatic free radicals generation, Cancer and Autoimmunity.

2. Sources of Angiotensin-(1–7)

2.1. Endo-peptidases

Angiotensinogen, aglycosylated protein that is primarily synthesized and secreted by the liver as well as other tissues is the sole precursor for angiotensin peptides (12). The only known substrate for the as partyl protease renin is angiotensinogen which is releases the decapeptide Ang I from the amino-terminal portion of the protein. AngI is then cleaved by ACE to form the bioac- tive peptide AngII. Early studies revealed that endogenous levels of both AngI and Ang-

(1–7)were markedly increased following the administration of ACE inhibitors (13),(14). The augmented response in Ang-(1–7) suggested that the circulating peptide may contribute to the beneficial actions of the inhibition of ACE pathway in addition to that of reducing endogenous levels of AngII. The increase in Ang-(1–7)in the presence of ACE blockadeneces- sitatesa processing pathway in dependent of the formation of Ang II. Several studies subsequently showed that the endopeptidase 3.4.24.11 (neprilysin) contributed to the circulating levels of Ang- (1–7) in animals chronically treated with various ACE inhibitors (15),(16),(17, 18). AngI infusion in normotensive WKY and hypertensive spontaneously hypertensive rat (SHR) treated with the ACE inhibitor lisinopril resulted in higher plasma levels of Ang-(1–7) and co-administration of then eprilysin inhibitor SCH39370 but not the prolyloligopeptidase (POP)inhibitor *z*-prolylprolinal abolished the circulating levels of the peptide (19). Moreover, acute infusion of a similar dose of AngII did not increase circulating Ang-(1–7) in either control or lisinopril-treated WKY and SHR. The increase in Ang-(1–7) following ACE blockade reflects both a reduction in Ang-(1–7) metabolism and alternative processing of AngI through tissue-specific endopeptidases (15). Inthis regard, Pereira etal. Recently demonstrated that the endopeptidase EC3.4.24.15 (thimetoligopeptidase) may contribute to formation of Ang-(1–7) in the rat hippocampus (20). Interestingly, these investigators reported higher expression of this peptidase and the Masreceptor in a rat model of temporal lobe epilepsy suggesting a possible role of the Ang-(1–7)-Masaxis in this central pathology (20). Indeed, the study supports earlier reports of the direct processing of AngI to Ang-(1–7) by thimetoligo peptidase in vascular smooth muscle cells and arathindlimb perfusion system (21),(22).

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2.2. ACE2

Apart from endopeptidases that process Ang I or Ang-(1–12) to Ang-(1–7), various mono-carboxy peptidases including prolyl carboxy peptidase (PCP), POP, and the ACE homolog ACE2 generate Ang-(1–7) directly from Ang II. Its hould be emphasized that PCP requires anacidic pH optimum for activity, but may contribute tolysosomal pathways for metabolism of internalized Ang II or to the processing of AngII to Ang-(1–7) in urine (23). ACE2 continues to be of primary focus given its ability to effectively metabolize AngII and generate Ang-(1–7) (24),(25). Renin cleaves the precursor protein angiotensinogen to angiotensin-(1–10) (AngI) which is further processed to the biologically active peptides Ang-(1–8) (AngII) by angiotensin converting enzyme (ACE) and Ang-(1–7) by endopeptidases such as neprilysin (NEP). AngII under goes further processing at the carboxyl terminus by the carboxypeptidase ACE2 to yield Ang-(1–7)(Ang7).Ang-(1–7)under goes decarboxylation (DC) of the aspartic acidresidue to form Ala1-Ang-(1–7)(Ala1-Ang 7).The dodeca peptide Ang-(1–12)is derived from the hydrolysis of the Tyr12-Tyr13 bond of rat angiotensinogen by an unknown enzymatic pathway. AngII recognizes both AT1 and AT2 receptors. Ang-(1–7) activates the Mas receptor and Ala1-Ang-(1–7) recognizes the Mas-D related receptor (Mrg). 100-fold higher than that of PC or POP; in this regard, the soluble form of ACE2 has been utilized as a therapeutic agent to reduce blood pressure and attenuate target organ damage in hypertensive and diabetic animal models (26),(27),(28),(29). ACE2 mRNA expression was increased in the brain medulla following long-

term AT1-receptor blockade (30). It is unclear whether the beneficial effects of ACE2 administration reflect the reduction in Ang II, the enhanced for- mation of Ang-(1–7) or the increased ratio of Ang-(1–7) toAng II. Moreover, Turner and colleagues report that soluble ACE2 attenu- ated the integrin-dependent stimulation of focal adhesion kinase (FAK) and increased the expression of the Akt kinase suggest- ing the peptidase may have direct cellular effects apart from its peptidase activity (31). In addition to the functional role of ACE2 that catalyzes the conversion of Ang II to Ang-(1–7), the peptidase may serve as a biomarker of renal and cardiac pathologies. Two studies in type I (streptozotocin-induced) and type II (db/db mice) dia- betic models reported an early increase in the urinary excretion of ACE2 (32),(33). The enhanced excretion of ACE2 in db/db mice closely correlated to the increase in albuminuria or proteinuria. Moreover, chronic treatmentwith insulin-sensitizing agent rosigli- tazone improved the metabolic balance in the db/db mice and reduced the excretion of both ACE2 and albumin (32). In con- trast to the reduction in urinary levels of ACE2, the increased renal expression of ACE2 in the db/db mouse was not altered by rosiglitazone which may reflect an added therapeutic benefit to maintain the peptidase in the diabetic kidney (32). An important aspect of the two latter studies suggests that in the diabetic kid- ney, the development of tissue injury should not necessarily be interpreted as arising from a deficit in ACE2 expression. Indeed, the increase in tissue and urinary levels of ACE2 in pathologi- cal conditions may reflect a compensatory response to alter the balance of Ang II and Ang-(1–7) pathways within a particular tissue or cell type (7). In this regard, the deleterious effects of an

ACE2 inhibitor or knock down of the enzyme may be particularly evident under conditions of enhanced ACE2 expression. The circulating levels of ACE2, which are typically low to not detectable, are also increased in experimental conditions of diabetes. We show in a model of diabetic hypertension that circulating ACE2 activity increased over five fold in female mRen2. Lewis rats (34). However, serum ACE activity also increased suggesting that the potential beneficial effects of higher ACE2 may be offset by ACE acting to increase AngII and metabolize Ang-(1–7).Indeed, plasma levels of Ang-(1–7) were not changed in the diabeticmRen2.Lewis despite them arked increase in ACE2 activity. Moreover, circulating ACE activity was substantially higher than that of ACE2 when assessed under similar incubation and substrate conditions for each enzyme (34). In the db/db mice, in fusion of exogenous ACE2 that markedly increased serum levels of the enzyme did not alter urinaryACE2 suggesting that the enzyme is not readily filtered by the glomerulus (33). One mechanism for the increase in urinary excretion of ACE2 is the regulated shedding of the enzyme from the apical face of the proximal tubules. Studies by Lambert and colleagues originally reported that the disintegrin and metalloproteinase (ADAM17) secretase was responsible for the release of ACE2 (35). A subsequent report identified a specific sequence of the juxta membrane stalk of ACE2 hydrolyzed that was by ADAM17 to release the peptidase from human pulmonary epithelial cells (36). In proximal epithelial cells of the db/db mouse kidney, there was extensive overlap of ACE2 and ADAM17 immuno staining (32). Moreover,rosiglitazone treatment at tenuated ADAM17 expression which may contribute to the reduced shedding of ACE2 in to the tubular

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fluid and subsequent excretion in the urine. In addition to the shedding ofACE2, ADAM17 may influence tissue damage by the release of the tethered inflammatory factors TNFa, EGF, and TGFa that subsequently activate their respective receptors in an autocrine or paracrine manner(37). IfexpressionofACE2on the apical membrane of the tubules contributes to the regulation of the local concentrations of Ang II,an increase in ADAM17 may lead to inflammatory and fibrotic events through enhanced AngII-AT1-receptor signaling, as well as increased cytokine and growth factor activation. Lazartigues and colleagues reportthatknockdownofADAM17inthebrainofDOCA-salt mice reduced blood pressure, and increased the tissue expression of ACE2 (37). In this model of neurogenic hypertension, the ben-efitofADAM17knockdownmayreflectareductionofAngII in brain ;however, the direct effects on the release of EGF and other cytokines cannot .Indeed, the transactivation of the EGF receptor (EGFR) and signaling pathways is a key signaling event of the AngII-AT1-receptorpathway(38). Theincreased sheddingofACE2mayalsoreducelevelsofAng-(1–7) and attenuate the inhibitory actions on the AngII-AT1-receptor axis and other pro-inflammatory and profibrotic pathways. Akhtar et al. Recently reported that Ang-(1–7) attenuated EGFR activation in response to Ang II, as well as reduced the extent of renalinjury in the diabetic SHR (39). Moreover, increasing evidence suggests that one of the primary pathways activated by Ang-(1–7) is the stimulation of various cellular phosphatases (PTP) including SHP-1 and DUSP-1 that may attenuate activated kinase-dependent pathways (40),(41),(42), (43),(44).

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3. Angiotensin-(1–12)

Nagata and colleagues identified a novel endogenous angiotensin peptide termed Ang-(1–12) that contains the first 12 amino acids of the N-terminal sequence of rat angiotensinogen(Asp1-Arg2- Val3-Tyr4-Ile5-His6-Pro7-Phe8-His9-Leu10-Leu11-Tyr12)(45). These investigators developed antibodies directed to the amino-and carboxyl-terminal sequences of Ang-(1–12) and demonstrated expression of Ang-(1–12) in essentially all tissues that contain AngII with the highest levels in the intestine, brain, heart, plasma, and kidney of rat. Differential expression of Ang- (1–12) was evident in the heart and kidney of the SHR and the normotensive control Wistar Kyotostrain (WKY) (55). Ananti- body specific to the C-terminals equence of rat Ang-(1–12) includ- ing Leu11-Tyr12 revealed selective staining in cardiacmyocytes and proximal tubule cells of the kidney.The site of hydrolysis for formation of Ang-(1–12) from rat angiotensinogen occurs at residuesTyr12-Tyr13 which is distinct from the Leu10-Leu11 sequence cleaved by renin to form AngI. Thus, the generation of Ang-(1–12) is likely through an on-ren independent pathway and maybe apparent in condition so flow or suppressed renin activity, particularly with the use of selective renin in hibitors. Similar to AngI, Ang-(1–12)can be hydrolyzed at the Phe8-His9 bond by ACE or chymaseto form AngII (45),(46),(47). The conversion of Ang-(1–12) to Ang II by ACE in the circulation is consistent with the acute increase in blood pressure following an infusion of Ang-(1–12) in normotensive rats, as well as the blockade of the pressor response by either ran ACE inhibitor or AT1-receptor antagonist. Arnol detal. Also find that central Ang-(1–12) administration attenuated baro reflexsen sitivity and the response was blocked by either an ACE inhibitor or AT1-receptor antagonist (47). Moreover, neutralization of

Ang-(1–12)by intra-cerebro-ventricular (ICV)infusion of anaffinity-purified antibody reduced blood pressure in the (mRen27)2 hypertensive rats consistent with the biochemical and immunocytochemical evidence for Ang-(1– 12) in the rat brain(48). To our knowledge, the latter study by Isa and colleagues is the only report to date that demonstrates an endogenous role for Ang-(1–12). As to the Ang-(1–7) axis, we recently demonstrated that Ang- (1–12) may be an alternative substrate for the generation Ang- (1–7) in the kidney(49). Isolated cortical membranes from the kidney of the hypertensive mRen2.Lewis rat processed Ang-(1– 12) to Ang-(1–7) and Ang-(1–4).We observed a similar pattern of metabolism using the recombinant forms of mouse and human neprilysin. The selective neprilysin inhibitor SCH39370 abolished the formation of Ang-(1–7). We noted a peak corresponding to Ang I in the processing of Ang-(1–12) by the cortical membranes that was also abolished by the neprilysin inhibitor suggesting the peptide may be an intermediate in the processing of Ang-(1–12) to Ang-(1–7)(49). In these studies, we also show that circulat- ing or renal renin did not metabolize Ang-(1–12) particularly in the presence of the ACE inhibitor lisinopril which implies that the peptide lacks the minimal sequence for recognition by renin(49). Bujak-Gizycka and colleagues demonstrated the generation of Ang-(1–12) in rat aorta homogenates by a serine peptidase using Ang-(1–14) as the substrate; however, the extent that this activity will process the angiotensinogen protein to Ang-(1–12) is not currently known(50). We did not detect the conversion of Ang-(1–12) to Ang-(1–7) in serum which would be consistent with the lack of soluble forms of neprilysin in the circulation, nor were there significant levels of Ang-(1–11) suggesting the absence of processing by ACE2 or other carboxypeptidases(49). It is fea- sible that Ang-(1–12) may be a potential substrate for Ang-(1–7) through the initial conversion to Ang-(1–11) by ACE2 and subse- quent processing to Ang-(1–9) and Ang-(1–7) by ACE. However, ACE activity is far higher in the circulation than ACE2 and Ang- (1–7) formation from Ang-(1–12) or Ang I more likely reflects endopeptidase activity. Although further studies are required to discern the endogenous pathways for the formation and process- ing of Ang-(1–12), the peptide constitutes a potential substrate for the conversion to either the active products Ang II or Ang-(1–7).

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4. Ala1-Angiotensin-(1–7) ANDPro1-Glu2-Ang II

In addition to the precursors to Ang-(1–7),the peptide itself may serve as a precursor tootheractive forms. Santosand colleagues recently identified an endogenous analog of Ang-(1–7) in which the as partic acid residue was decarboxylated to alanine (Ala)formingAla1-Ang-(1–7)(51). TheAla1-Ang-(1– 7) analog (alsotermedalmandine) may also potentially arise from the proteolytic processing of endogenous Ala1-AngII(AngA) byACE2(52). Similar toAng-(1–7),Ala1-Ang-(1–7)induced the relaxation of isolated aortic vessels and chronic infusion of the analog lowered blood pressure. Interestingly, the vascular effects ofAla1-Ang-(1–7) were not blocked by the typical receptor antagonist D-Ala7-Ang-(1–7) (A779) against the Masreceptor, but were attenuated by D-Pro7-Ang-(1–7) and the AT2 receptor antagonist PD123319.This study further showed that Ala7-Ang-(1–7) stimulated the Mas-related receptor (MrgD) and did not interact with the Mas receptor. Identification of Ala1-Ang-(1–7) in the human circulation and in an isolated heart perfusion system was achieved by aHPLC-Mass spectrometry approach. It is

worth noting that the available direct RIA or ELISA assays will not distinguishbetweentheAsp1-andAla1- forms of Ang-(1–7) since both is of or mss hare the identical C-terminals equence that is typically recognized by the immune reactive antibodies. Thus, an initial separation step such as HPLC combined with conventional immune-reactive assays will be required to routinely detectand quantify the different forms of Ang-(1–7) in the circulation and tissues.The potential importance of these findings may reflect the greater diversity of the Ang-(1–7) axis regarding the identification of both a novel ligand and receptor that contributes to vascular tone. Moreover, that the AT2 antagonist PD12319 antagonized the actions of Ala1-Ang-(1–7) at the MrgD receptor may explain the apparent interaction of Ang-(1–7)withtheAT2 receptor notedin several studies (53),(54),(55). Although distinct from either Ang-(1–7) orits Ala analog, Jan kows kietal. Identified another endogenous ligand to the AT7/ Mas receptor in human serum termed angioprotectin (56). This peptide resembles the octa peptide AngII but has substitutions of Pro and Gluat the first two N-terminal residues to form Pro1-Glu2-AngII. Despite the fact that the angio protectin contains both theTyr4 and Phe8 residues considered to be essential to the actions of AngII, the peptide lacked any vasoconstrictor activity in isolated a orticrings. However, the peptide induced a dose-dependent vaso-relaxation of isolated vessels that was absent in vessels from the Mas-knockout mice, as well as acutely reduced blood pressure in the SHR. Moreover, Pro1-Glu2-AngII stimulated NO formation in Mas-transfected CHO cells but notin the control cells. Finally, the study presented evidence for local formation of Pro1-Glu2-AngII from AngII in human endothelial cells that was enhanced by addition of exogenous proline and glutamicacid suggesting a post transcriptional modification of AngII. It is not known to what degree Pro1-Glu2-AngIIis processed by ACE2 or other carboxypeptidases to the Ang-(1–7) analog and whether Pro1-Glu2-Ang-(1–7) is functionally active at the either the Mas or Mrg D receptors. It is also unclear the extent conventional immune-reactive assays for Ang II will detect endogenous Pro1-Glu2-AngII in plasma or tissues given their identical C-terminal sequence. The circulating levels of Pro1- Glu2-AngII were 15% of AngII in humans, but the AngII analog increased fivefold in patients with endstage renal disease that may perhaps reflect a compensatory response in pathological conditions (56).

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5. Ang-(1–7) Metabolism

The endogenous levels of Ang-(1–7) are influenced by access to processing enzymes such as the carboxypeptidaseACE2or the endopeptidases neprilysin, thimet oligopeptidase, andpro-lylendopeptidase (oligopeptidase).The levels of Ang-(1–7)are also dependent on peptidases that metabolizethepeptide. Similar to bradykinin and substance P, ACE plays a significant role in the hydrolysis of Ang-(1–7) to the penta peptide Ang- (1–5) in the circulation and the proximal tubules of the kidney cortex (14),(57). ACE inhibition increased the half-life of Ang-(1–7) six fold in the circulation and is necessary to demonstrate the accumulation of Ang-(1–7) from both AngI-and AngII- dependent pathways in the renal proximal tubules (57),(57). Thus,the mechanism for the increased levels of Ang-(1–7) following ACE inhibitor treatment reflects both pro- tection of the peptide from ACE hydrolysis to Ang-(1–5) and shunting of AngI to Ang-(1–7) through endopeptidase path- ways such as neprilysinorthimet oligopeptidase (15). There is

relatively little information on other peptidases that participate in the metabolism of Ang-(1–7) other than ACE. We recently detected an endopeptidase activity in the cerebrospinal fluid (CSF) of sheep that metabolized Ang-(1–7)at the Tyr4-Ile5 bond to yield Ang-(1–4) and constituted the majority of Ang-(1–7) degrading activityin CSF (58),(59). Although the identity of the peptidase is currently unknown, the activity was insensitive to inhibitors against neprilysin, thimetoligopeptidase, orneurolysin (EC3.4.24.26) (59). The Ang-(1–7)peptidasea ctivity was abolished by the mercury-compounds *p*-chloromercuribenzoate (PCMB) and aminophenyl-mercuriacetate(APMA), as well as the chelating agents *o*-phenanthroline and EDTA, but not the cysteine epoxide inhibitor E-64 suggesting a metallo peptidase-like activity in CSF (59). The regulation of the CSF peptidase is described in the proceeding sectionon fetal programing.

6. Intracellular Ang-(1–7)-Mas Receptor System

The RAS was traditionally view edasanendocrine system where by circulating renin catalyzes an enzymatic cascade tof orm active peptide products; however, it is apparent that multiple tissues contain the necessary components for the local generation of angiotensin peptides(60),(61). These tissue systems may release the precursor angiotensinogen, the intermediate products AngI and Ang-(1–12), or the active peptides AngII and Ang-(1–7) to bind directly to cell surface receptors in an autocrine or paracrine manner.Roberts on and Khairallah reported over 40 years ago the localization of AngII binding ites on the chromatin fraction of vasculars mooth muscle cells and cardiomyocytes suggesting an intracellular site of action for AngII (62). Several laboratories sub- sequently identified AngII receptors using classical receptor bind ing techniques on nuclei isolated from liver(63),(64),(65). Eggena and colleagues demonstrated that AngII stimulated mRNA transcripts for angiotensinogen, renin, and PDGF from isolated liver nuclei suggesting that the nuclear binding sites were functional and capable of directly mediating gene expression (66),(67). Moreover,AT1 receptors were also evident on nuclei isolated from cortical and medullary areas of ther at kidney (68),(69),(70). AngII-AT1-receptor stimulation on isolated renal nuclei increased mRNA expression of angiotensinogen, the sodium-hydrogen exchanger(NHE3) and the cytokine monocytechemoattractant protein(MCP-1)(68). AngII also elicited animmediate increase in calcium by isolated corticalnucleiorviamicro injection of the peptide inintact epithelial cells (71). We find that AngII directly stimulates reactive oxygen species(ROS) as demonstrated by the enhanced fluores-centsig nature of dichlorofluoresce in (DCF);ROSformation was sensitive to the NAD(P) Hoxidase inhibitor diphenylene iodonium (DPI) and the AT1 antagonistlosartan(72). Blockade of phospho inositol3-kinase (PI3K) and proteinkinase C (PKC) abolished the AngII-AT1-receptor-dependent stimulation of ROS in renal nuclei.Inlieu of the nuclear localization of the NAD (P) Hoxidase iso form NOX4,activation of AT1 receptors may acutely stimulate ROS by a PI3K-PKC pathway and subsequent phosphorylation of NOX4(72),(73),(74),(75), The studies demonstrating nuclear AT1 receptors with in the kidney and other tissues clearly support an emerging view for the localization of various G-protein coupled receptors (GPRCs) to the nucleus (76),(77),(78),(79),(80),(81),(82),(83). Inregards to the Ang-(1–7)-Mas receptor system, O'Dowd and colleagues not edacanonical nuclear localiza tion sequence on the Masprotein in

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their studies on AT1-receptor trafficking and localization invascular smooth muscle cells (84). We under took a series of studies to establish an intracellular role for Ang-(1–7) in the cortical tissue and proximal tubules isolated from the sheep kidney.Immunoblotanalysis of nuclei isolated from sheep proximal tubules demonstrated a single immunoreactive band of 35kDa utilizing an affinity-purified antibody against the human Masprote in(85). Receptor binding studies with the non-selective antagonist 125I-(Sarcosine1, Threonine8)-Ang II (Sarthran) revealed significant competition by the AT7/Mas receptor antagonist D-Ala7-Ang-(1–7) in nuclei isolated from the renalcortex.Functional assessment of the nuclear AT7 receptor was then assessed with the sensitive NO fluorophore DAF in the presence or absence of the NO synthase inhibitor L-NAME.Ang- (1–7) dose-dependently increased thefluorescent signature for NO which was abolished by priortreatment with L-NAME or the Ang-(1–7) antagonist,but not antagonists to the AT1 or AT2 recep- tors. Consistent with the stimulation of NO by Ang-(1–7),protein expression for endothelial nitricoxide synthase (eNOS) and soluble guanylatecyclase (sGC) was evident in the isolated nucleiof sheep proximal tubules(85). These data further support previous studies that localized NOS ands GC to liver nuclei, as well as the stimulation of NO and cGMP by activation of the bradykininB2 receptor (79),(86). The exact function of the Ang-(1–7) axis of the RAS with in the nucleusis not known; however, we hypothesize this system may antagonize the intracellular actions of the AngII- AT1-receptor pathway.To address this possibility,we assessed the influence of the selective ACE2 inhibitor MLN4760 and the Mas receptor antagonist on the activation of ROS by AngIIin renal corticalnuclei.The AngII-AT1-receptor dependent increase in ROS was significantly augmented to a similar extent by treatment of nuclei with either the ACE2 inhibitor or the AT7 receptor antag- onist (87). That both MLN4760 and D-Ala7-Ang-(1–7) increased the stimulation of ROS suggests that the conversion toAng-(1–7) by ACE2 antagonizes the actions of the AngII-AT1-receptor axis on the nucleus. It is possible that that simply blocking the degradation of AngII with the ACE2 inhibitor may augment the actions of AngII; however, the comparable effects of the AT7 receptor antago- nist D-Ala7-Ang-(1–7) suggests a distinct role for Ang-(1–7).Since the Ang-(1–7) antagonistis apeptide and may potentially interact with ACE2,we further demonstrated that D-Ala7-Ang-(1–7) does not inhibit nuclear ACE2 activity as assessed by the HPLC- based conversion of AngII to Ang-(1–7).Moreover, our studies suggest that the processing of AngII to Ang-(1–7) by ACE2 on the nuclear membrane leads to the activation of signaling path- ways distinct from that of AngII (87). We donot know,however, whether the attenuation of ROS production by Ang-(1–7) involves the stimulation of NO or other signaling pathways. As previously discussed, Ang-(1–7)may attenuate the actions of AngII and other growth hormones by the activation of intracellular phosphatases such as the dual specificity phosphatasesMKP-1andSHP-1(41),(42). Several classes of phosphatases including MKP-1 traffic to the nucleus; however, it is unknown whether Ang-(1–7)can influence these enzymes to attenuate the actions of Ang II(88). Clearly, one issue regarding the intracellular RAS and other peptidergic systems is the localization of the components with in the cell. The nucleus is composed of two distinct bilayers termed the outer (OMN) and inner (INM) nuclear membranes. Nuclear pore proteins traverse both membrane domains and facilitate transport between the cytosol and the nuclear

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matrix which contains the chromatin-DNA complex. Portions of the ONM are continuous with the endoplasmic reticulum (ER) such that perinuclear spaceis shared with the ER.Then uclear envelope comprising both OMN and INM invaginates in to the nuclear matrix creating anuclear reticulum that is key in the regulated release of nuclear Ca2C (89),(90),(91). Although various studies have localized GPRCs primarily to the nuclear envelope and matrix, it is currently unclear how the peptide ligands target the nuclear GPRCs, as well as the precise coupling of the receptors to their signaling pathways with in the nucleus. Moreover, elucidation of the path-ways that deliver peptide ligands to the irrespective intracellular receptors, as well as the intracellular regulation under normal and pathological conditions has not been established. As to the intra- cellular expression of angiotensins in the kidney, there is evidence for expression and up take of angiotensinogen, as well as the uptake of Ang II and Ang-(1–7)by protein transporters such as megalin (60),(92),(93),(94),(95). In addition, AT1-receptor mediated internalization of Ang II may contribute to the intracellular content of the peptide (60),(78). In this regard, intracellular peptidases such as ACE2 may potentially process the internalized AngII to Ang-(1–7)as alternative pathway to attenuate AT1-receptor activity and stimulate the cellular actions of Ang-(1–7).Utilizing the renal epithelial NRK-52E cell line, we find evidence for the nuclear localization of angiotensinogen, left panels) consistent with earlier findings by Sherrod and colleagues regarding nuclear angiotensinogen in brain astrocytes and isolated nuclei of sheep proximal tubules (85),(96),(97). Interestingly, a second antibody directed to the Ang I sequence of angiotensinogen failed to detect the protein in the nucleus of the NRK-52E cells suggesting that enzymatic processing of the precursor may occur in this compartment (107). In support of an intra-cellular processing pathway, renin expression was also evident in the nucleus of the NRK cells, right panels).Isolated nuclei exhibited both renin and prorenin activity (following activation by trypsin)that was sensitive to the specific renin inhibitor aliskiren, bottom left panel),as well as immune reactive levels of Ang II and Ang-(1–7)(96). In addition, peptide metabolism studies in isolated nuclei revealed the direct conversion of Ang I to Ang-(1–7) that was essentially abolished by a selective inhibitor (CPP)of the metallo endopeptidase thimetoligo peptidase, bottom right panel).Others have reported the nuclear expression of thimetoligopeptidase in brain, as well as the identification of anuclear localization sequence for the human peptidase (98),(99). TheNRK-52E cells may constitute a relevant cell model to establish the pathways that contribute to the intracellular generation and actions of Ang II and Ang-(1–7) with inrenal epithelial cells. As analternative concept to intra- cellular formation, Ibarra and colleagues presented evidence for another model of nuclear signaling where by the plasma membrane invaginates to the peri-nuclear area that facilitates presentation of intracellular signals (IGF receptor coupled to IP3 formation) discretely to the nucleus in cardiomyocytes (100). The apparent advantages of this system may reflect a more selective activation of the signaling cascade and – eration of the peptide ligands. The latter study adds another potential mechanism to the complex pathways of the intracellular receptor system for angiotensins and other peptides, as well as emphasizes the need for additional studies to elucidate their organization and function. In the endeavor to elucidate the intracellular pathways, the importance of robust biochemical and molecular techniques to characterize the

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RAS cannot be overly emphasized. Several reports have raised concerns regarding the specificity of commercial AT1 and AT2 antibodies widely utilized for western immune blot and immunocytochemical distribution studies (101),(102),(103). Importantly, these studies find that receptor protein and sat the appropriate molecular weights were not abolished in AT1- orAT2-deleted cell and tissues amples.We have utilized antibodies to both AT1 and AT2 receptors to establish the ir molecular weight in the nuclear fraction as this pertains to the maturation or processing of the receptor protein. However, studies by our laboratory and others also in corporate peptide binding assays to quantitate receptor density and affinity, as well as various antagonists to identify the receptor subtype. The receptor binding assays also parallel the demonstration of functional signaling pathways (ROS,NO) on nuclei and the sensitivity to receptor antagonists. Reliance on the assessment of mRNA for the receptor may not equate to protein expression and certainly does not reveal the discrete intracellular distribution of the receptor. Antibodies to angiotensin receptors or other RAS components are useful and convenient tools to characterize this system; however, parallel approaches to establish the expression and regulation of the RAS particularly with in the cell are clearly warranted.

7. Angiotensin II and MAPK

RAS is a very wide and important pathway which coordinate several biological functions like regulation of blood pressure, cellular growth, production of extra cellular matrix, stimulation of proinflammatory or inflammatory cytokines and initiates apoptosis if necessary (104). This pathway is generally activated once renin is available inside circulation. Renin, which is a protease enzyme produced from the kidney, converts liver angiotensinogen to angiotensin I. Furthermore, angiotensinI is converted to Angiotensin I and Angiotensin II when eitherpulmonary ACE or tissue chymase or cathepsin are available via G protein-coupled receptor (105). So far, several types of angiotensin (I-XII) have been isolated from various models and those are activated through AT1R and AT2R (106). Angiotensin can be activated in both circulation and tissue due to the availability of its receptors throughout the body (107). It has been also noticed that activation of AT2R often brings some protective effects like reduced inflammation via epoxyeicosatrienoic acid and by inhibiting nuclear factor –κβ (108). Another study explained that Ang II induce arterial pressure can be reduced by the activation of AT2R in rat model (109). A cross study has also been found which explained that deletion of AT2R may protect from dietinduced obesity and insulin resistance in rats (110). On the other hand it is highly established that Ang II-AT1R binding mostly signals through MAPK pathway that promotes cellular growth and inflammation (111). However, MAPK often serves a huge number of fundamental cellular processes to exchange extracellular and intracellular information (112). Within the endoplasmic reticulum (ER), generally three core types of MAPK are found which are MAP3K, MAPKK, and MAPK. Beside this, downstream kinase (MAPKAPK) and upstream kinase (MAP4K) are also noticed. Inside the mammalian MAPK cascade, four different kinds of MAPK were isolated and named as MAPK p38, ERK5, cJun Nterminal kinase (JNK) and extracellular signalregulated kinase 1 and 2 (ERK1/2) respectively (113).

8. Ang II and MAPK in hepatic free radicals generation

Free radicals are highly reactive molecules which directly damage cell membrane, cytoplasm as well as nucleus(114). The prime members of this family are reactive oxygen species and reactive nitrogenous species that hamper the normal cellular activity and produces oxidative stress(115). However, free radical as well as oxidative stress mediated hepatic dysfunction has been noticed enormously by disturbing liver Cytochrome P450 system (116). Oxidative stress markers have also been identified inside the serums who were suffering from chronic hepatitis C (117). Furthermore, hepatic tissue injuries are very common when free radicals like •OH, •O2-, and H2O2 are generated inside liver (118). Although Ang II is mainly responsible for controlling blood pressure, it also significantly generates free radical when it is attached with AT1R (119). Study also suggested that Ang II-AT1R interaction stimulates NAD(P)H oxidase (NOX) and generates ROS and RNS which further stimulates several proinflammatory cytokines (120),(121). Mitogen activate protein kinase also participates in generation of free radicals which is mostly noticed via NAD(P)H oxidase (NOX)4 and AKT1-AKT2 (protein kinase B) pathway (122),(123). Another study explained that activation of p38MAPK was observed in ATP depleted hepatic stellete cells (HSCs) culture. The study also described that p38MAPK depended reactive oxygen species (ROS) declined number of normal HSCs (124),(125). One of the crucial studies explored that reactive oxygen species contribute significantly in development of several cancers by stimulating different cytokines (126).

9. Ang II and MAPK in diabetes

Diabetes, a heterogeneous metabolic disorders which not only affects pancreas but also hampers normal functioning of liver(127). Nonalcoholic liver diseases and diabetes are the two major components of metabolic syndrome(128). Inside the liver, high glucose concentration may alter cellular homeostasis(129), and might induce several pathological events(130). Taken together, the complication of diabetes can activate hepatic Ang II(131), which further induces MAPK family(132), that possesses several inflammatory cytokines like activated protein-1,tumor necrosis factor-α, and nuclear factor-κβ, and many others(133). Inflammatory cytokines further responsible for the development of liver cirrhosis, cancer and liver failure if not treated with care(134). However, chronic diabetic status also triggers collagen and extracellular matrix production in liver which further develop hepatic fibrosis(135),(136).

10. Ang II and MAKP in hepatic inflammation

Biological subjects are always exposed to its surrounding environment, at the same time they also need food and air to survive. Unfortunately, environment carries several foreign harmful elements which often invade inside biological system and activates host immunity(137). On the other hand, liver serves various protective roles by producing several growth factors, antibody and other immune components to fight against harmful stimuli (138) Once those foreign elements invade into hepatic tissue, liver immediately attracts neutrophil, Tcell, local macrophages, βintegrin and natural killer cells(139). The evidences for the role of Ang II and MAPKs in the development of hepatic inflammation**.** It is highly suggested that Angiotensin II often stimulates immune cells by activating kupffer cell to invite monocytes, killer cells, tumor necrosis factor and interleukins(140). Local hepatic renin angiotensin system is also regulated by chronic liver injury which simultaneously activates some events such as recruitment of inflammatory cells and generation of free radicals(141). Angiotensin related hepatic inflammation often showed elevated level of liver marker such as AST, ALP and ALT

which confirms hepatic tissue injury(142). Study also explored that MAPK which is activated by Ang II solely participates in hepatic inflammation(143). Another study disclosed that MAPK-JNKs remarkably serve inflammation(144). A hypothetical mechanism for Ang II and MAPKs mediated inflammatory response. also restored the development and cellular proliferation in developing liver(145). However, these newly developed molecules showed promising results in various in vitro and in vivo assays, their side effects and adverse drug reaction profiles are not established fully. A selective inhibitor of p38 mitogenactivated protein kinase, BIRB796, is such a molecule which activates thenuclear factor (erythroidderived 2) like 2 signaling pathway(146). However, a reactive intermediate of BIRB796 could be found both in mouse and human liver microsomes which is responsible for the development of BIRB796's hepatotoxicity(146).

11. New promising molecules against MAPK family

Researchers always try hard to develop a potent molecule against any kind of pathogenesis. Various molecules have been tested against angiotensin and related family. There is no such potent MAPK inhibitor has been established yet. Some good activities have been showed by few molecules but they need proper trial (147),(148), and their effects on various experimental animal models. BI78D3, an inhibitor of JNK showed prevention of JNK phosphorylation and ameliorates JNK dependent liver damage (149). Another JNK inhibitor SP600125 showed promising effect on preventing the JNK expression and its activity in HeLa cells (150). Furthermore, animal studies also showed that CNI1493 may prevent p38 MAP kinase signaling cascade and reduced TNF-α level in collagen induced arthritis rats (151). Inhibition of p38 MAP kinase signalling cascade by SB203580

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12. Angiotensin II as a New Potential Therapeutic Target

12.1 Angiotensin II and the Renin-angiotensin System

Angiotensin II (AngII) has been initially identified as the major biologically active peptide of the reninangiotensin system (RAS), but it is now well established that other peptides derived from the RAS, namely Ang1-7, AngIII and AngIV, also display biological activities(152),(153),(154). All angiotensin peptides derive from a unique precursor, angiotensinogen (AGT), synthesized and released from the liver. In response to blood changes (such as decrease in blood pressure or plasma sodium level), the kidneys produce and release the renin protease which cleaves AGT into a decapeptide designated Angiotensin I (AngI). AngI is in turn cleaved by Angiotensin Converting Enzyme (ACE) to produce the octapeptide AngII. AngII can then be processed by either the Angiotensin Converting Enzyme 2 (ACE2) to produce Ang1-7, or by aminopeptidase A and N to produce AngIII and Ang IV, respectively(154). (153). Angiotensin peptides, and in particular AngII, are produced in the plasma but also in several organs where a local RAS is active(155). Interestingly, angiotensin peptides exert diverse biological effects, such as vasoconstriction /vasodilatation, inflammation, proliferation and apoptosis, through binding to different receptors, namely AT1R, AT2R, AT4R and MAS-R(154),(156). Both AngII and its cleavage product Ang1-7 have been shown to contribute to cancer processes, by different mechanisms. Studies reporting an effect of Ang1-7 acting though the MAS receptor have been recently reviewed(154), and their role in cancer have first been described by Tallant's group(157),(158),(159). In this chapter, we will mainly focus on the effects of AngII in cancer progression.

13. **Conclusion**

Ang II-induced activation of the AT1R reflects its extraordinarily diverse repertoire of signaling mechanisms and pathways, including some that require stringent counter regulatory control systems to prevent excessive activity leading to deleterious cellular growth and proliferation. This is indicative of the scope and biological significance of angiotensin's numerous actions, and the roles of critical signaling mechanisms that are susceptible to dysfunction leading to progressive inflammatory and degenerative conditions that underlie major disease entities. On the other hand, the availability of highly effective agents for inhibition of Ang II formation, and selective blockade of the AT1R, have led to the identification of many of the hitherto unrecognized pathological actions of Ang II

Ang II induced hepatic inflammation via MAPK suggests several pathways. Firstly, Ang II interacts with AT1R, stimulates Gprotein couple receptor (GPCR) and then activates MAPK, resulted free radicals generation, later sends stimulation for inflammatory and proinflammatory molecules and production of other growth factors which finally contribute hepatic inflammation or hepatitis. MAPKs are important signaling molecules in several pathways in liver physiology and disease pathogensis. In many pathologic processes, JNK1 is found responsible

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over the past two decades have highlighted important effects of the Ang II vasoactive peptide in cancer, acting both on tumor cells and the host micro-environment. In this chapter, we summarize our recent studies indicating that Ang II facilitates breast cancer metastasis by contributing to the cross-talk between cancer cells and the host stroma. While AT1 receptor blockade by ARBs is clearly beneficial in animal models, relevance to human cancer still remains to be evaluated and further studies should focus on selected populations of tumors overexpressing RAS components.

the recent literature on the non-classical oralternativeACE2-Ang-(1–7)-Mas receptor axis of the RAS. The mounting biochemical and functional evidence clearly supports the tenet that this pathway may antagonizetheACE-AngII-AT1-receptorarmoftheRASeither directly through metabolism of Ang II to Ang-(1–7) by ACE2 or via distinct pathways that limit the activation of AngII-AT1- receptor signaling. Indeed, the demonstration of an intracellular ACE2-Ang-(1–7)-Mas axis that attenuates the AngII-dependent stimulation of ROS on renal nucleiis in keeping with the concept of a balanced RAS even with in the cell and importance of targeting the intracellular system as a therapeutic approach to enhance the functional ratio of Ang-(1–7) to Ang II. The evidence that an altered Ang-(1–7)system with in the brain and the kidney following antenatal glucocorticoid exposure implicates an interaction between the -(1–7)

pathways that contribute or promote the cardiovascular dysfunction associated with fetal programing events.

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