

Review

Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease

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Abstract

The development of diabetic nephropathy in patients with Type I (insulin-dependent) and Type II (non-insulin-dependent) diabetes mellitus is still a huge clinical problem associated with increased morbidity and mortality. The mechanisms underlying the development of diabetic kidney disease are extremely complex and yet not completely understood. Among many potential pathogenic mechanisms responsible for the development of diabetic kidney disease, various growth factors have been suggested to be important players. In particular, growth hormone (GH)/insulin-like growth factors (IGFs), transforming growth factor β (TGF- β), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) have measurable effects on the development of experimental diabetic kidney disease through complex intra-renal systems. Recent findings that these growth factors

might initiate the early diabetic renal changes have provided insight into processes that might be relevant for future development of new drugs useful in the treatment of diabetic kidney disease. As will appear from the present review, enhanced understanding of the cellular mechanisms responsible for the development of diabetic kidney disease has already allowed the design of specific antagonists of pathophysiologically increased growth factors. Recent studies have shown that treating experimental diabetic models with such antagonists is followed by renoprotection. [Diabetologia (2000) 43: 1205–1223]

Keywords Growth hormone, insulin-like growth factors, transforming growth factor β , vascular endothelial growth factor, epidermal growth factor, antagonist, angiotensin converting enzyme inhibition, protein kinase C inhibition, nephropathy, somatostatin analogue.

Introduction

Diabetic nephropathy is one of the most common causes of end-stage renal failure in the Western world [1–5]. The characteristic early changes of the diabetic

kidney disease are increased renal size, glomerular volume and hyperfiltration. Later on in the incipient stage an increase in albumin excretion rate (AER) is followed by development of mesangial proliferation,

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Abbreviations: ACEi, Angiotensin converting enzyme inhibitor; bGH, bovine GH; DAG, diacylglycerol; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; GH, growth hormone; GHBP, GH binding protein; GHR, GH receptor; IGF-BPs, IGF binding proteins; IGF-IR, IGF-I recep-

tor; IGF-II/man-6-PR, IGF-II/mannose-6-phosphate receptor; KO, knockout; MAP kinase, mitogen-activated protein kinase; NF- κ B, nuclear factor- κ B; NOD, non-obese diabetic; NSILA, non-suppressible insulin-like activity; OLETF Rat, Otsuka-Long-Evans-Tokushima Fatty Rat; PKC, protein kinase C; SF, sulphation factor; STZ, streptozotocin; TG, transgenic; TGF- β type RI, TGF- β type I receptor; TGF- β type RII, TGF- β type II receptor; TGF- β type RIII, TGF- β type III receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

glomerular extracellular matrix (ECM) accumulation and glomerular sclerosis. Overt diabetic nephropathy is clinically characterized by proteinuria, hypertension and a progressive renal insufficiency [1–5]. The search for important pathogenic mechanisms in diabetic kidney disease has focused on the early events, at the time when the above mentioned pathophysiological changes take place. Several metabolic, functional and structural renal changes in diabetic rodents have fundamental similarities to those occurring in diabetic patients and accordingly rats and mice with spontaneously developing diabetes or diabetes induced by streptozotocin (STZ) have been used extensively in diabetes research aiming at explaining the pathogenesis of diabetic kidney disease. These diabetic rodents develop renal enlargement, glomerular hypertrophy and renal hyperfiltration within weeks after diabetes debut and increased AER, glomerular ECM accumulation, increased glomerular basement membrane thickness and mesangial proliferation within months [6].

Due to their growth-promoting and proliferative effects, growth factors and cytokines have attracted attention in various aspects of diabetes research including conceivable effects on functional and structural changes in the development of diabetic kidney disease. The term growth factor is used as a generic designation of any substance capable of inducing cellular proliferation and differentiation and it embraces an ever increasing number of peptides found in the circulation and in different tissues, including the kidney. In their role as mediators, certain growth factors extend the known cascade that begins with hypothalamic releasing factors, that flows through the pituitary trophic hormones to endocrine gland hormones released into the circulation, and culminates with peripheral tissue growth. Some growth factors do not, however, mediate their actions as hormones because their effects are mediated directly through paracrine/autocrine mechanisms.

Viewed historically, growth hormone (GH) is the growth factor with the longest association to diabetes. The anterior pituitary became interesting when Young in 1937 showed that anterior pituitary extracts precipitated diabetes in dogs [7] and attention was focussed on GH 50 years ago, when it was shown that daily injections of highly purified GH made dogs permanently diabetic [8]. The discovery that induced further interest in GH and diabetes was that diabetic patients present with substantial GH hypersecretion [9, 10]. At the same time the 'GH-hypothesis' that GH plays an important part in the development of diabetic microangiopathy was launched [11]. The role of insulin-like growth factors (IGFs) and diabetes seems to be much shorter, although as early as 30 years ago studies on sulphation factor (SF) or non-suppressible insulin-like activity (NSILA) in diabetic patients were done oblivious of their partial identity with

IGFs [12]. Yde was the first to show that the SF was abnormal in diabetic serum, with a negative correlation with metabolic control [12]. Today it is generally believed that metabolic deterioration in diabetes first decreases hepatic IGF-I formation and serum IGF-I concentration, which then secondarily induces GH hypersecretion through an intact feedback mechanism [13, 14]. Increased circulating GH concentrations are then believed to stimulate local IGF-I concentrations in non-hepatic tissues (e.g. in the kidney) as discussed in detail below.

In the last 10 to 15 years great progress and new information have been generated in a constantly increasing number of publications on the possible relation between changes in growth factors and diabetic complications. This progress has mainly been facilitated by the development of new techniques allowing isolation and purification of growth factors and because modern molecular and cellular techniques have been developed. This review will present an update of the published evidence for a pathophysiological relation between changes in circulating and intrarenal growth factors and development of early diabetic renal changes in animal models. The first part of the review presents data on a definitive role of the GH/IGF system in the pathogenesis of the renal changes in experimental diabetes focussing on the renoprotective effects of long-acting somatostatin analogues and GH-receptor (GHR) antagonists. In the second part data suggesting a causal role for transforming growth factor β (TGF- β) in the pathogenesis of diabetic renal changes is presented, with emphasis on the possible close relation between TGF- β and the renin angiotensin and protein kinase C (PKC) systems. In the third and fourth part evidence for the roles of vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) being involved in the development of diabetic kidney disease is given. The present review will focus on data obtained in experimental diabetic models as only few clinical studies have been published on this topic.

Growth hormone (GH) and insulin-like growth factors (IGFs)

The GH/IGF system

The GH/IGF system constitutes a complex system of peptides in the circulation, extracellular space and in most tissues (Fig. 1). The classic endocrine effect of pituitary secreted GH is the induction of IGF-I synthesis in various organs through activation of specific GHRs. The GHR consists of intracellular, transmembrane and extracellular components. In humans the latter is identical to the GH binding protein (GHBP), produced by a post-translational cleavage of the extracellular component, which is shed to the

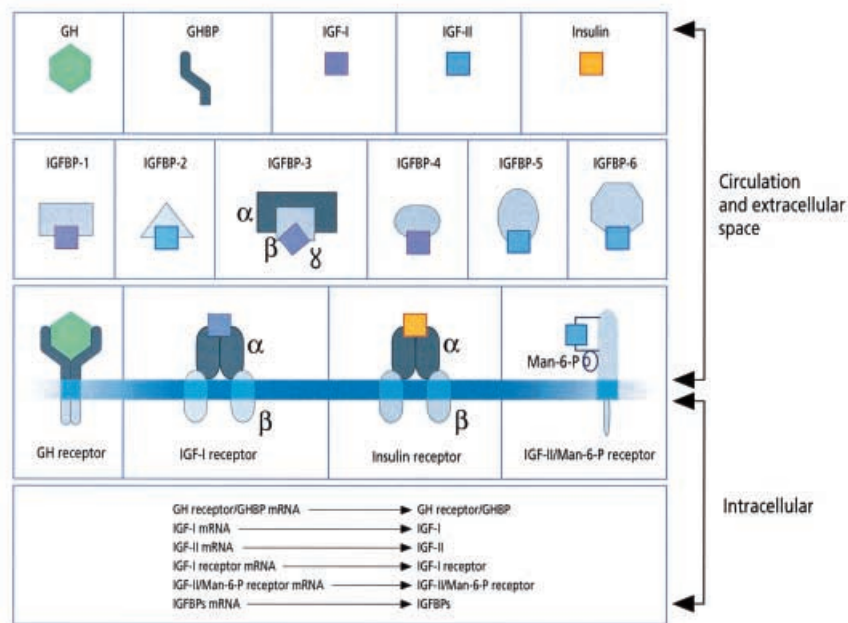


Fig. 1. Schematic depiction of the growth hormone (GH)/insulin-like growth factor (IGF) axis. GHBP: GH binding protein; IGF-I: insulin-like growth factor I; IGF-II: insulin-like growth factor II; IGFBP-1 to -6: IGF binding protein-1 to -6; IGF-II/man-6PR: IGF-II/mannose-6-phosphate receptor; mRNA: messenger RNA. For further explanation and references see text

circulation. In rodents both GHR and GHBP are, however, products of alternate splicing of a single gene [15, 16]. The effects of IGF-I and IGF-II are mediated through two specific IGF receptors [17–19]. The IGF-I receptor (IGF-IR) (also known as the type 1 IGF receptor) has a high degree of homology with the insulin receptor, with two extracellular IGF-I binding α -subunits, two transmembrane β -subunits linked together by disulphide bonds and with intracellular tyrosine kinase activity. The IGF-II/mannose 6-phosphate receptor (IGF-II/man-6-PR) (also known as the type 2 IGF receptor) binds IGF-II and man-6-P and plays a part in trafficking of lysosomal enzymes. Finally, IGFs are bound to specific IGF-binding proteins (IGFBPs) of which six high-affinity (IGFBP-1 to IGFBP-6) [20, 21] and several low-affinity IGFBP-related proteins (IGFBP-rP) have been characterized [22]. In the circulation IGFBPs are believed to prolong the half-life of IGFs as well as to regulate the endocrine effects of IGFs, whereas at the cellular level the IGFBPs are believed to modulate local actions of IGFs both in an inhibitory and stimulatory fashion.

Expression of the GH/IGF system in the normal kidney

The GH/IGF-system is expressed in the normal kidney, ranging from messenger RNA (mRNA) expression for the GHR and GHBP [15, 16], IGF-I and IGF-II [23, 25], the respective IGF-IR and IGF-II/man-6-PR [17–19] and all six specific IGFBPs [20–22]. As described previously in detail [15–28], each member of the GH/IGF axis has its specific localization in the nephron and thereby constitutes a system in which GH, GHBP and IGFs in the circulation and in the kidney could affect the nephron both through endocrine and autocrine/paracrine mechanisms.

In vitro evidence for GH/IGF effects on kidney cells

Insulin-like growth factor I has been shown to exert biological effects on murine and human mesangial cell lines in vitro. A dose-dependent increase in thymidine uptake and cell number IGF-I induced by has been observed in murine mesangial cells exposed to IGF-I under serum-free conditions [29]. In addition, IGF-I has been shown to be a progression factor for human mesangial cell growth in vitro [30]. Further, IGF-I stimulates proteoglycan production by human fetal mesangial cells [31] and vascular endothelial cells [32] in culture. Notably, mesangial cells isolated from non-obese diabetic (NOD) mice (an experimental model of Type I (insulin-dependent) diabetes mellitus) showed a robust phenotypic switch in vitro, consisting of increased IGF-I synthesis and proliferation when compared with the IGF-I effect of mesangial cells from non-diabetic animals [33]. Recent in vitro data obtained in mesangial cells from

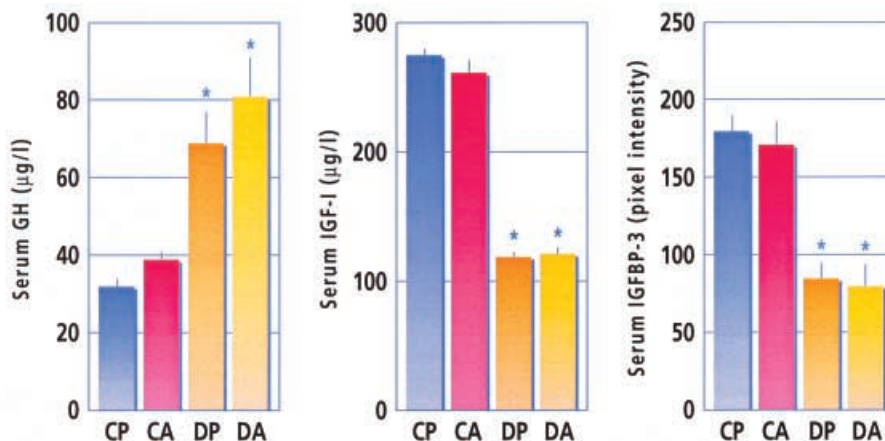


Fig. 2. Changes in serum growth hormone (GH), insulin-like growth factor I (IGF-I) and IGF binding protein 3 (IGFBP-3) in non-diabetic mice treated with placebo (CP) or GH receptor (GHR) antagonist (CA) and diabetic mice treated with placebo (DP) or GHR antagonist (DA) for 1 month. Note the increased serum GH concentrations, low serum IGF-I and IGFBP-3 concentrations in diabetic animals compared with non-diabetic controls. Adapted from [37] with permission

NOD mice, showed that excessive IGF-I production contributes to the altered ECM accumulation in NOD mice, largely through a reduction in metalloproteinase 2 (MMP-2) activity [34], giving further evidence for IGF-I being a major contributor to the development of diabetic glomerulopathy.

In vivo evidence for a role of GH/IGF in diabetic kidney disease

As described above it is well known that poorly controlled diabetes in humans is characterized by GH hypersecretion [9–14] whereas the most widely used experimental animal model for Type I diabetes, the STZ-diabetic rat, is characterized by suppressed circulating GH concentrations, with loss of the characteristic pulsatility early after induction of diabetes [35]. The reason for this discrepancy is still controversial [36]. Just recently we discovered that diabetic mice present with GH hypersecretion and low circulating IGF-I concentrations [37], indicating that this animal strain might be a better model than the diabetic rat for the circulating perturbations in the GH/IGF axis seen in human diabetes (Fig. 2). It seems, however, evident that the difference in the GH/IGF axis between experimental diabetes in rats and diabetes in humans is restricted to GH. Similar changes have been reported for other elements in the GH/IGF axis in poorly controlled diabetic rats and humans, including changes in circulating concentrations of GHBP, IGF-I and IGFBPs [26–28, 38–40]. In addition, specific changes occur locally in the diabetic kid-

ney, involving a number of complex cellular mechanisms with changes in renal GHBP, IGF receptors and IGFBPs.

Decreased serum GHBP concentrations and low hepatic GHR number are well described features in experimental diabetes [35, 38, 39, 41]. In contrast only few data have been published on the renal expression of GHR and GHBP in experimental diabetes. In one study GHR mRNA expression was measured 4 days after induction of STZ-diabetes and unchanged levels were reported, despite decreasing expression in hepatic GHR mRNA [41]. In a recent study including both short-term and long-term diabetic rats, differential changes in kidney GHR and GHBP mRNA were observed [16]. In the cortex, no change was seen in the GHR mRNA throughout the 6 months of observation, but a statistically significant increase in the GHBP mRNA was observed 1 month after induction of diabetes and sustained for the rest of the study period [16]. No changes were seen in GHR or GHBP mRNA in the medullary regions [16]. These data indicate that although the GHR and GHBP mRNAs originate from the same gene, they are differentially regulated during the development of experimental diabetic kidney disease and furthermore imply a specific functional role for GHBP. Whether the increase in renal GHBP mRNA actually enhances renal GH availability to the GHR and thereby enhances a pathophysiological role of GH is still not known.

It is well established that the rapid increase in renal growth and function seen in STZ diabetes is preceded by a rise in renal tissue concentration of IGF-I reaching a maximum at 1–2 days after induction of diabetes and returning to basal concentrations after about 4 days [24, 26–28, 40–47]. The increase in renal IGF-I has recently been confirmed in STZ-diabetic mice [37] and in the spontaneous hyperglycaemic, NOD mouse [48]. The degree of kidney IGF-I accumulation 48 h after injection of STZ is directly proportional to the prevailing blood glucose concentration in animals with varying degrees of metabolic control [47] and strict insulin treatment abolishes

both the increase in kidney IGF-I and renal hypertrophy [42, 43]. Finally, IGF-I infusion into diabetic rats, commencing after the initial renal growth rate has abated, re-accelerates diabetic renal hypertrophy adding further weight to the hypothesis that IGF-I acts as a renotropic growth factor in early experimental diabetes [49].

Further evidence that IGF-I, with the modulating effect of GH, could be involved in both the short-term and long-term renal changes is given in a series of experiments in diabetic dwarf rats. The dwarf rat strain used in these experiments is characterized by having an inherited autosomally recessive gene [50]. Homozygous dwarf rats present with an isolated GH deficiency with about 5–10% of normal pituitary GH content, low circulating GH concentrations and reduced circulating and tissue concentrations of IGF-I but otherwise normal pituitary function [50]. In short-term experiments, STZ-diabetic dwarf rats have slower and lesser initial renal and glomerular hypertrophy as well as a smaller rise in kidney IGF-I than diabetic controls with intact pituitary, indicating that GH itself could be involved in the modulation of renal enlargement [42]. Furthermore, long-term diabetic dwarf rats that have had diabetes for 6 months, have a smaller degree of renal and glomerular hypertrophy and rise in AER compared with the changes in pituitary intact diabetic rats [51]. One possible explanation for the early renal IGF-I accumulation in STZ diabetes is an increased local kidney IGF-I production. Support for this theory comes from a single report in short-term experimental diabetes of a short-lived increase in IGF-I mRNA [52]. This has not, however, been a consistent finding [24] and in a 6-month study in STZ-diabetic rats [23], decreased renal IGF-I mRNA expression was seen from the time of diabetes induction and was sustained for the whole study period as measured by *in situ* hybridization [26]. These findings indicate that the increase in renal IGF-I protein is probably not due to local IGF-I production and that accumulation of renal IGF-I is more likely to be caused by changes in renal IGF receptors and IGFBPs as discussed below.

As indicated above part of the biological actions of IGFs are mediated through their specific IGF receptors. In STZ diabetes no statistically significant changes are seen in the renal IGF-IR mRNA [18, 53] or receptor binding [54] within the first days after induction of diabetes. When focusing on the later phases of the diabetic glomerulopathy taking place in the months after induction of STZ diabetes, a sustained increase in kidney IGF-IR mRNA is, however, seen for up to 3 months [53]. In contrast to the IGF-IR, early increased IGF-II/man-6-PR protein concentrations and mRNA expressions [55] have been shown within the first days after induction of diabetes. These changes are specific for the kidney as no changes occur in the IGF-II/man-6-PR mRNA in lung or heart

over the same period [55]. In long-term diabetes for up to 6 months no changes are, however, seen in the renal IGF-II/man-6-PR mRNA. In conclusion, these results suggest that the two structurally and functionally unrelated IGF receptors show specific time course changes over a time span of 6 months in experimental diabetes and play different parts in the development of the diabetic kidney disease. New results have shown the early renal changes in experimental diabetes are all abolished when diabetic rats are treated with a specific IGF-IR inhibitor (see below).

Concomitantly with the rise in endogenous kidney IGF-I in early STZ diabetes, an increase in kidney IGFBP species is seen [56]. This finding is corroborated by the finding of IGF-I binding in the diabetic kidney to low-molecular-weight material that could represent IGFBPs [18, 46]. In a study describing the renal IGFBP mRNA expression in both short-term and long-term diabetes, very prominent and complex alterations in the renal IGFBP mRNAs and distribution were seen [26]. These changes included a pronounced shift in the distribution in the IGFBP-1 expression [26]. Immediately after induction of diabetes, the cortical IGFBP-1 mRNA expression showed a pronounced increase persisting for up to 6 months whereas the medullary IGFBP-1 mRNA was greatly reduced [26] (Fig. 3). In addition, a sustained increase in the medullary IGFBP-5 mRNA expression was seen but the cortical IGFBP-5 expression was decreased [26]. No great changes were seen in IGFBP-2 or IGFBP-3 mRNAs although there was a sustained decrease in IGFBP-4 mRNA over the 6 months studied [26]. The pathophysiological effects of these changes in renal IGFBP expression in diabetes are not known. The striking and sustained redistribution of renal IGFBP-1 mRNA from medulla to cortex is, however, of note because recent studies have suggested that some IGFBPs, in addition to acting as carriers for IGFs, also operate as local modulators of IGF action. Such mechanisms could in particular be relevant for IGFBP-1 because it contains an Arg-Gly-Asp motif near the C-terminal, which could enable it to interact with cell surfaces and deliver IGF to adjacent IGF receptors, enhancing the subsequent binding and action of IGFs on the cell. In other studies IGFBP-1 has, however, been found to inhibit cellular IGF-I binding and action. The changes in IGFBP mRNA described above [18, 26, 56] occurred in the very early stages of the diabetes-associated renal changes, thereby preceding the early renal and glomerular hypertrophy. Furthermore, the changes were sustained for up to 6 months after induction of diabetes during the period when the development of diabetic glomerulopathy takes place. The IGFBP mRNA changes support the notion, both in duration and localization, that some IGFBPs are involved in the development of the renal changes in experimental diabetes. New results have shown the early rises

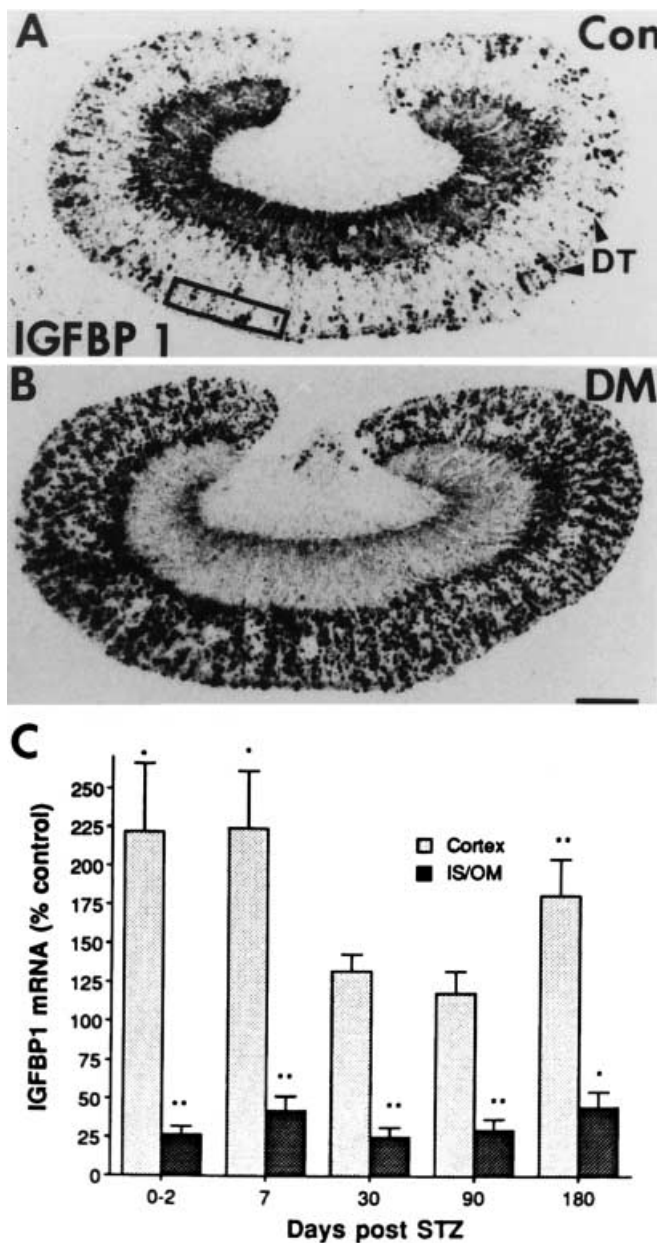


Fig.3A–C. Insulin-like growth factor binding protein 1 (IGFBP-1) messenger RNA (mRNA) expressions in control (Con) and streptozotocin (STZ) diabetic (DM) rat kidneys over a period of 180 days as shown by in situ hybridization. **A** and **B**, representative autoradiographs of longitudinal sections of kidneys from control and day 2 diabetic animals. **C**, quantitative densitometry results indicating the effects of STZ diabetes on renal IGFBP-1 mRNA. At each time-point concentrations in diabetic animals are expressed as a percent of the concentrations measured in the non-diabetic control group. Adapted from [26] with permission. DT, distal tubule; IS/OM, inner stripe/outer medulla

in renal IGFBP-1 mRNA, renal IGF-I and renal hypertrophy are all abolished when diabetic animal models are treated with long-acting somatostatin analogues, GHR antagonists or an inhibitor of advanced glycation end products (AGEs) (see below).

Table 1. Potential new therapeutic agents in the treatment of diabetic kidney disease: antagonists of the pathophysiologically altered growth hormone (GH)/insulin-like growth factor (IGF) system

Antagonists of the GH/IGF system

- 1 Long-acting somatostatin analogues
- 2 GH receptor antagonists
- 3 IGF-I receptor antagonists
- 4 AGE inhibition
- 5 ACE inhibition

For further explanation and references please see text

Agents with effects on the altered GH/IGF axis in diabetic kidney disease

Agents that have been shown to affect the GH/IGF axis and exert beneficial renal effects in diabetic kidney disease in experimental animal models are given in Table 1. Each agent is discussed in detail below.

Long-acting somatostatin analogues. The hypothesis that somatostatin and its long-acting analogues could be used in the treatment of subjects with diabetes was initially based on the expected benefit of suppressing increased circulating GH concentrations in order to minimize their deleterious effects on diabetic metabolic aberration and to prevent development of long-term diabetic complications. The biological half-life of native somatostatin is too short to allow its use in clinical therapy and accordingly, long-acting somatostatin analogues (e.g. octreotide and lanreotide) have been developed and opened the possibility of doing experimental and clinical trials. In short-term experimental diabetes, octreotide treatment for 7 days from diabetes onset completely inhibits the initial renal hypertrophy and the kidney IGF-I accumulation in rats [40, 44]. Further, lanreotide in a dose similar to the octreotide dose given above has been shown to inhibit renal glomerular growth as well [57]. When a lower octreotide dose is used a partial inhibition of the diabetes-associated renal growth is seen, which suggests a dose dependent effect of octreotide on renal IGF-I accumulation and diabetic renal growth [58]. A series of recent experiments has shown that early intervention with somatostatin analogues after diabetes onset seems crucial for achievement of full inhibitory effects on morphological chan-

ges. When postponing initiation of octreotide treatment 3 to 9 days after diabetes induction the early diabetic renal hypertrophy is only partly inhibited, indicating that early intervention with somatostatin analogues is important [59]. Similar results were observed in a study in which octreotide treatment was initiated for 3 weeks after a period of untreated diabetes for 3 or 6 months, as a tendency only towards a reduction in renal volume was observed [60]. The effects of somatostatin analogues on circulating and local IGFBPs in diabetes have only been examined to a limited extent. Lanreotide has been shown to inhibit the diabetes-associated increase in serum 30000 M_r IGFBP species (containing IGFBP-1) in rats and further to reduce serum IGFBP-3 [57]. In addition, octreotide inhibits the increased renal IGFBP-1 mRNA expressions in early experimental diabetes, with no effect on hepatic IGFBP-1 mRNA expressions [61]. This is in contrast to the effect of octreotide on IGFBP-1 mRNA expressions in non-diabetic rats, in which a dose-dependent stimulatory effect of octreotide on hepatic IGFBP-1 mRNA is seen along with unchanged renal IGFBP-1 mRNA expressions [62]. Accordingly, it can be hypothesized that somatostatin analogues prevent some of the classic early diabetic renal changes by inhibiting the renal IGF-I accumulation through mechanisms involving effects on renal IGFBPs.

The effects of somatostatin analogue treatment on diabetic renal morphological and functional changes have also been examined in long-term experimental diabetes. Treatment with octreotide for 6 months from the day of diabetes induction is followed by statistically significant reductions of increase in kidney weight, kidney IGF-I concentrations and AER compared with untreated diabetic rats [63]. In addition, diabetic rats treated for 5 weeks with octreotide from diabetes onset showed a reduction in renal glomerular growth [64]. When octreotide treatment for 14 weeks was initiated in unilaterally nephrectomized diabetic rats on the day of diabetes induction 3 days after the nephrectomy, no effect of octreotide treatment on renal hypertrophy, AER or glomerular hyperfiltration was, however, observed [64]. In some studies [65, 66] octreotide treatment for 2–3 weeks was followed by diminished kidney hypertrophy and albuminuria in uninephrectomized-diabetic rats after 15 weeks of untreated diabetes. A recent study from our group aimed at examining the effect of 3 weeks of treatment with octreotide and an angiotensin-converting enzyme inhibitor (ACEi) (captopril) either alone or in combination after 3 months of untreated diabetes [67]. Octreotide treatment alone and in combination with captopril induced a statistically significant reduction in kidney weight compared with diabetic animals treated with placebo [67]. Further, the combined treatment of octreotide and captopril was followed by a statistically significant reduction in

AER compared with diabetic rats treated with placebo giving evidence for a definite effect of the combined treatment with octreotide and captopril on manifest experimental diabetic renal changes [67].

GH-receptor antagonists. A series of highly specific antagonists of the GH action has recently been developed for the potential therapeutic use in various pathophysiological conditions, including diabetes. Initially it was shown that alteration of single amino acids in the third α -helix of bovine (b)GH (residues 109–126) results in a GH antagonist [68, 69]. In vitro experiments showed that this new group of GH antagonists binds to the GH receptor with the same affinity as native GH but in vivo a phenotypic dwarf animal characterized by low circulating IGF-I concentrations and a proportional body composition develops when the GH antagonist is expressed in transgenic (TG) mice [68, 69]. Studies in long-term diabetic GH antagonist TG mice, that express the GH antagonist (bGH-G119R or hGH-G120R), have shown that these animals are protected against development of diabetic renal changes [70, 71]. Compared with TG diabetic mice expressing wild-type bGH or bGH, the diabetic mice expressing GH antagonists showed less glomerular damage, no increase in total urine protein, no glomerular hypertrophy and no increase in glomerular α 1 type IV collagen mRNAs [70, 71]. The inhibitory effects of GH antagonists in transgenic mice were seen without alteration in glycaemic control as similar concentrations in blood glucose, insulin and HbA_{1c} were seen in the different diabetic animals expressing wild-type bGH, GH antagonists or bGH [70, 71]. These results are further supported by data obtained in a recent study in diabetic *GHR/GHBP* knockout (KO) mice, where disruption of the *GHR/GHBP* gene was followed by protection against diabetes-induced renal changes, despite equivalent levels of hyperglycaemia [72]. Theoretically GH antagonist TG mice and *GHR/GHBP* KO mice could, however, be less susceptible to diabetic renal changes because of effects of GH receptor blockade and low circulating IGF-I concentrations before the induction of diabetes. Furthermore, the renal effects might be mediated indirectly through the low serum IGF-I concentrations themselves at the time of diabetes induction. To investigate the potential usefulness of GH antagonists as a therapeutic agent in diabetic kidney disease for tolerance and specificity, we recently did a series of studies with exogenous treatment of a long-acting GH antagonist (G120K-PEG) to diabetic mice [37, 73, 74]. In the initial experiment non-diabetic and STZ-diabetic mice were treated with the GH antagonist for 1 month after diabetes induction [37]. In GH antagonist-treated diabetic mice, renal IGF-I accumulation, renal enlargement and glomerular hypertrophy were abolished and the diabetes-associated increase in AER

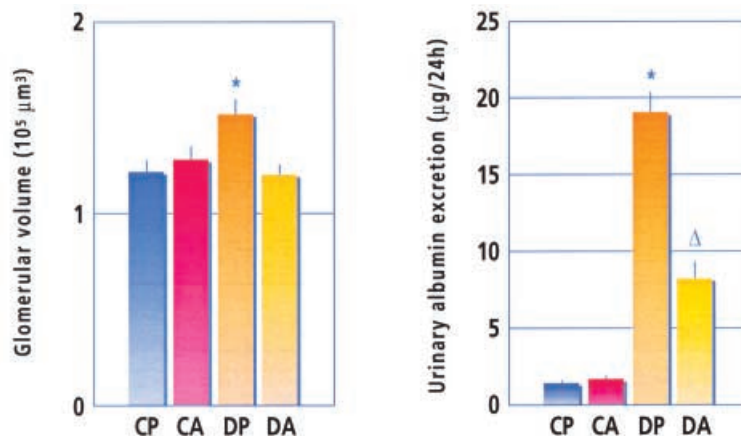


Fig. 4. Inhibitory effect of a specific growth hormone receptor (GHR) antagonist of the diabetes associated glomerular hypertrophy and rise in urinary albumin excretion (UAE) in non-diabetic mice treated with placebo (CP) or GHR antagonist (CA) and diabetic mice treated with placebo (DP) or GHR antagonist (DA). Adapted from [37] with permission

was also reduced [37] (Fig. 4). These effects were achieved through a specific mechanism at the renal GHR level, as no effect of treatment was seen on changes in body weight, food consumption, metabolic control, serum GH or IGF-I [37] (Fig. 2). These results have been confirmed in NOD mice [73]. In a recently conducted experiment the effect of GH antagonism alone or in combination with ACEi treatment was examined on manifest renal changes in NOD mice [74]. Preliminary results showed that GH antagonist treatment was as potent as ACEi treatment in reducing AER [74].

IGF-I receptor antagonists. Through a three-dimensional modelling of the IGF-I protein, the synthesis of selective antagonists of the IGF-I receptor (by mimicking the D-domain of IGF-I) have been designed [75]. The series of IGF-I receptor antagonists include 12 amino-acid cyclic peptides one of which (JB3) is synthesized from D-isomer rather than L-isomer to reduce susceptibility to proteolytic degradation [75]. The JB3 peptide can therefore be used in vivo and in a recent study in short-term experimental diabetes in rats, the diabetes-induced rise in renal size, kidney protein and DNA were all normalized by JB3 treatment [77]. The effects of long-term JB3 treatment in experimental models of diabetes are not known. Notably, in a recent study using a model of non-diabetic retinopathy in rats, treatment with JB3 suppressed retinal neovascularization and in addition, suppressed the rise in retinal VEGF suggesting VEGF is a downstream mediator of IGF-I effects [78] (see also below). Further, both IGF-I [79] and VEGF [80] have been shown to stimulate nuclear fac-

tor- κ B (NF- κ B) concentrations in cultured endothelial cells, adding more weight to the hypothesis of an interrelation between the two systems in retinal pathology. Whether these pathways are also operative in mediating the GH and IGF-I effects in diabetic renal changes is not known.

Advanced glycation end-product (AGE) inhibition. Increased formation of advanced glycation end products (AGEs) is a common phenomenon in diabetes with possible implications in the development of diabetic complications, including diabetic kidney disease. The formation of AGEs arise from a non-enzymatic reaction between glucose and biologically relevant structural proteins. Advanced glycation end products are known to mediate their effects both through receptor-dependent and receptor-independent mechanisms and in vitro evidence seems to indicate that AGEs also stimulate the synthesis of various growth factors, including IGF-I and TGF- β (see also below). Notably, a recent study in long-term STZ-diabetic rats [81] showed the classic changes in the intra-renal IGF axis, with decreased IGF-I mRNA [24, 26] and IGFBP-4 mRNA [26] and increased IGFBP-1 mRNA expression over a diabetes duration of 8 months [26]. Oral treatment with aminoguanidine, a potent inhibitor of AGE formation with renoprotective actions [81], reduced renal IGFBP-1 mRNA expression to normal, along with partial restoration to normal of diabetes-associated changes in IGFBP-4 and IGF-I mRNA [81]. The partial restoration to normal of the diabetes-associated changes in the IGF system could contribute to the aminoguanidine-induced renoprotection.

Angiotensin converting enzyme (ACE) inhibition. The possible effects of ACEi on the GH/IGF axis in diabetic kidney disease have not been examined extensively. In one short-term experimental study, trandolapril treatment for 1 week had no effect on renal IGF-I accumulation or renal changes, despite a pronounced reduction in renal ACE activity [82]. Still as mentioned above, late intervention with an ACEi

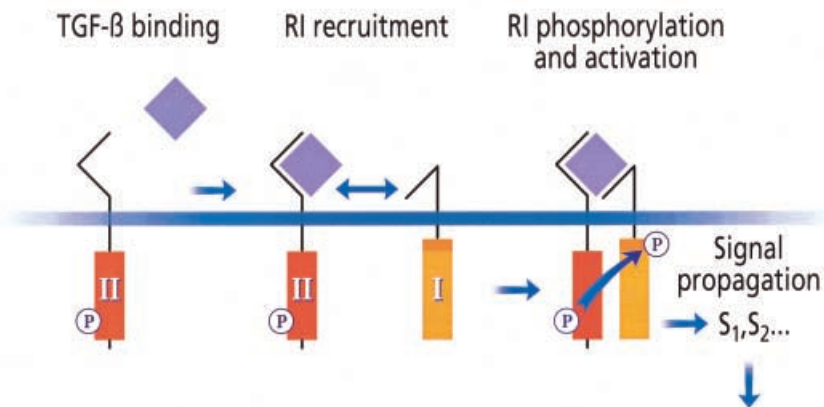


Fig. 5. Schematic depiction of signalling through the transforming growth factor β (TGF- β) receptors. After initial binding of TGF- β to the TGF- β Type II receptor (II), the TGF- β /TGF- β Type II receptor complex binds to the TGF- β Type I receptor (I) with subsequent phosphorylation of the latter and intracellular signalling. Adapted from [84] with permission

rectly to the TGF- β type II receptor (TGF- β type IIR) which is a constitutively active kinase. The TGF- β bound to the type II receptor is then recognized by the TGF- β type I receptor (TGF- β type IR) which is recruited to form a ternary complex with subsequent phosphorylation of the TGF- β type IR and induction of intracellular signalling [84].

and a long-acting somatostatin analogue, octreotide, given either alone or in combination, monotherapy with either drug had no effects on renal IGF-I concentrations or functional variables although attenuating effects of these drugs were seen when given in combination [67]. These data indicate that, although the renoprotective effects of ACEis on early renal changes in diabetes might not be mediated through the IGF-I system, they could still be effective, even on manifest renal changes, when the two different therapeutic regimens are applied [67].

Transforming growth factor β (TGF- β)

The TGF- β system

The TGF- β s belong to a superfamily of several related proteins, including three mammalian TGF- β isoforms (TGF- β 1, TGF- β 2, and TGF- β 3), activins, inhibins and bone morphogenic proteins. The TGF- β isoforms are members of a family of cell regulatory proteins that are unique among growth factors in their broad effects on extracellular matrix. Transforming growth factor- β 1 is a 25000 M_r homodimer polypeptide, synthesized as an inactive precursor protein which possibly binds to a TGF- β -binding protein of 125000 M_r and is proteolytically changed into its active form [83]. The two other TGF- β isoforms (TGF- β 2 and TGF- β 3) are believed to be activated the same way as TGF- β 1. The manner in which TGF- β isoforms induce intracellular signalling through a complex cascade of receptor binding involving two different types of TGF- β receptors has been shown experimentally [84] (Fig. 5). In brief, TGF- β binds di-

Expression of the TGF- β system in the normal kidney

The kidney is a site of TGF- β production and a target of TGF- β action, as both mRNA for TGF- β isoforms and receptors and the active TGF- β proteins have been shown in all cell types of the glomerulus [84–88] and proximal tubular cells, including localization in both nuclei and cytoplasm [88, 89]. In a recent study from our group a full characterization of the renal distribution of all three TGF- β isoforms and the three TGF- β Rs was done in normal and diabetic rats [88] (see also below).

In vitro evidence for TGF- β effects on kidney cells

In vitro, TGF- β has been shown to modulate extracellular matrix production and notably both glomerular mesangial and epithelial cells increase synthesis of extracellular matrix proteins including proteoglycans, fibronectin, type IV collagen and laminin in response to TGF- β [90–92]. In addition, TGF- β inhibits the synthesis of collagenases and stimulates tissue production of metalloproteinase inhibitors [93, 94]. Both these mechanisms could lead to a reduced degradation of ECM and possibly contribute to matrix accumulation. Evidence has been published recently for TGF- β playing a part in different forms of kidney diseases characterized by changes in structure and ECM. In cultured glomerular cells obtained from immunologically induced glomerulonephritis, increased amounts of active TGF- β can be detected [95].

In addition, there is in vitro evidence for TGF- β playing a part in the development of diabetic nephropathy. High glucose concentrations increase TGF-

Table 2. Potential new therapeutic agents in the treatment of diabetic kidney disease: antagonists of the pathophysiologicaly altered transforming growth factor β (TGF- β) system

Antagonists of the TGF- β system

- 1 Neutralizing TGF- β antibodies
- 2 ACE inhibition
- 3 PKC β inhibition
- 4 AGE inhibition

For further explanation and references please see text

β 1 mRNA expression in both cultured mesangial cells and proximal tubular cells in vitro [85–89]. Further, increased glucose concentrations in vitro stimulate TGF- β mRNA expression and bioactivity, cellular hypertrophy and collagen transcription in proximal tubules [89] in addition to increased mesangial production of heparan sulphate proteoglycan [96].

In vivo evidence for a role of the TGF- β system in diabetic kidney disease

Other than the in vitro studies mentioned above reports have been published recently on changes in renal TGF- β in experimental short-term diabetes in vivo. In a study in STZ-diabetic rats, an increase in glomerular TGF- β 1 mRNA was reported as early as 24 hours after the onset of hyperglycaemia [97]. The animals were followed for up to 2 weeks with a sustained rise for the whole period and a threefold increase in glomerular TGF- β 1 concentrations could be shown by use of immunohistochemistry [97]. Intensive insulin treatment to restore blood glucose concentrations to normal attenuated the rise in glomerular TGF- β expression [97]. Increased renal expressions of TGF- β 1 mRNA in the early course of diabetes-associated renal hypertrophy have also been described in the NOD mouse [98, 99] and the diabetic BB rat (an experimental model of Type I diabetes) [97]. In addition, a sustained increase in glomerular TGF- β 1 mRNA expressions in long-term STZ-diabetic rats with a diabetes duration for up to 24 weeks has been described [85]. Further, in a non-diabetic transgenic mouse model overexpressing glomerular TGF- β 1, accumulation of extracellular matrix components (i.e. laminin and collagen IV) was observed [100]. Previous studies [85, 97–99], have focussed on changes in only one TGF- β isoforms (i.e. TGF- β 1)

and, in most studies, only the mRNA expressions were examined. This provided us with the impetus to carry out a rigorous characterization of the changes in the whole TGF- β system [i.e. TGF- β 1, TGF- β 2, TGF- β 3 isoforms and TGF- β type RI, RII and TGF- β type III receptors (RIII's)] in acute and chronic diabetes [88]. The results showed that of all the components of the TGF- β axis studied, the TGF- β 1 and TGF- β 2 isoforms and the TGF- β type RII proved to be the most responsive elements within the glomerulus and tubules in response to diabetes induction [88]. Although concentrations of TGF- β 1 protein decreased in the glomerulus, increased concentrations could be detected in the tubules by immunohistochemistry [88]. This observation along with the TGF- β 1 mRNA and TGF- β 1 protein concentrations in cortical homogenates being increased in diabetes, indicates a pronounced compartmentalization of the changes in TGF- β 1 in diabetes. By contrast to the changes in glomerular TGF- β 1 concentrations, the glomerular TGF- β 2 isoform concentrations increased statistically significantly in response to induction of diabetes [88]. Of further note the spatio-temporal rise in TGF- β 2 corresponded exactly with that of pro-collagen-1C peptide, a marker of renal fibrosis [88]. Concentrations of glomerular TGF- β type RII protein were also considerably increased during development of diabetes [88]. This observation is of note because the TGF- β type RII is a necessary receptor for signalling the fibrogenic response to the nucleus as described above [84] (Fig.5). It seems that glomerular TGF- β type RII is coordinately up-regulated with the glomerular TGF- β 2 isoform in diabetes, suggesting that fibrogenic signalling is up-regulated, which could result in the increased rate of fibrosis observed in the diabetic kidney. These experimental studies support the hypothesis that the glucose-induced rise in renal TGF- β expression and peptide in the kidney are responsible for some of the renal changes that precede the development of diabetic nephropathy.

Agents with effects on the altered TGF- β axis in diabetic kidney disease

Agents that have been shown to affect the TGF- β axis and exert beneficial renal effects in diabetic kidney disease in experimental animal models are given in Table 2. Each agent is discussed in detail below.

Neutralizing antibodies. Data supporting the hypothesis that the glucose-induced rise in renal TGF- β expression and peptide is responsible for some of the renal changes that follow the development of diabetic nephropathy have been published in studies using neutralizing antibodies. In vitro the glucose-mediated increase in type IV collagen synthesis in mesangial

cells is dependent on the autocrine action of TGF- β 1 because neutralizing antibodies to TGF- β attenuate the rise [101]. Further, in vivo treatment of STZ-diabetic mice for 9 days with a neutralizing TGF- β 1, TGF- β 2, TGF- β 3 antibody attenuated the increased renal TGF- β 1 and TGF- β type IIR mRNA expressions and reduced the diabetes-associated renal/glomerular growth and enhanced renal expression of collagen IV and fibronectin [102].

Angiotensin converting enzyme (ACE) inhibition. It has been suggested that activation of the renal TGF- β system in diabetes is mediated through, apart from a direct stimulatory effect of hyperglycaemia itself, an activation of the renin-angiotensin system because exposure of mesangial cells in vitro to angiotensin II stimulates the expression of TGF- β and extracellular matrix proteins [103]. Further a recent study examined the effect of an ACEi (captopril) on changes induced by high glucose concentrations in the TGF- β system and growth in LLC-PK₁ cells, a porcine kidney cell line analogous to the proximal tubule cell [104]. In this cell system high-glucose concentrations increased TGF- β 1 mRNA, TGF- β Type IR and IIR protein expressions and cellular hypertrophy whereas cellular mitogenesis was inhibited. Captopril dose-dependently decreased TGF- β Type IR and IIR protein expressions and cellular hypertrophy, increased cellular hyperplasia but TGF- β mRNA was unchanged [104]. Another study examined the effects of ACE inhibition (ramipril) in vivo in diabetic rats but looked only at changes in TGF- β 1 mRNA and protein in the renal tubulointerstitium and not at possible changes in the glomeruli [105]. Using different techniques it was shown that the diabetes-associated tubular-interstitial increase in TGF- β 1 and collagen IV (both mRNA and protein) was prevented by ACE inhibition. A recent study from our group aimed at examining the possible effect of ACE-inhibition on the intra-renal (in particular the glomerular) changes in all TGF- β isoforms (TGF- β 1, TGF- β 2, TGF- β 3) and TGF- β receptors (type I, type II, type III) in experimental diabetes [106]. Streptozotocin-diabetic and non-diabetic rats were treated for up to 4 weeks with enalapril or placebo. Enalapril partially prevented the diabetes associated renal hypertrophy and fully prevented the increase in AER. Further, enalapril treatment decreased the glomerular concentrations of TGF- β type IR and TGF- β type IIR isoforms to values below those of non-diabetic control and decreased the glomerular TGF- β type IIR to almost undetectable concentrations. The glomerular expression of the TGF- β isoforms were not greatly influenced by treatment [106]. These findings suggest that the TGF- β axis operating through a complex intra-renal system, are a statistically significant mediator of the renal changes observed in experimental diabetes. Moreover, ACE inhibition has pro-

nounced inhibitory effects on the increased concentrations of the TGF- β receptors required for intracellular signalling through this growth factor system (Fig. 5). These experimental findings suggest a possible new mechanism of action for ACEi through regulation of a pathophysiologically enhanced circulating and renal TGF- β system.

Protein kinase C (PKC) β inhibition. Hyperglycaemia-induced activation of PKC and diacylglycerol (DAG) has been suggested to be an important mechanism in the endothelial dysfunction and long-term vascular complications in diabetes [107]. Based on in vitro experiments, it has been proposed that PKC activation in glomerular cells induced by high glucose concentrations is followed by increased TGF- β activity [108] and mitogen-activated protein (MAP) kinase activity [109]. In a recent study, treating mesangial cells exposed to high glucose concentrations with antioxidant α -tocopherol blocked the increase in PKC, TGF- β and matrix accumulation but did not alter the effect of TGF- β -induced matrix production [108]. In vivo experiments in which a specific PKC β isoform inhibitor (LY 333 531) was given to STZ-diabetic rats for 3 months the diabetes-associated increase in glomerular TGF- β 1 mRNA, matrix accumulation and vascular dysfunctions [i.e. increased glomerular filtration rate (GFR) and AER], were abolished suggesting that hyperglycaemia-induced PKC β activation is involved in the mediation of abnormal glomerular and mesangial functions, such as overproduction of TGF- β and matrix components [110, 111]. Further, in a recent long-term study in diabetic *db/db* mice (an experimental model of Type II (non-insulin-dependent) diabetes mellitus), PKC β inhibition reduced long-term renal changes, including mesangial expansion [112]. Glomerular TGF- β 1 immunostaining was increased in placebo-treated *db/db* mice and partly restored to normal in mice treated with PKC β inhibitor [112]. In a recent study treating STZ-diabetic rats for 1 month with the same specific PKC β isoform inhibitor (LY 333 531) showed, however, renoprotective effects, without effect on any components of the intra-renal TGF- β system [113]. In contrast to other studies [110, 112], this study included a full characterization of changes in all TGF- β isoforms and receptors, thus indicating that the renoprotective effects of PKC β inhibition in vivo might not strictly be mediated through the TGF- β system.

Advanced glycation end-product (AGE) inhibition. In an experimental model of Type II diabetes [the Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat] renal TGF- β 1 mRNA and glomerular TGF- β 1 immunoreactivity was reported to be increased over a diabetes duration of 9 to 68 weeks [114]. Oral treatment with a new AGE-inhibitor (OPB-9195) for up to 68 weeks reduced the increased renal expressions of

TGF- β 1 mRNA and concentrations of protein to normal, along with restoring diabetes-associated renal collagen IV accumulation to normal and diminishing the increased AER [114].

Vascular endothelial growth factor (VEGF)

The VEGF system

The group of VEGFs consists of at least five different isoforms of homodimeric glycoproteins [115–122]. Further, at least two high-affinity VEGF receptors, (VEGFR-1) and VEGFR-2, have been described [118]. Vascular endothelial growth factor is a 46000 M_r homodimeric glycoprotein with pronounced angiogenic actions [116, 119–121] and effects on vascular permeability [115, 119, 124]. The expression of VEGF was initially described to be considerably increased in highly vascularized, rapidly growing tumours [123] and VEGF has been shown to be a potent mitogenic factor for endothelial cells [121, 122]. The two best described VEGF receptors (VEGFR-1 and VEGFR-2), also known as the *fms*-like tyrosine kinase (Flt) and fetal liver kinase 1 (Flk-1), are high-affinity transmembrane, tyrosine kinase receptors [118]. The VEGFs and their receptors are crucial for embryologic development because disruption of the VEGF system, as seen in KO animals, is lethal [125, 126].

Expression of VEGF system in the normal kidney

Both VEGF and the two VEGFRs are expressed in the kidney [127–133]. Vascular endothelial growth factor expression and specific VEGF binding has been described in rat [127] and human kidney [128–131]. It has been localized to the epithelial glomerular cells [131, 132], the podocytes [132, 133] distal tubules and renal collecting ducts [129, 132]. Further, the VEGFR-2 has been localized mainly to the glomerular endothelial cells and cortical interstitial fibroblasts [132].

In vitro evidence for VEGF effects on kidney cells

In vitro mesangial cells, glomerular endothelial cells, vascular smooth muscle cells, proximal and distal tubular cells are capable of producing VEGF [133–135]. The most potent stimulator of VEGF that has been found is hypoxia [124, 136] but in mesangial cells angiotensin II has been shown to stimulate VEGF, a process that is blocked by co-incubation of losartan, an angiotensin 1 receptor antagonist [133–135]. High glucose concentration has been shown to stimulate VEGF expression in vascular

smooth muscle cells [137], probably through PKC stimulation [138]. In addition, phorbol esters stimulate mesangial VEGF production [139] and notably, inhibition of PKC blocks VEGF signalling [140]. Further, various growth factors and cytokines, including TGF- β [141, 142], IGF-I [143], basic fibroblastic growth factor (bFGF) [144] and platelet derived growth factor (PDGF) [136, 145] have been shown to stimulate VEGF production in various non-renal cell types. Finally, as mentioned above, both IGF-I [79] and VEGF [80] stimulate NF- κ B concentrations in endothelial cells in vitro.

In vivo evidence for a role of the VEGF system in diabetic kidney disease

In the recent study in OLETF rats described above [114], renal VEGF mRNA and glomerular VEGF immunoreactivity was reported to be increased over a diabetes duration of 9 to 68 weeks [114]. In another purely descriptive study, changes in renal VEGF concentrations were described in STZ-diabetic rats with a diabetes duration of 3 and 32 weeks [132]. Vascular endothelial growth factor mRNA and protein were localized mainly to the glomerular epithelial cells and VEGFR-2 mRNA mainly to glomerular endothelial cells [132]. Vascular endothelial growth factor mRNA and peptide were increased in diabetic animals at both time-points examined but the expression of VEGFR-2 and VEGF receptor binding were increased at 3 weeks only [132].

Agents with effect on the altered VEGF axis in diabetic kidney disease

Although the area of identifying and developing specific antagonists of a pathophysiologically enhanced VEGF system in oncology and different eye diseases has attracted increasing interest in the last few years [146], only a few studies have been published on the effect of VEGF antagonists in diabetic kidney disease. These studies are described in detail below along with some promising new candidates.

Neutralizing antibodies. Data supportive for the hypothesis that an enhanced renal VEGF system is responsible for the diabetes-associated renal changes, have been published in a study using an anti-VEGF monoclonal neutralizing antibody in STZ-diabetic rats [147]. Treatment for 6 weeks with the VEGF antibody fully abolished the diabetes-associated hyperfiltration and partially abolished the increase in AER [147]. Treatment with VEGF antibody had no effect on metabolic control in diabetic animals and no renal effects of treatment were seen in non-diabetic controls [147].

Advanced glycation end-product (AGE) inhibition. In the study described above in an animal model of Type II diabetes [114], it was shown that long-term AGE-inhibitor (OPB-9195) treatment of diabetic animals abolished the enhanced renal VEGF mRNA and glomerular VEGF immunoreactivity along with renoprotection, by restoring diabetes-induced renal collagen IV accumulation to normal and reducing the rise in AER [114].

Other potential treatments. Although not proved, several of the available agents that have beneficial renal effects in diabetic animal models could turn out in the near future to involve VEGF as a downstream cytokine. As mentioned above VEGF has been shown to be stimulated by IGF-I [143] and further, IGF-IR blockade in an ischaemia-induced retinopathy model has been shown to lower enhanced retinal VEGF expression along with reducing retinal neovascularization [78]. In addition, blockade of PKC β activity with a specific inhibitor (LY333531) suppresses the VEGF-induced alterations in retinal leakage, retinal blood flow and ischaemia-induced retinal neovascularization [148–150]. Future studies are warranted to fully explain the role of VEGF as a downstream mediator for some of the pathways leading to diabetic renal damage.

Epidermal growth factor (EGF)

The EGF system

The EGF family is a group of peptide growth factor ligands and receptors which is being expanded by continuing studies [151–153]. At present the EGF family comprises four receptors, the EGF receptor (EGFr), erb-B2, erb-B3 and erb-B4 [153]. These receptors are activated by EGF ligand binding followed by homodimerisation and heterodimerisation of the receptors [153]. The EGFr, which is a transmembrane tyrosine kinase receptor, is the most explored receptor of the EGF family and it has been shown that other than activation by EGF, the EGFr is also stimulated by other related ligands such as transforming growth factor α , heparin-binding EGF, amphiregulin, betaregulin and epiregulin.

Expression of the EGF system in the normal kidney

The kidney is one of the main sites of EGF synthesis and several studies have suggested that the kidney is also a target organ for ligands of the EGF family [154]. Epidermal growth factor receptors are present in mesangial cells, in medullary interstitial cells and in the basolateral cell membrane of proximal tubular cells and in the collecting ducts [155–159]. Epidermal

growth factor is produced in the thick ascending limb of Henles and in the distal tubules and accordingly it is unlikely that EGF itself stimulates the renal EGFr. It is therefore believed that it is not EGF but the other ligands of the EGF family, produced locally in the kidney, which are responsible for stimulation of the EGFr.

In vitro evidence for EGF effects on kidney cells

In cell culture systems EGF has been shown to stimulate proliferation of renal tubular cells, with a possible effect through the pentose phosphate pathway and the Na⁺/H⁺ antiporter [157, 160]. Further, EGF has been shown to stimulate phosphorylation and to inhibit gluconeogenesis in rat proximal tubule cells [159]. Finally, the EGF-induced mitogenesis in proximal tubular cells is enhanced by angiotensin II [161], thereby suggesting a possible interaction between the EGF and the renin angiotensin systems.

In vivo evidence for a role of EGF in diabetic kidney disease

In vivo treatment with EGF systemically or directly into the renal artery increases urine flow and urinary sodium and potassium excretion [162]. The finding that EGF has short-term effects on renal function [163, 164] opens some possibilities for a physiological role of EGF in the kidney. Although one study [148] reported reduced urinary EGF excretion after reduction of renal mass, others reported increased urinary EGF excretion [165], increased renal EGF synthesis and a transiently increased amount of immunoreactive EGF in distal kidney tubules after unilateral nephrectomy [166]. In experimental diabetes a possible role for EGF in the *early* renal enlargement has been proposed. In one study [167] a pronounced increase in diurnal urine EGF excretion was seen but no statistically significant change in renal EGF within the first 7 days after induction of diabetes. These findings were confirmed in a recent study [168] where unchanged concentrations of kidney and plasma EGF were reported, within the first week after induction of diabetes, along with a threefold increase in urinary EGF excretion. In addition, renal EGF mRNA as measured both by northern blotting and in situ hybridization, was increased by 4–8 times [168, 169]. After a diabetes duration of 2–3 weeks the urinary EGF excretion was restored to normal or even tended to reach subnormal levels compared with non-diabetic animals [169, 170]. These studies implicate that EGF plays a part as a renotropic factor in early experimental diabetes. So far no studies have been published on changes in the renal EGF content or EGF excretion in long-term experimental diabetes.

Agents with effects on the altered EGF axis in diabetic kidney disease

As is the case for VEGF only few studies have been published on pharmacological manipulation of the altered EGF axis in diabetic kidney disease. No specific EGF inhibitors have been designed. Some antihypertensive drugs have, however, proved effects on the EGF production or excretion or both in experimental diabetes as indicated below.

Adrenergic blockade. In a recent study in experimental diabetes the effect of α -adrenergic blockade (prazosin), β -adrenergic blockade (propranolol) or combined treatment was examined in STZ-diabetic rats [170]. Treatment with prazosin or propranolol alone or both drugs in combination, reduced diabetic renal hypertrophy, the rise in renal EGF content and the rise in urinary EGF excretion to the same extent, without altering metabolic control of the diabetic animals [170]. These results seem to indicate a newly discovered relation between the sympathetic nervous system, EGF and early renal enlargement in diabetes.

Conclusions

Despite beneficial effects of intensified metabolic control, early treatment with ACEi in microalbuminuric Type I diabetic patients and intensified antihypertensive treatment in Type II diabetic patients, the development of diabetic nephropathy is still a huge clinical problem. The pathogenesis of diabetic kidney disease is believed to be multifactorial and various pathophysiological mechanisms have been suggested, apart from the well-known effects of hyperglycaemia and hypertension themselves. This article has reviewed recent evidence of the importance of growth factors and cytokines in the development of diabetic kidney disease. The knowledge we have today indicates that growth factors and cytokines, through complex endocrine and paracrine systems could be responsible for early and late renal changes in experimental and human diabetes in the absence of sufficient insulin action. Insight into the regulation of growth factors and cytokines in diabetic kidney disease have allowed specific antagonists of these systems to be developed and recent studies have shown that treating with such antagonists in experimental diabetes models is followed by renoprotection. Notable observations in recent years are that there is most likely an important interaction or cross-talk among various growth factors that could promote the development of diabetic kidney disease and that combined inhibition of several of these potential pathogenic pathways towards the development of diabetic kidney disease could be more efficient than application of a single antagonism. This would imply that strate-

gies which involve multiple effects on the development of diabetic kidney disease could be more effective than drugs which influence one pathway. The combination of inhibitors with different points of action have the potential to provide new therapeutic strategies in the treatment of diabetic kidney disease.

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