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Molecular Basis of Renal Disease

Molecular mechanisms of angiotensin II in the kidney: emerging role in the progression of renal disease: beyond haemodynamics

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Introduction

Franz Volhard (1872–1950), one of the most influential German clinicians in the first half of this century and probably best known for his seminal classification of nephritis [1], postulated as early as 1923 the existence of a renal-derived factor as the cause of vasospasm of the then so-called pale hypertension [2]. Hartwich, one of Volhard's disciples, demonstrated in 1930 that ligation of the renal artery in dogs caused a transient rise in blood pressure [3]. Although not widely appreciated at that time, these studies were the precursors of Goldblatt's authoritative experiments establishing that renal ischaemia causes persistent hypertension [4]. Until the work of Braun-Menéndez [5], and independently of Page [6], it was believed that a saline extract, first prepared from rabbit kidneys by Tigerstedt and Bergmann in 1898 and named renin [7], was sufficient to induce vasoconstriction. Page noticed that further purification of a crude renal preparation abolished its vasoconstrictive abilities whereas mixing of this purified renin with plasma restored the effect, suggesting that renin generates a vasoactive substance from plasma [6]. In parallel experiments, Braun-Menéndez and Fasciolo discovered that a vasoconstrictor of identical properties, as found in the venous blood from a kidney with experimentally induced renal artery stenosis, is produced by incubating renin with plasma [5]. These fundamental investigations, together with the discovery of an enzyme in plasma capable of converting vasoactive factors, later named angiotensin-converting enzyme (ACE), by Skeggs and associates [8], laid the groundwork for the many subsequent studies on the renin–angiotensin system (RAS). The synthesis of angiotensin II (ANG II) enabled investigators to test directly the effect of this peptide on many diverse functions of the organism [9,10]. The discovery that a venom of the Brazilian viper was able to inhibit ACE and the synthesis of competitive peptide analogues of ANG II such as saralasin in the early 1970s provided the tools to interfere with the activation of the RAS [11,12]. *In vivo* binding studies in several organs including the kidney using saralasin provided convincing evidence that ANG II-receptors are heterogeneous in expression and function with high- and low-affinity binding characteristics [10,13]. The development of specific competitive non-peptide ANG II-receptor antagonists [14], whose applications confirmed the existence of at least two types of ANG II receptors, has been pivotal in the cloning of the AT_1 receptor in 1991 [15,16]. The AT_2 receptor was more recently cloned, and it is currently the last member of the RAS whose molecular structure has been identified [17].

The functions of ANG II to induce vasoconstriction and to stimulate aldosterone release from the adrenal gland were well established in the 1950s [18]. Micropuncture studies in the 1970s firmly demonstrated the role of ANG II in the regulation of glomerular haemodynamics [19,20]. In parallel, evidence accumulated that mesangial cells express ANG II receptors, contract after challenge with the octapeptide, and increase the uptake and processing of macromolecules in the presence of ANG II [21,22]. However, it became clear that ANG II effects were not limited to the glomerular circulation, and several investigators showed that the peptide modulates proximal tubular sodium and water transport [24,25].

Anderson and co-workers published their landmark study in 1985 showing that an ACE inhibitor reduced proteinuria and limited glomerular injury in rats with experimentally induced reduction of renal mass [26,27]. These beneficial effects of the ACE inhibitor were then solely attributed to the normalization of glomerular hypertension [26]. It took almost a decade until the validation that ACE inhibitors also provided protection against the progression of renal insufficiency in humans with various renal diseases [28,29]. There is strong evidence that these protective effects of the ACE

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inhibitors may also be attributable to mechanisms other than a reduction in blood pressure [28,29].

During recent years, a myriad of data has accumulated from observations in the kidney as well as other organs that ANG II exerts diverse effects such as growth stimulation, induction of fibrogenesis, and immunomodulation, which are clearly beyond the commonly appreciated classical function of this vasoactive peptide. A review describing these more recently recognized functions of ANG II has been consequently entitled 'Angiotensin actions in the kidney: renewed insight into the old hormone' [30]. An overview of some, but not all, of the newer functions of ANG II is given in Figure 1.

This revitalized interest into the vasoactive peptide emanated from different sources. First of all, the cloning of the diverse classes of ANG II receptors and the pharmacological tools to block these receptors offer the opportunity to dissect the various signal transduction pathways involved subsequent to receptor activation, and to better understand the function of ANG II on a molecular level including activation or suppression of target genes [14–16]. Although the RAS has been traditionally considered as an endocrine system with only renin being synthesized by the kidney, there is now convincing evidence that all components of a local RAS resides within the kidney and all elements of a functional RAS may be even present in proximal tubular cells [31–34]. ANG II is indeed secreted in nanomolar concentrations into the tubular fluid, and cells along the nephron come in contact with much higher concentrations of the peptide than previously thought [35]. Equally important, the local renal RAS may be independently regulated from its systemic counterpart, and commonly used systemic concentrations of ACE-inhibitors may not necessarily inactivate the renal RAS. The recent identification of new angiotensin peptides, such as angiotensin IV and angiotensin (1–7) with distinct functions and probably separate putative receptors, and the accompanying enzymatic machinery to generate these peptides as well as the previously underestimated role of ANG II metabolism in contributing to local peptide concentrations, have added considerable complexity to the RAS [36–38]. Finally, many of the newer functions attributed to ANG II such as compensatory growth responses, stimulation of fibrogenesis, and initiation of monocyte/macrophage (M/M) influx into the kidney may be involved in the progressive loss of renal function in chronic disease [30,39–41]. Furthermore, ANG II may function as a bridge linking haemodynamic mechanisms which take place during the functional adaptation of surviving nephrons after renal injury to the associated morphological remodelling of renal architecture [40]. The current review will first describe how ANG II modulates renal growth and fibrogenesis and will then focus on potential immunomodulatory effects of the peptide. Mechanisms of ANG II-mediated changes in glomerular haemodynamics and ultrafiltration, although important in the appreciation of this peptide as a pivotal factor in the progression of renal disease, will not be covered, and have been reviewed elsewhere [42–45].

ANG-II-mediated induction of cytokines and growth factors

Many of the diverse effects of ANG II delineated in detail below are not directly caused by the vasopeptide itself but are rather mediated through the ANG II-induced production of a wide array of different cytokines and growth factors [30,40,46,47]. In such a scheme, ANG II may stimulate a particular factor in a distinct renal cell. This factor, most probably but not necessarily subsequent to secretion, may then bind and activate either nearby cells in a paracrine or the same cells in an autocrine manner. Since the majority of information of ANG II potential to induce other factors is derived from culture experiments with homologous cells, the actual *in vivo* situation is much more complex and the identification of which local cytokine may be induced by ANG II is very difficult, if not impossible. An overview of the various cytokines and

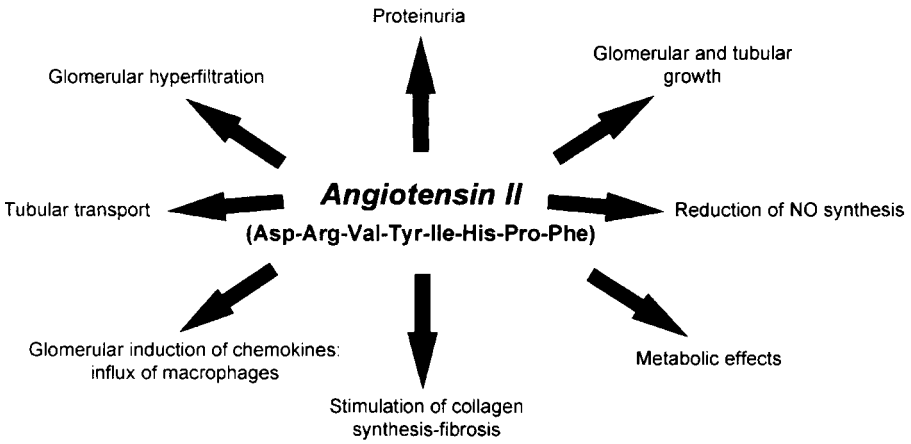

Fig. 1. ANG II has many recently discovered effects which may all contribute to the progression of chronic renal disease towards irreversible scarring.

growth factors induced in renal cells is provided in Table 1.

ANG II as a renal growth factor

Although the growth-promoting effects of ANG II on vascular smooth-muscle cells have been relatively well defined [41], it was only more recently that several groups have drawn their attention to the capacity of ANG II to modify renal growth processes. Norman *et al.* were among the first to formally investigate the potential growth effects of ANG II on proximal tubular cells, describing that the octapeptide clearly potentiated proliferation induced by epidermal growth factor [48,49]. Our group was the first to demonstrate that ANG II stimulates hypertrophy of a murine proximal tubular cell line (MCT cells), as determined by stimulating protein synthesis and increased cell size without accompanying DNA synthesis [50]. We have subsequently shown that ANG II induces a hypertrophic response in LLC-PK_1 cells through activation of AT_1 receptors [51]. These findings have been confirmed in primary cultures of rabbit and rat proximal tubular cells [52,53]. Pertussis toxin and agents increasing intracellular cAMP abolished the ANG II-induced protein synthesis, indicating that the AT_1 receptor is coupled to adenylate cyclase by a pertussis-toxin-sensitive inhibitory G-protein [54,55–57]. Subsequent activation of cytosolic S6 kinase appears to be essential in this process [52,56]. ANG II stimulates bioactivation and expression of transforming growth factor-β (TGF-β) in tubular MCT cells [58]. This ANG-II-mediated expression of TGF-β is due to an increase in transcriptional activity [59]. A neutralizing anti-TGF-β antibody attenuates the ANG-II-induced increase in protein synthesis in MCT cells, suggesting that the hypertrophy is mediated by synthesis and activation of endogenous TGF-β [89]. Interestingly, transfection of MCT cells with the *c-mas* oncogene converts the hypertrophic growth response of ANG II into proliferation [60]. This change is associated with decreased TGF-β transcription and synthesis in *c-mas* transfected cells, suggesting that ANG-II-mediated TGF-β transcription is pivotal for the hypertrophic growth response in proximal tubular cells [59]. Proximal tubular cells undergoing ANG-II-mediated hypertrophy are arrested in the G_1 phase of the cell cycle and express typical G_1-phase-associated genes [50,54,57]. Induction of such G_1-phase-associated early growth response genes have been also described *in vivo* after infusion of ANG II into the renal artery [61]. This G_1 phase arrest depends on the induction of the cyclin-dependent kinase (CdK) inhibitor $p27^{Kip1}$ [62]. $p27^{Kip1}$ expression is stimulated after incubation of LLC-PK_1 cells with ANG II or TGF-β and binds to cyclin D1-CdK4 complexes, inhibits their kinase activity, and hampers G_1-phase exit [62]. In addition to G_1-phase arrest and stimulation of *de novo* protein synthesis, there is also evidence, at least in LLC-PK_1 cells, that ANG II reduces protein degradation, suggesting that these effects are additive in the induction of tubular hypertrophy [63]. An overview of the molecular mechanisms of how ANG II induces hypertrophy of proximal tubular cells is shown in Figure 2. In contrast to proximal tubular cells, ANG II is mitogenic for a mouse cell line derived from medullary thick ascending limb of Henle's loop (mTAL) [64]. This mitogenic response is associated with failure of ANG II to stimulate TGF-β synthesis [64], a finding that may explain the induction of a proliferative growth response in these mTAL cells.

We reported in 1992 that ANG II causes a small but significant proliferation of a murine mesangial cell line in serum-free medium in the absence of other factors [65]. Although several investigators have subsequently corroborated these findings in cultured mesangial cells from different species [66–73], not all found evidence of ANG II-mediated mitosis [74–76]. In fact it has been proposed that ANG II induces hypertrophy rather than proliferation of mesangial cells [74–76], and there may exist differences in the kinetic pattern of induction of mitogen-activated protein (MAP) kinases between strong proliferative stimuli such as platelet-derived growth factor (PDGF) and ANG II [76]. However, the differences in the growth pattern (proliferation *versus* hypertrophy) are most probably due to variance in culture conditions, addition of supplementary growth factors, species differences, and the methods used to assess proliferation. There is some evidence that ANG-II-induced proliferation of mesangial cells may be mediated by autogenous release of other cytokines such as interleukin 6, PDGF or endothelin-1 [66,67,73]. Nevertheless, infusion of this peptide into naive rats causes a small proliferation of mesangial cells [77,78], although it remains to be established whether this effect is directly generated by ANG II and not by hypertension.

Interestingly, the *in vitro* proliferative effects of ANG II on mesangial cells are inhibited in the presence of atrial natriuretic peptide (ANP) or supplementation of the medium with the nitric oxide precursor L-arginine, suggesting that an increase in intracellular cGMP inhibits mitosis [70,79]. Other effects, however, like ANP-mediated synthesis of TGF-β may play additional roles in this process [80]. ANG-II-mediated

Table 1. ANG-II-mediated induction of cytokines and growth factors in various renal cells

Factor	Cell type	Reference
Endothelin–1	Mesangial cell	66
	Glomerular endothelial cell	82
Interleukin 6	Mesangial cell	67
MCP–1	Mesangial cell	136, 137
Platelet-activating factor (PAF)	Mesangial cell	46
PA-1	Mesangial cell	121
PDGF	Mesangial cell	73
RANTES	Glomerular endothelial cell	135
TGF-β	Proximal tubule cell	58
	Mesangial cell	116

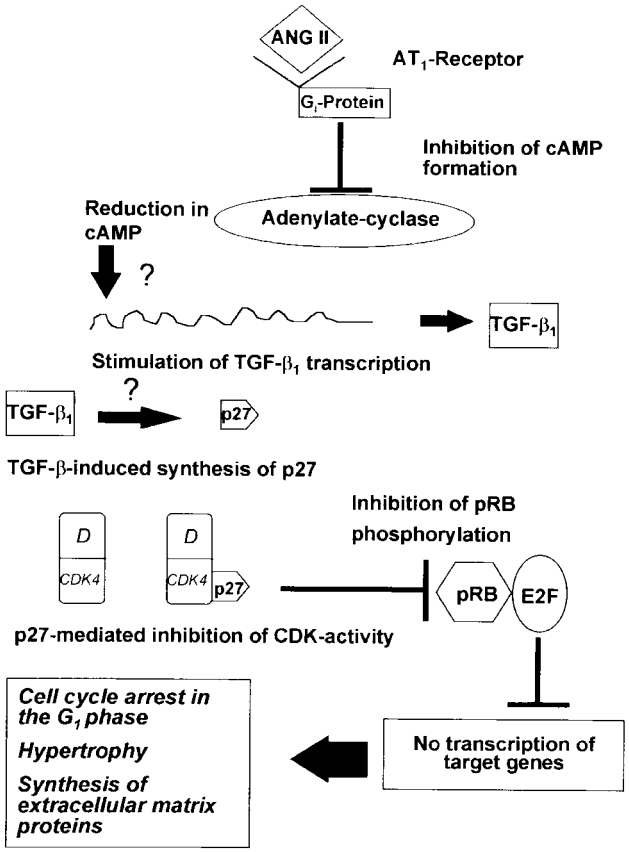

Fig. 2. Overview of the complex mechanisms of how ANG II may stimulate hypertrophy of cultured proximal tubular cells. After binding to AT_1 receptors, an inhibitory G protein leads to inhibition of adenylate cyclase, resulting in a decrease in cAMP. This decrease, in addition to other putative signal transduction pathways, may stimulate transcription and synthesis of TGF-β1. TGF-β1 expression itself, as well as TGF-β-independent effects of ANG II, stimulate translation, but not transcription, of the CdK inhibitor $p27^{Kip1}$. This protein associates with CdK4-cyclin D complexes and inhibits their kinase activity. The result is a decrease in phosphorylation of the protein product of the retinoblastoma gene (PRB) which retains the transcription factor E2F. The lack of transcription of various target genes (which are different from immediate early genes being induced by ANG II) facilitates cell cycle arrest in the G_1 phase, with resulatnt hypertrophy. This arrest as well as direct effects of TGF-β1 stimulate protein synthesis including extracellular matrix proteins. $\Leftarrow$ indicates stimulation; $\perp$ refers to inhibition; **?** marks potential mechanisms which have not yet been tested.

proliferation has been also described in rat renomedullary interstitial and glomerular endothelial cells [81,82].

ACE inhibitors partly attenuate compensatory renal growth in different models, independently of concomitant haemodynamic changes [30,40,83–85]. For example, a high dose of enalapril abolished compensatory renal hypertrophy and glomerular sclerosis better than a low dose of this ACE inhibitor in subtotally nephrectomized rats, despite a similar reduction in glomerular capillary hydraulic pressure [86]. Treatment of weaning rats for 6 weeks with frusemide activates the RAS and stimulates tubular and glomerular hypertrophy as measured by morphometry [87]. Treatment with enalapril preventes this compensatory renal hypertrophy [87]. Amann and co-workers [78] showed in a subtotal nephrectomy model in rats that only ACE inhibitors, but not other antihypertensive drugs, prevented mesangial proliferation and podocyte hypertrophy despite comparable reduction in systolic blood pressure. Although this finding may not rule out a potential protective effect of ACE-inhibitor-mediated reduction in glomerular haemodynamics, these investigators persuasively showed that the beneficial effects of interference with the RAS are not caused by a decrease in hypertension.

A strong activation of the RAS occurs in the kidney during fetal and early postnatal life, resulting in high levels of ANG II [88–90]. Inhibition of the RAS causes an arrest in nephrovascular maturation and in renal growth and development [88]. Moreover, mice deficient in ACE exhibit abnormal vessels and tubules [91,92]. Although not supported by data from some investigators [93], these findings suggest that ANG II may be an important factor in nephrogenesis and renal maturation and growth.

Studies performed in coronary endothelial cells and rat phaeochromocytoma cells which both express the AT_2 subtype of the ANG-II receptor suggest that engagement of ANG II with this receptor inhibits cell proliferation [94]. Along this line, further studies indicate that activation of the AT_2 receptors exerts antiproliferation by increasing programmed cell death (apoptosis) through activation of a protein–tyrosine–phosphatase [95]. This activation leads to an underphosphorylation and inhibition of Bcl-2 protein, a key factor preventing apoptosis [95]. Despite this fascinating result and the potential therapeutic effect of overexpressing AT_2 receptors, there is currently little information that similar mechanisms are operative in the kidney [82,97].

Profibrogenic role of ANG II

End-stage kidneys, independent of the primary renal disease, are characterized by the irreversible development of glomerulosclerosis and tubulointerstitial fibrosis [98,99]. Net accumulation of extracellular matrix proteins in the glomerular tuft and/or the tubulointerstitium is the result of an increase in the synthesis of these substances, a decrease in turnover, or a combination of both [98,99]. Although a wide variety of diverse factors including systemic and glomerular hypertension, proteinuria, metabolic factors such as acidosis, and influx of circulating monocytes may all contribute to the fibrogenic process in end-stage kidneys, there is increasing evidence that many of these factors induce profibrogenic cytokines such as TGF-β, which in turn stimulates production and inhibits turnover of extracellular matrix proteins [100]. Moreover, in the dynamic mechanisms of fibrogenesis, growth processes such as proliferation of intrinsic glomerular cells and compensatory tubular hypertrophy may precede the later development of glomerulosclerosis and tubulointerstitial fibrosis [101–104]. Consequently, potential effects of ANG II on synthesis and turnover of extracellular

matrix have been investigated, mainly in cultured proximal tubular and mesangial cells. ANG II stimulates transcription of collagen type IV in MCT cells [55]. This stimulation is mediated by endogenous synthesis and autocrine action of TGF-β because a neutralizing anti-TGF-β antibody as well as TGF-β antisense oligonucleotides attenuate ANG II-induced collagen type IV transcription and synthesis [105].

More indirect evidence that ANG II plays an important role in tubulointerstitial fibrosis *in vivo* has been obtained from comprehensive studies in rats with unilateral ureteral ligation [106–108]. Tubular TGF-β expression precedes the development of tubulointerstitial fibrosis and was partly attenuated when animals were treated with an ACE inhibitor [106,107]. Enalapril administration reduces TGF-β expression and halts tubulointerstitial fibrosis even when the drug is given later during the course of obstructive nephropathy [108]. Another model in which a close relationship between activated RAS, TGF-β, and the subsequent development of tubulointerstitial fibrosis has been appreciated is chronic cyclosporin nephrotoxicity. Administration of cyclosporin to animals as well as direct treatment of cultured proximal tubular cells stimulates expression of TGF-β and collagen synthesis [109–112]. Treatment of rats with enalapril or an AT_1-receptor antagonist prevents cyclosporin-induced TGF-β and α_1(I)procollagen mRNA expression as well as tubulointerstitial fibrosis, convincingly demonstrating that ANG II induces TGF-β, which in turn activates extracellular matrix synthesis [113,114]. Enalapril, but not a calcium antagonist, also attenuates tubulointerstitial fibrosis in ageing mice of 2 years [115].

In addition, ANG II stimulates fibronectin and collagen type I synthesis in cultured mesangial cells [65,71,72]. It appears that some of these changes are also mediated by secondary effects of induced TGF-β [116]. ANG II induced mesangial-cell activation of the cyclic adenosine monophosphate response element binding protein (CREB) transcription factor, whose activation seems to be a necessary prerequisite for fibronectin transcription [117]. After subtotal nephrectomy, rats develop glomerulosclerosis and tubulointerstitial fibrosis, leading to impairment of renal function [118]. These changes are associated with a 2.5-fold increase in TGF-β gene expression, localized to sclerotic glomeruli and areas of tubulointerstitial damage [118]. Administration of the ACE inhibitor ramipril and the AT_1-receptor blocker valsartan blunt the increase in TGF-β mRNA and attenuate the structural manifestations of injury, indicating an *in vivo* relationship between RAS, TGF-β, and subsequent development of renal scarring [118]. In a separate study, even delayed ACE treatment started 8 weeks after 5/6 nephrectomy reduces glomerulosclerosis compared to untreated rats [119]. Lee *et al.* [120] have also provided clear evidence in a model of progressive glomerulosclerosis that endothelial and mesangial RAS is essential in TGF-β induction and subsequent synthesis of extracellular matrix proteins.

Complementary to ANG-II-stimulated increase in extracellular matrix proteins, recent findings indicate that the peptide also modulates the plasmin protease system [121–124]. Plasmin degrades extracellular matrix itself and additionally activates metalloproteinases which further degrade collagens. Plasmin synthesis itself is regulated by the balance between plasminogen activators (PA) and their inhibitors (PAI-1,2). Kagami *et al.* [121] have recently demonstrated in cultured rat mesangial cells that ANG II upregulates PAI-1 production, partly through induction of TGF-β. ANG-II-mediated expression of PAI-1 depends on activation of protein kinase C. [122]. Since PAI-1 is an inhibitor of plasmin-mediated extracellular matrix degradation, ANG-II-mediated PAI-1 induction may contribute to the development of renal fibrosis [124]. Surprising recent studies disclosed that renin may also bind directly to mesangial cells and increases PAI-1 expression [123]. *In vivo* studies utilizing a model of radiation-induced glomerulosclerosis have shown a decrease in PAI-1 expression when animals were treated with ACE inhibitor or AT_1-receptor blocker [124]. This PAI decrease was associated with accelerating fibrinolysis, suggesting that ANG II may indeed accelerate renal fibrosis through interaction with protease systems [124].

Probably the most direct evidence that the RAS is involved in renal scarring may stem from investigations designed to overexpress renin and angiotensinogen locally in rat glomeruli by using haemagglutinating virus of Japan [125]. Seven days after transfection, extracellular matrix was expanded and α-smooth-muscle actin was expressed in mesangial cells [125]. No significant difference in the blood pressure was observed between the different groups, indicating that systemic hypertension did not contribute to the glomerulosclerosis in renin–angiotensinogen overexpressing glomeruli.

Immunomodulatory effects of ANG II

Glomerular and interstitial infiltration with M/M is a common feature in many immune-mediated and non-immune-mediated renal diseases, and it is believed that this infiltration is crucial in the progression of renal disease [126]. Diamond and Anderson [127] treated rats with experimentally induced chronic aminonucleoside nephrosis with the ACE inhibitor enalapril and observed a decrease in tubulointerstitial cellular infiltrates and in the development of interstitial fibrosis compared with untreated controls. An infiltration of M/M has been reported in the unclipped kidneys of rats with two-kidney one-clip hypertension, a classical model of an initially strongly activated RAS [128,129]. ACE inhibitor or AT_1 treatment also reduces renal M/M in uninephrectomized spontaneously hypertensive rats (SHR) [130]. Although these effects could be due to a reduction of proteinuria and normalization of glomerular hyperfiltration, they may additionally indicate the tantalizing possibility that ANG II exerts

immunomodulatory properties by promoting renal M/M influx. Indeed studies more than a decade ago suggest that the peptide may have chemotactic activity for various immunocompetent cells. It has been reported, for example, that ANG II stimulates the chemotactic response of human mononuclear cells *in vitro* [131], and activates phagocytosis of granuloma-derived macrophages [132]. Farber *et al.* [133] showed that ANG II influences neutrophil accumulation via production of chemoattractant activity by vascular endothelial cells. However, this neutrophil chemoattractant was not further identified, but it was implied that it is not identical with known eicosanoids [133]. More recent studies have demonstrated that ANG II induces adhesion of M/M, but not of neutrophils, to aortic endothelial cells without upregulation of adhesion molecules [134].

We have studied in a recent series of experiments whether ANG II may modulate M/M recruitment in the kidney [135]. ANG II dose-dependently stimulated mRNA and protein expression of RANTES, a member of the C-C chemokine subfamily with chemoattractant properties for M/M, eosinophil, and basophil granulocytes as well as T cells, in cultured glomerular endothelial cells of the rat (GER), but not in syngeneic mesangial cells [135]. Chemotactic assays revealed that the produced RANTES and not ANG II itself was actually chemotactic for human monocytes. Surprisingly, the ANG II-stimulated RANTES expression was transduced by AT_2 and not AT_1 receptors [135]. Intraperitoneal infusion of ANG II (500 ng/h) into normal rats for 4 days significantly stimulated glomerular RANTES mRNA and protein expression [135]. Immunohistochemistry revealed induction of RANTES protein mainly in glomerular endothelial cells and small capillaries (see Figure 3A,B). ANG-II-infused animals demonstrated an increase in glomerular M/M staining, an effect attenuated by oral treatment with an AT_2-receptor blocker [135]. These studies demonstrated that ANG II may play an important role in glomerular chemotaxis of M/M through the local induction of the chemokine RANTES in endothelial cells. The astonishing observation that the ANG-II-mediated induction of RANTES is transduced by AT_2 rather than AT_1 receptors may influence the decision as to which substances should be used for the therapeutic interference with the RAS. However, RANTES may not be the only chemokine whose expression is induced by ANG II in the kidney. Preliminary reports from two independent groups suggest that ANG II stimulates monocyte chemoattractant protein-1 (MCP-1) expression in cultured mesangial cells [136,137]. Since reactive oxygen intermediates have been recently implicated in transcription of MCP-1 by the transcription factor family of NF-κB [138], and ANG II may induce and support the generation of such reactive oxygen species [139], at least in some tissues this pathway offers an interesting explanation of how the peptide could stimulate MCP-1 expression. Since nitric oxide (NO), whose tubular synthesis is impaired by ANG II [140], inhibits NF-κB DNA binding [141], an ANG II-mediated reduction in NO may attenuate this negative feedback mechanism and further facilitate MCP-1 transcription.

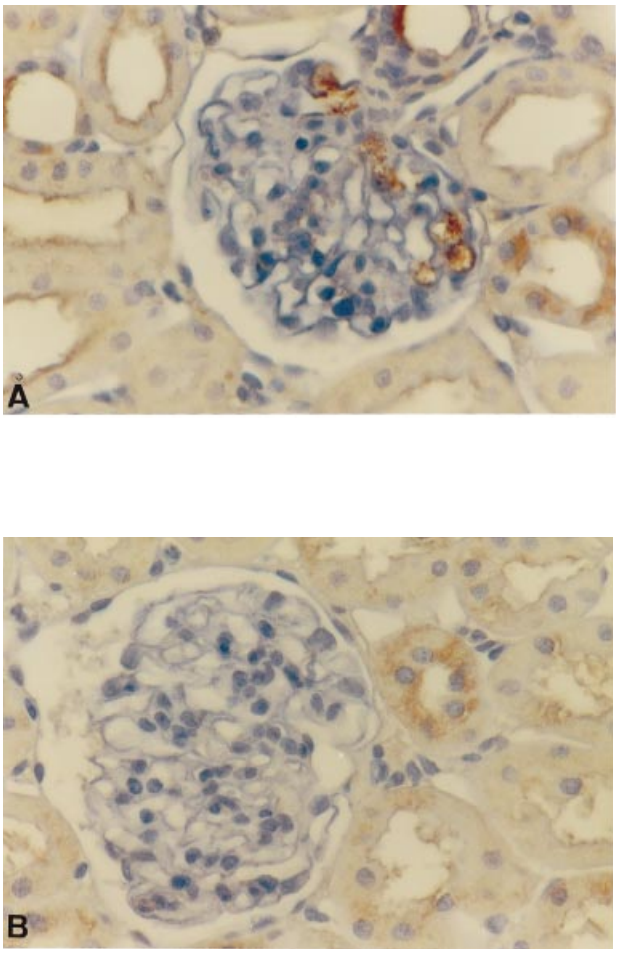

Fig. 3 A, B. Evidence for immunoregulatory effects of ANG II *in vivo*. **A.** Kidney section of a rat infused for 4 days with ANG II showed positive staining for chemokine RANTES, mainly in glomerular endothelial cells. **B.** Rats infused with solvent (no ANG II) revealed only a slight staining of tubular cells with no detection of RANTES protein in the glomerular tuft. Magnification $\times 200$. For details see reference 135.

We have addressed in a very recent study the effect of two AT_1-receptor antagonists, losartan and irbesartan, on glomerular MCP-1 expression as well as influx of M/M in a model of mesangioproliferative nephritis induced in rats by injection of an anti-thy 1 antibody [142]. Both AT_1-receptor antagonists caused a significant, but not total, reduction of MCP-1 mRNA and protein expression 24 h after injection of antithymocyte serum. Treatment with losartan or irbesartan also reduced chemotactic activity of isolated glomeruli from nephritic animals [142]. Quantification of ED1-positive cells revealed that losartan as well as irbesartan reduced glomerular M/M infiltration in nephritic rats by approximately 30–50% [142]. These data indicate that short-term antagonism of AT_1 receptors abolished the early glomerular MCP-1 expression and M/M influx

in a model of experimentally induced glomerulonephritis.

After loss of functional renal tissue, surviving proximal tubules have to increase their acid secretion through enhanced bicarbonate reabsorption and generation of ammonia to maintain acid–base homeostasis [143]. This response is partly mediated by ANG-II-induced stimulation of ammoniagenesis [144]. Such an increase in ammonia production may lead to the activation of complement C3, provoking further the influx of M/M into the kidney [145].

Osteopontin is an arginine–glycine–aspartate-rich protein recently shown to induce M/M influx [146]. A marked increase in osteopontin expression was observed in distal tubule and collecting ducts of ANG-II-infused rats, preceding other pathological changes [146]. These observations may suggest that ANG-II-induced osteopontin overexpression facilitates M/M accumulation at the sites of tubulointerstitial injury.

Provocative recent studies demonstrated renin and ANG II expression in M/M [147,148]. ANG II release from infiltrating M/M may, on the one hand, further stimulate chemoattraction of these cells, but may, on the other, additionally influence renal haemodynamic, growth and fibrogeneic processes through a local increase in ANG II. A preliminary report suggests that ANG II augments anti-CD3-antibody-induced proliferation of a murine nephritogenic T cell clone [149]. This clonal expansion was transduced through AT_1 receptors [149]. These compelling studies indicate that ANG II may foster expansion of nephritogenic T cells.

What does this all mean for the treatment of renal patients?

After elaborating in detail on the myriad of ANG II effects in the kidney, one may ask how does this cause any paradigm change in the clinical treatment of patients with chronic renal disease. Patients suffering from diabetic nephropathy were among the first to be treated with ACE inhibitors after the landmark experimental studies by Anderson and co-workers showed a protective effect of these drugs on renal function [150,151]. Many studies have since convincingly demonstrated the protective effects of ACE inhibitors in the prevention of diabetic nephropathy [152–156]. In addition, several studies are also completed indicating that various ACE inhibitors prevent the progression of chronic renal failure in patients with different primary renal diseases [28,29,157,158]. Patients suffering from autosomal dominant cystic kidney disease may be the exception [29]. Almost all studies indicate that this beneficial effect of ACE inhibitors is independent of a reduction in blood pressure [28,29,150–158]. Although some critical voices have been raised suggesting that bias may prevent the publication of negative studies [159], which exist, I nevertheless believe that there is no doubt that ACE inhibitors effectively attenuate the loss of function in chronic renal disease. However, which patients should be treated? A recent study suggests that patients with chronic nephropathies and a proteinuria $\geqslant 3$ g/24 h derive the most benefit from ACE-inhibitor treatment [158]. Other studies found also a protective effect in patients with lesser proteinuria [28,29]. A specific high-risk group in which ACE inhibitors may be less effective are patients with the ACE DD genotype, although the significance of this genotype is currently the subject of intensive research and is heavily disputed. It may be wise to look at the evolution of ACE-inhibitor treatment in diabetic patients. Initially, only diabetic patients with hypertension and proteinuria have been treated [150]. Subsequently, a protective effect of ACE inhibitors has been documented in normotensive diabetics with microalbuminuria [152]. Quite recently the EUCLID study demonstrated protective effects of ACE-inhibitor treatment on the progression of renal disease in patients with early stages of diabetes mellitus, even in the absence of microalbuminuria and hypertension, although the greatest clinical effect was observed in those with microalbuminuria [153]. This change in the treatment strategy in diabetes mellitus may serve as a model of how patients with other chronic renal diseases should be treated. Eventually all patients might be treated after the diagnosis of chronic renal insufficiency is made, irrespective of concomitant hypertension or proteinuria. Therefore ACE inhibitors could be ultimately considered as renoprotective drugs.

The recent introduction of AT_1-receptor blockers as antihypertensive drugs has opened new avenues to how the effects of ANG II may be more specifically antagonized, and the theoretical advantages of this new class of drugs over ACE-inhibitors have been reviewed [160–162]. So far, at least in animal models, AT_1-receptor antagonists are comparable to ACE inhibitors in their protection of renal structure [163–166]. Although AT_1-receptor antagonists may have fewer bradykinin-mediated side-effects, such as cough, than ACE inhibitors [167], whether these substances are superior to ACE inhibitors in the slowing of renal function deterioration in humans, similar to findings reported in the extremely controversial ELITE study in patients with myocardial infarction, is the subject of ongoing investigations [168]. However, some experimental observations suggest that unopposed binding of ANG II to AT_2 receptor initiate proinflammatory responses in the kidney [135].

Conclusion

ANG II has emerged as a multifunctional factor exhibiting such diverse actions as influencing renal haemodynamics and tubular transport, acting as a growth factor and a profibrogenic cytokine, and even having inflammatory properties. Cell-culture studies have provided clear evidence that many of these functions are independent of haemodynamic changes and may be pivotally involved in the progression of chronic renal disease. Naturally the contribution of such non-haemodynamic effects *in vivo* is much more complic-

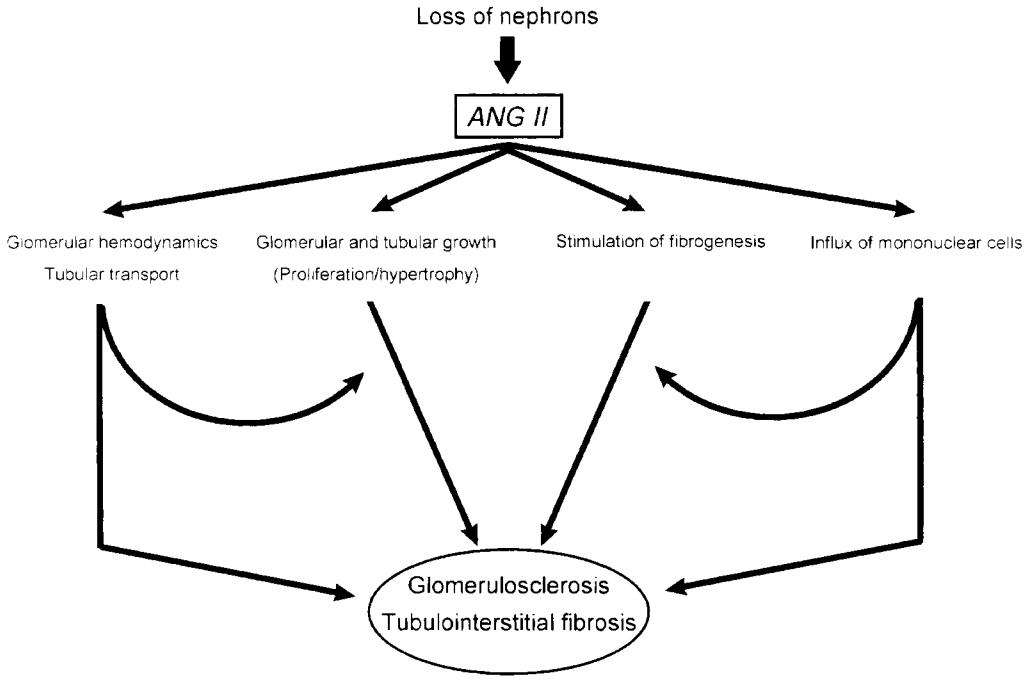

Fig. 4. Integrative concept of how haemodynamic as well as non-haemodynamic effects of ANG II may jointly contribute to the development of glomerulosclerosis and tubulointerstitial fibrosis. After loss of functioning renal tissue, a local and/or systemic activation of the RAS occurs, resulting in an increase in ANG II levels. On the one hand, ANG II modulates the adaptive haemodynamic changes such as increasing glomerular hydraulic pressure and tubular reabsorption to maintain fluid and electrolyte homeostasis; on the other, ANG II stimulates compensatory glomerular and tubular growth processes. The octapeptide also stimulates fibrogenesis by increasing extracellular matrix synthesis and reduction in turnover of these proteins. Part of this profibrogenic effect of ANG II may be mediated by the induction of TGF-β. Finally, ANG II may facilitate recruitment of monocytes into the kidney through complement activation and induction of chemoattractants such as MCP-1, RANTES, and osteopontin. These diverse actions of ANG II are closely interrelated: mechanical stretch caused by glomerular hyperfiltration or a stimulated proximal tubular reabsorption of solutes may initiate or enhance compensatory growth processes. Infiltrating monocytes release various cytokines, growth factors and toxic oxygen products which may all further promote fibrogenesis. All these diverse actions of ANG II may eventually give rise to the irreversible structural changes of glomerulosclerosis and tubulointerstitial fibrosis, which are the common endpoints of all chronic renal diseases. Taking into account the pivotal role of ANG II in these processes, it is obvious that therapeutic interventions to antagonize ANG II may slow the development of end-stage renal disease.

ated to assess, and it may ultimately be impossible to dissect haemodynamic from other effects of ANG II in the whole organism. However, haemodynamic effects, for example glomerular stretch, may further augment non-haemodynamic actions of ANG II such as renal growth or fibrogenesis [169]. The connection between haemodynamic and non-haemodynamic actions of ANG II jointly contributing to the development of renal scarring are schematized in Figure 4. Experimental investigations as well as clinical studies have provided clear evidence that ACE inhibitors slow the loss of renal function in various diseases. Although such effects may be partly due to the normalization of glomerular hyperfiltration and the reduction of proteinuria, it is wise to assume that interference with the multiple actions of ANG II, as described above, may contribute to the renoprotective capacity of ACE inhibitors. Franz Volhard, who assumed the existence of ANG II based almost exclusively on clinical observations, and who envisioned so many things in advance, would not be surprised at all.

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