INTRODUCTION

Type 2 diabetes is the commonest form of diabetes constituting 90% of the diabetic population. The global prevalence of diabetes is estimated to increase to 5.4% by the year 2025. The World Health Organization has predicted that the major burden will occur in the developing countries. There will be a 42% increase from 51 to 72 million in the developed countries and 170% increase from 84 to 228 million, in the developing countries. The countries with the largest number of diabetic people are, and will be in the year 2025, India, China and United States. 1 Diabetes mellitus, a metabolic disorder characterised by high levels of blood glucose, is associated with several vascular complications. Although insulin treatment, oral medications, dietary regulations and exercise can delay the development of diabetic microangiopathy, the development of macroangiopathy cannot be prevented solely by glycaemic control. Diabetic retinopathy and nephropathy leading to blindness and renal failure are the hallmark of microangiopathy. However, diabetic-macroangiopathy refers mainly to an accelerated form of atherosclerosis. This in turn affects both the coronary and cerebral vasculature, thus increasing the risk of myocardial infarction, angina pectoris and cerebrovascular accidents. Indeed, coronary heart disease and peripheral vascular disease are the leading causes of morbidity and mortality in diabetes mellitus. 2

Long-standing diabetes mellitus is associated with an increased prevalence of microvascular and macrovascular diseases. With the rising prevalence of diabetes, the number suffering from the vascular complications of diabetes will also increase. Diabetic nephropathy is one of the leading causes of chronic renal failure in India. Among 4837 patients with chronic renal failure seen over a period of 10 years, the prevalence of diabetic nephropathy was 30.3% followed by chronic interstitial nephritis (23%) and chronic glomerulonephritis (17.7%). 3

Influence of diabetes on renal oxygen tension and oxygen consumption

Reduced renal medullary oxygen tension

The in vivo PO2 in any tissue is the result of net delivery of oxygen and oxygen consumption within that specific tissue. Altering any of these two parameters will undoubtedly affect the PO2. Decreased PO2 in renal medulla has been proposed as a mechanism involved in progression of nephropathy. Diabetes-induced increase in renal medullary hydrogen ion concentration increases the Bohr Effect when acidic blood in ascending vasa recta comes in the vicinity of arterial blood in descending vasa recta. Shunting of oxygen (from descending to ascending vessels) increases, and the net result was even further reduced oxygen delivery to renal medulla. Decreased oxygen delivery to medullary structures occurs despite the fact that total blood perfusion might be unaffected, or even increased.

Nishikawa and co-workers 4 showed that hyperglycaemia induces excessive formation of ROS from the electron transport chain located in the mitochondrial membrane. Hyperglycaemia-induced increase in substrate available for the electron transport chain increased electrochemical potential gradient over across the mitochondrial membrane. This stabilized superoxide generating intermediates in electrode transport chain, resulting in increased formation of ROS. Formation of ROS lead to reduced renal PO2. Treatments with antioxidants such as α-tocopherol, pycnogenol, β-carotene, and α-lipoic acid have been shown to affect oxidative stress. Antioxidant treatment reduced the diabetes-induced increase of the antioxidant enzyme hemeoxygenase-1 and increased the activity of glutathione and glutathione redox enzyme. It should be noted that mechanisms which mediated these alterations are highly dependent of the nature of the antioxidant used. In one of recent studies, treatment of diabetic animals with the free radical scavenger α-tocopherol fully prevented diabetes-induced decrease in renal PO2. 5

ABSTRACT

Diabetic nephropathy, a progressive development of renal insufficiency in the setting of hyperglycaemia is the major single cause of chronic renal failure (CRF) in which hypoxia plays a critical role. Over the recent past years, views of Angiotensin II (Ang II) have changed from being a simple vasoconstrictor to that of a complex growth factor mediating effects through diverse signalling pathways. Through increased generation of ROS and activation of redox-sensitive transcription factors, Ang II exerts diverse role in endothelial damage. The mechanisms involved in endothelial dysfunction are not entirely understood. Mechanisms underlying the cellular effects of Ang II seem to occur at the post-receptor level and appear to be associated with hyperactivity of Ang II-stimulated G protein-coupled phospholipases, tyrosine kinase-, and MAP kinase-dependent pathways, as well as with oxidative stress. Interactions between these cascades are highly complex, and dysregulation at any level could manifest as pathological functional sequelae and structural vascular changes. Angiotensin II induces oxidative stress via the activation of NADPH oxidase which damages endothelial cells directly, and results in relative hypoxia due to inefficient cellular respiration. Thus the involvement of hypoxia inducible factor in regulation of these pathophysiological actions seems very promising. There are compelling evidence which show that chronic hypoxia final pathway to ESRD (End stage renal disease), therapeutic approaches which target the chronic hypoxia should prove effective against a broad range of renal diseases and associated vascular dysfunction.

Keywords: Angiotensin II, Vascular dysfunction, Reactive oxygen species, Nitric oxide, Hypoxia inducible factor
Increased oxygen consumption

A previously reported adaptation of the renal medulla to a low \( \text{PO}_2 \) is a decrease in enzyme activity, which rapidly and profoundly decreases metabolic activity, thereby decreasing oxygen demands. In a recent study, however, renal oxygen consumption was found to be increased in cells isolated from diabetic animals. This finding is in conjunction with previous reports and is linked to increased \( \text{Na}^+ / \text{K}^- \text{ATPase} \), since the ouabain sensitive oxygen consumption was significantly increased. It is well known that glomerular hyperfiltration occurs during the early onset of hyperglycemia, both in diabetic patients and in animal models of experimental diabetes. Increased glomerular filtration increased tubular load of electrolytes, resulting in increased tubular reabsorption and increased oxygen consumption. This is certainly a contributing mechanism, but a large part of the diabetes-induced increase in medullary oxygen consumption is unrelated to active transport since oxygen consumption is independent of glomerular filtration rate (GFR). This suggests that other mechanisms apart from an altered tubular sodium load are involved in the increased oxygen consumption. Increased flux through the \( \text{Na}^+ / \text{glucose-linked transporters (SGLT)} \), as a result of excessive tubular load of glucose due to the increased blood glucose concentration, has been shown to increase \( \text{Na}^+ / \text{K}^- \text{ATPase} \) activity. Another possible mechanism is a decreased inhibition of the oxygen consumption by NO, which would occur in a dose-dependent manner. Sustained hyperglycemia is known to induce enhanced expression of uncoupling protein-2, increase gluconeogenesis and also increase fatty acid metabolism, all resulting in increased oxygen consumption. During normoglycemic conditions there are no insulin independent \( \text{Na}^+ / \text{glucose} \) transporters in the renal medulla, but the low affinity GLUT-2 has been found in significant amounts all along the nephron. A challenging speculation is that increased medullary tubular glucose load can induce protein expression of SGLT, resulting not only in increased intracellular glucose concentrations, but also in increased cellular energy demand and subsequent oxygen consumption.\(^7\)

Changes in vascular endothelium in diabetes

The endothelium, once considered a simple monolayer of cells covering the entire inner surface of all the blood vessels, has recently been established as a strategically-located multifunctional organ. It lies between circulating blood and the vascular smooth muscle and plays many pivotal roles in the regulation of vascular tone and endothelial integrity as well as in the maintenance of blood fluidity and homeostasis. To perform such a wide range of functions, the endothelium synthesises or releases several vasoactive substances, including the vasodilators NO, prostacyclin and endothelium-derived hyperpolarising factors (EDHFs) and the vasoconstrictors angiotensin II and endothelin-1. Under physiological conditions, the endothelium acts as an inhibitory regulator of vascular contraction, leukocyte adhesion, vascular smooth muscle cell growth and platelet aggregation. However, the characteristics of the endothelium change in response to local or systemic changes such as trauma, hyperglycaemia or dyslipidaemia and dysfunction of endothelium is considered present when normal organ function can no longer be preserved either in the basal state or in response to any given physical, humoral or chemical stimuli. Diabetes mellitus in humans and animal models of diabetes\(^8\) are associated with impaired endothelium-dependent relaxation i.e. endothelial dysfunction. The term “endothelial dysfunction” in fact refers to impairment of many significant functions of the endothelium including anti-inflammatory and antiproliferative characteristics as well as vasodilatation. Several factors including increased synthesis of vasoconstrictor agents through the cyclooxygenase (COX) pathway and dysregulation of the gene encoding endothelial type of nitric oxide synthase (eNOS)\(^9\) in endothelium have been proposed to account for this defect in diabetes. However, in recent years, reduced bioavailability of nitric oxide (NO), the most important endogenous vasodilator agent, due to excessive synthesis/release or diminished destruction of reactive oxygen species (ROS) has been implicated in the pathogenesis of this defect.\(^10\)

CHRONIC HYPOXIA IN THE KIDNEY

A variety of methods have been successfully employed to demonstrate renal hypoxia in experimental animals with chronic kidney disease. A study previously employed a pencil lens probe CCD intravital video microscopic system to evaluate renal microcirculation and observed stagnation of peritubular capillaries at an early stage of a model of progressive glomerulonephritis induced by uinephrectomy and repeated injection of anti-mesangial Thy1 antibody Pimonidazole, a water-soluble small molecule which binds only to cells at oxygen tensions less than 10 mmHg \( \text{in vivo} \), allowed demonstrating hypoxia of the kidney in association with reduction in peritubular capillary blood flow. Chronic hypoxia of the diabetic kidney and polycystic kidney disease (PKD) was also demonstrated by pimonidazole.\(^11\) A decrease in oxygen tension of the diabetic kidney induced by streptozotocin was also demonstrated by measurement with a microelectrode.\(^12\) Oxygen molecules diffuse across the end of microelectrodes, generating an electrical current proportional to the oxygen concentration and allowing us its estimation. Early transplantation of pancreatic islets to streptozotocin-treated animals prevented hypoxia of the kidney, showing that the decrease in renal oxygen tension was due to the long-term diabetic condition and could not be ascribed to direct toxic effects of streptozotocin.\(^13\) Measurement of intrarenal oxygen tensions utilizing a micro-needle electrode also showed hypoxia of the kidney in spontaneously hypertensive rats and in the clipped kidney of the early 2-kidney, 1-clip angiotensin II-dependent model.\(^14\) Blood-oxygen level dependent (BOLD) MRI also revealed renal hypoxia in streptozotocin-induced diabetic rats. Histological studies of human kidneys and animal models have shown that extensive tubulointerstitial injury is associated with damage to renal arterioles as well as with loss of peritubular capillaries, leading to a decrease in capillary blood supply and oxygenation to the corresponding region. Sequential follow-up of experimental animals revealed that the loss of peritubular capillaries precedes the development of tubulointerstitial fibrosis. In addition to loss of peritubular capillaries, in appropriate and sustained activation of the endothelium under hypoxic conditions results in leukostasis and can compromise peritubular flow, aggravating regional hypoxia.

Hypoxia plays a pathogenic role even in the early stages of kidney disease without structural tubulointerstitial injury. Peritubular capillaries occur downstream of the glomerular efferent arterioles, and impairment of the parent glomerular capillary bed, as occurs in glomerulosclerosis, automatically results in a decrease in peritubular perfusion and tubular oxygen supply. Imbalances in vasoactive substances and
associated intrarenal vasoconstriction can also cause chronic hypoxia in the kidney in the early stage of kidney disease. Among various vasoactive substances, local activation of the renin-angiotensin system (RAS) is especially important because it can lead to constriction of efferent arterioles, hyperperfusion of post-glomerular peritubular capillaries, and subsequent hypoxia of the tubulointerstitium in the downstream compartment. To support this notion, estimation of the peritubular capillary blood flow by analyzing the velocity of fluorescein in isothiocyanate-labeled erythrocytes showed that intravenous infusion of angiotensin II decreased total renal blood flow and peritubular capillary erythrocyte velocity. In addition, angiotensin II induces oxidative stress via the activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase). Oxidative stress damages endothelial cells directly, causing the loss of phosphate metabolism of resident renal cells. These fibrotic changes aggravate regional hypoxia and result in further obliteration and loss of peritubular capillaries with progression of fibrosis. Thus, hypoxic changes combine to institute a vicious cycle of regional hypoxia and progressive kidney injury, leading to eventual ESRD.

**Therapeutic approaches targeting hypoxia in the kidney**

Because chronic hypoxia is a final common pathway to ESRD, therapeutic approaches which target the chronic hypoxia should prove effective against a broad range of renal diseases. Today’s best modality for the treatment of kidney disease is blockade of RAS. One important mechanism of the blood pressure-independent renoprotective effect of this blockade is amelioration of chronic hypoxia of the kidney, because activation of RAS induces hypoxia of the kidney via multiple mechanisms. Administration of an angiotensin receptor blocker at the early phase of remnant kidney rats, classically characterized by chronic renal failure with glomerular sclerosis and hypertension, improved renal oxygenation, which was associated with preservation of peritubular capillary flow.

On the other hand, HIF stimulation holds promise as a future therapy. Gene transfer of constitutively active HIF protected the kidney against ischemic injury. However, as HIF levels are determined by its hydroxylation-induced degradation, hydroxylases involved in this reaction may be good targets for therapy against kidney disease. As PHD require iron as a cofactor to hydroxylate the critical prolines on HIF-α, the finding that some of the best-established activators of HIF-1 are chelators of iron is reasonable. Recent studies have demonstrated that L-mimosine and ethyl 3,4-dihydroxybenzoate (3,4-DHB) activate the HIF pathway primarily through iron chelation and induce angiogenesis in a sponge model. Desferoxamine and cobalt chloride are among the most well-established iron chelators in the activation of HIF. More than half a century ago, oral administration of cobaltous chloride was shown to be effective in treatment of renal anemia. A study has demonstrated the renoprotective effects of chemical pre-conditioning with cobaltous chloride in an ischemic model of renal injury. Administration of cobalt induced up-regulation of HIF-regulated genes, and subsequently protected the kidney against the tubulointerstitial damage induced by hypoxia. Activation of HIF by pretreatment with either carbon monoxide or the novel PHD inhibitor FG-4487 also ameliorated acute kidney failure induced by ischemia/reperfusion in rats. Collectively these data proved that preconditional activation of the HIF system protects the kidney against acute ischemic injury. Activation of HIF was also effective in nephrotic acute kidney injury such as cisplatin nephropathy and in an acute rat glomerulonephritis model induced by co-administration of angiotensin II and Habu snake venom. A study demonstrated that cobalt treatment mediated improvement in the tubulointerstitial injury as well as preservation of glomerular and peritubular capillary networks with no evidence of vascular leakage in remnant kidney model. Recent studies also demonstrated that cobalt ameliorated disease manifestations of type 2 diabetic nephropathy in rats. Success of cobalt treatment has been reported in various models of kidney diseases as described above. However, long-lasting iron chelation by cobalt may also lead to severe side effects, because iron is a necessary cofactor for a host of important cellular functions, including oxidative phosphorylation and arachidonic acid signaling. Therefore, while these studies clearly provided rationale and promise of HIF activating therapies in kidney diseases, potential side effects may hinder the therapeutic use of iron chelators. A systematic examination of the relative efficacy of PHD...
ANGIOTENSIN II IN DIABETIC NEPHROPATHY AND VASCULAR DYSFUNCTION

Angiotensin II is a critical, active peptide of the renin-angiotensin system (RAS) that is primarily involved in blood pressure regulation and fluid homeostasis. Recently, RAS has been recognized as more than a circulating hormone system, in particular, some organs, i.e. pancreas, adipose, skeletal muscle and liver, which have their own local RAS with distinct functions. An emerging body of evidence suggests that Ang II also possesses multiple functions concerning other tissue and organ systems, thus it can be associated with diseases other than hypertension. Moreover, Ang II has been implicated in type 2 diabetes, but the mechanisms underlying its involvement remain largely unknown. Given that Ang II is a potent vasoconstrictor, it has long been implicated in hypertension. Universal treatments for hypertension include administering an Ang II receptor blocker (ARB) or angiotensin converting enzyme (ACE) inhibitor. Incidentally, extensive clinical studies have demonstrated that an ARB or ACE inhibitor abolished Ang II action and reduced the occurrence of type 2 diabetes in high risk patients by 25%, where preservation of β-cell function and/or improvement of insulin sensitivity are suggested to be the protective mechanisms. This finding prompted investigations into a possible novel role of Ang II in type 2 diabetes and brought new insight into clinical implication. Type 2 diabetes is a complicated disease that is influenced by many factors. The primary causes for its development are insulin resistance and β-cell dysfunction/apoptosis. Multiple peripheral organs are involved in type 2 diabetes and may interact with each other to produce complicated circumstances. A study showed that prolonged angiotensin II administration reduced renal cortical oxygen tension in association with a reduction of oxygen consumption efficiency and concurrent infusions of tempol, a permeant superoxide dismutase mimetic, blunt these changes. Thus, angiotensin II induces renal hypoxia through both hemodynamic as well as nonhemodynamic mechanisms.

Angiotensin II and Renin Angiotensin System (RAS)

Ang II (Asp1-Arg2-Val3-Tyr4-Ile5-His6-Pro7-Phe8) is an important peptide of the RAS, which is derived from the precursor, angiotensinogen, via the enzymes, renin and ACE. It is a full agonist acting at the type I Ang receptor (AT1R) and binds with high affinity to the type II Ang receptor (AT2R). Angiotensinogen is synthesized mainly by the liver and then secreted into the blood. It is hydrolyzed by renin in the juxtaglomerular cells of the kidney to form Ang I. Ang I is cleaved by pulmonary membrane- bound ACE to generate Ang II which exerts many physiological actions primarily via AT1R binding. AT1R and AT2R are both G protein-coupled receptors with seven trans-membrane domains and are located in numerous cells and tissues; AT1R is about 30% homologous to AT2R. Normally, the latter is highly expressed during fetal development and is much less abundant in adult tissues unless it is up-regulated during pathological conditions. AT2R mediates divergent cellular responses including cell growth and proliferation, while AT1R is suggested to counterbalance its effects.

Angiotensin II and renal failure

Angiotensin II plays a central role in the pathophysiology of renal diseases. In addition to its hemodynamic actions, Ang II exerts several nonhemodynamic effects. Ang II causes mesangial cell proliferation and regulates the expression of several genes that are involved in intracellular signaling cascades in glomerular mesangial cells. Recently, it has been shown that Ang II is involved in the process of tissue destruction in chronic renal diseases and that angiotensin-converting enzyme inhibitors slow the progress of renal diseases.

The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in regulating physiologic and pathophysiologic processes in the kidney. Although different components of the RAAS, such as renin, aldosterone, and various angiotensin fragments, can initiate renal impairment on their own, angiotensin II (Ang II) is the primary effector of this system. Intensive research in the past 15 yr has provided convincing evidence that AngII is a key contributor to progression of renal disease by stimulating growth, inflammation, and fibrosis of the kidney. AngII binds to specific receptors to mediate its particular effects. The angiotensin type 1 (AT1) and type 2 (AT2) receptors are the best characterized receptors on a molecular level, but additional types may exist. Most of the known physiologic and pathophysiologic effects of AngII are transduced by the AT1 receptor, a 359-amino acid protein that belongs to the seven-membrane superfamily of G-protein–associated receptors. After the binding of Ang II to the AT1 receptors, a series of signaling cascades is activated. Although traditionally divided into G-protein– and non-G-protein–related signaling, there are so many interactions between these subgroups of Ang II–induced signaling pathways that a strict distinction becomes difficult. An example of a G-protein– dependent pathway is activation of phospholipase C with the subsequent production of inositol 1,4,5-phosphate and diacylglycerol. Non–G-protein pathways induced by Ang II are phosphorylation and the activation of various tyrosine kinases. Ang II is an important mediator of oxidative stress, and reactive oxygen species (ROS) induced by Ang II are chief signal intermediates in several signal transduction pathways involved in renal pathophysiology. Moreover, Ang II–induced ROS are important for renal growth processes, inflammation, and fibrosis.

Angiotensin II and vascular dysfunction

Ang II is an important growth modulator of blood vessels and renal organogenesis during development and plays a critical role in regulating blood pressure and fluid homeostasis in physiological conditions. In pathological conditions, through its vasoconstrictor, mitogenic, pro-inflammatory, and profibrotic actions, Ang II contributes to altered vascular tone, endothelial dysfunction, structural remodeling, and vascular inflammation, characteristic features of vascular damage in hypertension, atherosclerosis, vasculitis, and diabetes. The subcellular mechanisms and signaling pathways whereby Ang II mediates its physiological and pathophysiologic vascular effects are complex. Growing evidence indicates that production of ROS and activation of reduction-oxidation (redox)-dependent signaling cascades are critically and centrally involved in Ang II–induced actions. All vascular cell types, including endothelial cells, smooth muscle cells, adventitial fibroblasts, and resident macrophages, produce ROS.

A previous study showed that early application of combined Ang II blockade with losartan and captopril improves renal hemodynamics and normalizes Qo/ \( T_{\text{no}} \). In further study, it was demonstrated that induction of HIF, as demonstrated in kidney tissue by Western blot and indexed by the expression...
of VEGF, HO-1, Epo (Erythropoietin), and GLUT1 mediates the beneficial effects of Ang II blockade in CKD by utilizing two different mechanisms of HIF induction, administration of cobalt chloride (CoCl₂) and dimethylxalulidine (DMOG). This hypothesis seems less likely given the prior observations from this laboratory in this model that combined Ang II blockade normalized kidney oxygen consumption factored by Na reabsorption, thereby eliminating the proximate cause of hypoxia, a presumed stimulus to generation of HIF-1 and related proteins, but stimulation by growth factors have also been postulated to increase HIF. In a study, the effects of HIF induction on renal metabolism and hemodynamics were investigated to compare with those observed with Ang II blockade in ablation/infarction (A/I) and examined the expression of HIF-induced proteins with both treatments to explain the observed effects by utilizing both cobalt and DMOG therapy to induce HIF and produced similar metabolic and hemodynamic outcomes. The results suggest that both Ang II blockade and persistent activation of the HIF pathway are highly effective and improve renal metabolic and hemodynamic functions but by mechanisms that are similar in some respects but differ significantly in other important molecular expressions.

Angiotensin II and Reactive oxygen species

Recent evidence suggests that Ang II stimulates mitochondrial ROS generation through the opening of mitochondrial K<sub>ATP</sub> channels, leading to redox sensitive activation of mitogen-activated protein kinases (MAPK). It is interesting that a process of Ang II-mediated preconditioning has been described, at least in cardiac myocytes, in which NAD(P)H oxidase-derived O<sub>2</sub> stimulated K<sub>ATP</sub> channels, facilitating the efflux of large mitochondrial-derived amounts of O<sub>2</sub> into the cytoplasm. The relationship between generation of O<sub>2</sub> in the mitochondrial respiratory chain and NAD(P)H oxidase is complex, and it has been shown that mitochondrial inhibitors suppress the induction of Nox1. A more indirect effect of Ang II on ROS formation may be mediated through the hypoxia-inducible factor 1-α (HIF-1α). A study has showed that Ang II stimulates HIF-1 α expression through AT<sub>2</sub> receptors via suppression of prolyl hydroxylase 3, an enzyme that hydroxylates HIF-1 α with the consequence of inducing degradation of this factor. In heterogeneous mice partially deficient in HIF-1 α, ROS formation induced by chronic intermittent hypoxia was, in contrast to wild-type mice, absent, indicating a role of HIF-1 α in the formation of ROS. However, a vice versa pathway has also been described, because ROS interact with and inhibit prolyl hydroxylase, leading to a decrease in HIF-1 α hydroxylation and stabilization of this transcription factor.

Angiotensin II and Hypoxia inducible factor

Although systemic RAS is activated by hypoxia, the role of PHD in the regulation of RAS remains uncertain. In a study, Matsuura et al. examined whether PHD inhibition affects AT1R expression and the Ang II signaling pathway in the vascular smooth muscle cell (VSMC) and vascular remodeling process. The study demonstrated a direct inhibitory effect of CoCl₂ on AT<sub>1</sub>R expression in VSMCs. However, the study could not exclude the possible indirect effects of CoCl₂ on the Ang II signaling in vivo. Recent studies showed that inhibition of PHD, using different PHD inhibitors, lowered the levels of proinflammatory cytokines, such as tumor necrosis factor-α, in different models. Tumor necrosis factor-α and interleukin 1β have been reported to transcriptionally enhance the AT(R gene expression in cardiac fibroblasts. Therefore, reduction of Ang II–induced cytokine production may also be responsible for CoCl₂-induced AT<sub>1</sub>R downregulation in vivo. The study also demonstrated that PHD inhibition downregulates AT<sub>1</sub>R expression, reduces the cellular response to Ang II, and attenuates the profibrotic effect of Ang II on the coronary arteries. These data revealed necessity of further studies to determine the detailed molecular mechanism for AT<sub>1</sub>R downregulation, PHD inhibition may be beneficial for the treatment of cardiovascular diseases, in which activation of RAS plays a critical role.

MECHANISMS UNDERLYING DIABETIC NEPHROPATHY

Involvement of hyperglycemia in diabetic nephropathy

The magnitude of hyperglycemia correlates with the functional and structural changes of diabetic nephropathy. Clinically, strict glycemic control inhibits both the functional decline in GFR and the formation of characteristic structural lesions. The restoration of euglycemia reverses structural changes. Exposure to high glucose causes an increase in matrix protein generation and cell cycle arrest by cultured cells. Increased intracellular glucose initiates changes in the mesangial cell phenotype. This is made possible by the unrestricted entry of glucose in the presence of increased extracellular glucose. The inability to restrict glucose entry is a characteristic of mesangial cells and other tissues that are especially susceptible to the microvascular complications of diabetes, including the capillary endothelial cells of the retina and peripheral neurons. Whereas glucose entry into many tissues, like skeletal muscle, is tightly regulated and does not increase in the presence of increased extracellular glucose, high glucose causes an increase in glucose uptake by mesangial cells. Regulation of the expression of facilitative glucose transporters (GLUTs) underlies this distinguishing characteristic of mesangial cells and other susceptible cell types. Glucose transport into all mammalian cells occurs via glucose transporters, including the sodium-coupled and facilitative transporters.

Once inside the cell, glucose is metabolized via multiple pathways that increase the generation of reactive oxygen species (ROS), enhance cell susceptibility to oxidative injury by reducing glutathione, and generate metabolites that activate protein kinase C (PKC) or directly induce the expression of transforming growth factor beta-1 (TGF-β1). The majority of intracellular glucose is metabolized in the cytosol via glycolysis, which generates pyruvate. Pyruvate is oxidized in the mitochondria via the tricarboxylic acid (TCA) cycle. Both glycolysis and the TCA cycle generate nicotinamide adenine dinucleotide (NADH) that acts as an electron donor for mitochondrial oxidative phosphorylation. Excessive electron transfer caused by increased intracellular glucose generates a high electrochemical potential difference that prolongs the half-life of superoxide-generating electron intermediates in the mitochondria. Some argue that the generation of superoxide and other ROS underlie most, if not all, the pathogenetic effects of intracellular glucose.

Clearly, ROS generation plays a significant role in altering the phenotype of mesangial cells exposed to high glucose. Perhaps, most importantly, ROS formation alters glucose metabolism such that alternative pathways to glycolysis are increased, resulting in the accumulation of metabolites that initiate pathogenetic signaling. First, ROS accumulation depletes cells of NAD because of the massive ROS-triggered activation of the NAD-consuming DNA repair enzyme, poly-adenosine diphosphate (ADP)-ribose polymerase-1 (PARP-1). This alters the NADH/NAD ratio of the cell that shuts
down the NAD dependent enzyme, glyceraldehyde-3-phosphate dehydrogenase, and thus inhibits the formation of pyruvate. As a result, upstream pyruvate precursors accumulate, forcing their metabolism via alternative pathways. For instance, glyceraldehyde-3-phosphate is metabolized to diacylglycerol (DAG) and phosphatidic acid and both of these metabolites activate PKC isoforms.41 PKC induces TGF-b via activation of the transcription factor, AP-1, and increases eicosanoid generation. The pyruvate precursor, glucose, is also shunted from the glycolytic to the polyl pathway. Aldose reductase is the first and rate-limiting enzyme of the polyl pathway and generates sorbitol that is oxidized to fructose. Excessive activity of aldose reductase depletes NAD-phosphate (NADPH), which is essential for the regeneration of glutathione, thus enhancing cell susceptibility to oxidative injury. Furthermore, the oxidation of sorbitol to fructose worsens the NADH/NAD ratio, exacerbating the inhibition of pyruvate formation. The increased expression of aldose reductase has been demonstrated in cells derived from diabetic patients. Finally, increases in glucose and in metabolites of the polyl and glycolytic pathways favor the formation of advanced glycation endproducts (AGEs). AGEs are proteins and lipids that have been modified by the nonenzymatic covalent addition of a sugar residue via a series of biochemical reactions collectively termed the “maillard reaction”.42 Levels of AGEs, while detectable under physiologic conditions, are markedly increased in diabetic patients and studies in experimental diabetic animals have suggested that AGEs contribute to lesions in mesangial cells and podocytes. The inhibition of AGE formation prevents mesangial expansion and decreases albuminuria, and the intraperitoneal injection of AGE-modified albumin increases the expression of glomerular collagen and TGF-b1 in the streptozotocin-induced diabetic rat.43 Critical studies have delineated the contribution of hemodynamic changes to diabetic nephropathy; the interruption of the renin-angiotensin system has become a cornerstone in the treatment of diabetic nephropathy. However, whether the glomerular hemodynamic changes are causal or secondary to mesangial lesions remains controversial.

**DIABETES-INDUCED ALTERATIONS IN THE NITRIC OXIDE SYSTEM**

**Production and Bioavailability of Nitric Oxide**

NO bioavailability is of importance not only for hemodynamic regulation and delivery of oxygen to renal medulla, but also for regulation of oxygen consumption. Furthermore, if mitochondrial NO reacts with superoxide radicals peroxynitrite will form, which induces oxidative stress and cause irreversible changes and modifications of mitochondrial targets. Even during normal physiological conditions, the influence of NO inhibition of mitochondrial respiration is likely to be significant in renal medulla due to normally low PO2. It has been demonstrated that NO bioavailability in STZ-diabetic animals is markedly lower compared to normoglycemic animals. The reason for the decreased NO activity is a reduced plasma L-arginine concentration, which limits NO production. Intravenous administration of L-arginine caused a pronounced increase in bioavailable NO specifically in diabetic animals. This might influence the mitochondrial oxygen consumption rate and, thus, the renal PO2. Acidosis is thought to influence NO production. A study by Prabhakar et al. showed an 80% reduction of inducible NOS (iNOS) activity in mesangial cells during low pH, despite close to normal NOS mRNA and protein levels. This decrease could not be reversed by L-arginine supplementation, but NO production resumed when pH was returned to normal. Density and distribution of NOS binding sites are decreased in kidneys from diabetic rabbits, implying a role in pathogenesis of renal disease. Renal cortex from STZ treated diabetic rats displays a 50% reduction in total NOS activity All alterations in NOS expression and activity were totally restored by intense insulin treatment. In another study, performed on bovine aortic endothelial cells, hyperglycemia and treatment with glucosamine reduced eNOS activity. Blocking the hexosamine pathway via inhibition of its ratelimiting enzyme glutamine:fructose-6-phosphate amidotransferase, reversed these changes. This chronic impairment of eNOS activity may partly explain the accelerated atherosclerosis seen in diabetic patients. Recently, mitochondrial NOS (mtNOS) have been reported.44

**Prevention and reversal of diabetic endothelial dysfunction**

The close link between hyperglycaemia and endothelial dysfunction is supported by both in vitro and in vivo studies. The adverse effect of hyperglycaemia on vascular function in diabetes may be due to the consequences of the impaired L-arginine/NO pathway, oxidative stress and increased formation of AGEs as a result. Hence several therapies have been proposed for preventing and to a certain extent reversing endothelial dysfunction in diabetic state by directly targeting these pathogenetic mechanisms. Antioxidant defences may also be impaired in diabetes thereby contributing to net oxidative stress.45 Indeed a variety of defects in serum antioxidant status has been reported in diabetic patients compared to healthy subjects. Hence it has consequently been suggested that diabetic patients might benefit from supplementation with antioxidant vitamins (vitamin C and vitamin E) to prevent free radical oxidation and endothelial dysfunction as a result. Protein kinase C β II induction has been implicated in vasoconstrictive effects of several hormones such as angiotensin II. It has been shown in animal models of diabetes that vitamin E treatment improves coronary and aortic vascular endothelial function and prevents diabetes induced abnormalities although the opposite has been reported in mesenteric arterioles. Increased superoxide may however directly inactivate NO with the formation of the highly toxic oxidant, peroxynitrite. Endothelial dysfunction not only occurs in overt diabetes but can also be induced by simple exposure of isolated vessels to high glucose media in vitro. Pretreatment of rat aorta with SOD produces significantly greater relaxations in aortic rings incubated in high glucose. Likewise pretreatment with SOD plus catase or an inhibitor of hydroxyl radical formation (DETPAC) has been shown to improve endothelial dysfunction in aortic rings of streptozotocin-induced diabetic rats suggesting that vascular production of both O2- and hydroxyl radicals may contribute to endothelial dysfunction in this model.46 However, elevated ambient glucose concentrations in diabetes mellitus may result in glycosylation of native superoxide dismutase leading to impairment of its enzymatic activity. In addition, the changes in intracellular cell signalling may impair appropriate activation of NOS in response to neurohumoral or mechanical stimuli.47

**Nitric oxide and Hypoxia Inducible factor**

Classically, studies into the mechanisms underlying diabetic nephropathy have focused on glomerular injury and the development of albuminuria; however, changes intubulointerstitial structure and function are also evident.
evenduring the early stages in diabetes. Changes in proximal tubule structure consistent with hypertrophy (increases in cell height, tubule diameter, and length) area prominent component of diabetic renal hypertrophy, with other nephron segments also displaying changes in tubule length. There is evidence of altered renal handling of electrolytes, including Na\(^+\), and increased renal Na\(^+\)-K\(^-\)-ATPase (NKA) activity has been widely reported in diabetes. The changes in NKA activity accompanying type 1 diabetes mellitus coincide with, and thus may play a role in, the development of hypertrophy. On the other hand, as NKA mediated ion transport is the major consumer of metabolic energy in the kidney, the early and pronounced increase in tubular NKA activity in diabetes has been proposed to represent an important adaptive response to the osmotic diuresis and/or the chronic increase in filtered Na\(^+\) load. Upregulation of NKA activity is particularly evident in the outer medulla, where low blood flow limits O\(_2\) supply despite high O\(_2\) consumption coupled with reabsorptive Na\(^+\) transport. As O\(_2\) extraction is almost maximal under normal conditions in the outer medulla, the increased NKA activity linked to increased Na\(^+\) transport during diabetes is accompanied by reduced PO\(_2\) in this region that normally exists near the brink of hypoxia. Thus diabetes would promote chronic hypoxia, which may be a common pathway leading to ESRD.\(^9\) In the healthy kidney, the renal medulla has high concentrations of nitric oxide (NO). Reduced NO bioavailability has been shown to result in increased O\(_2\) consumption and, therefore, increased sodium reabsorption in the renal medulla. Diabetes is known to be a condition of oxidative stress and reduced NO bioavailability. Little information is available on the literature pertaining to the status of NO and O\(_2\) availability in the renal medulla during diabetes. Palm and colleagues\(^48\) have begun to unravel the complex mechanisms involved in the interrelationship between reduced NO bioavailability and hypoxia in the renal medulla in the early stages of diabetes. Palm et al. has reported that reduced renal medullary NO levels in diabetes are due to decreased plasma L-arginine and unrelated to diabetes-induced oxidative stress, while the reduction in medullary PO\(_2\) was restored by L-arginine administration or antioxidant treatment. The study also showed that the O\(_2\) availability in both normal and diabetic rats was independent of blood flow alterations. These observations underscore the potential importance of diabetes-induced renal metabolic alterations and their functional consequences. At physiological concentrations, NO inhibits the mitochondrial enzyme cytochrome c oxidase (complex IV) in competition with O\(_2\), thereby impeding mitochondrial respiration. When NO levels are decreased, such as in diabetes, this regulatory mechanism is dysfunctional, thus allowing increased mitochondrial respiration and O\(_2\) consumption. Thus, in addition to effects of diabetes to increase Na\(^+\) transport dependent O\(_2\) consumption and NKA activity in the outer medulla, altered NO bioavailability may increase mitochondrial O\(_2\) consumption and contribute to reduced PO\(_2\) under these conditions. Although the validity of this scenario remains speculative, the data from Palm et al. established a link between NO synthase substrate availability and the changes in medullary PO\(_2\) accompanying diabetes. Hypoxia induces regulatory mechanisms via its influence on gene expression, specifically through a family of transcription factors known as hypoxia inducible factors (HIFs). Most HIF-induced responses confer protection against hypoxic injury; however, renal profibrotic genes have also been shown to be directly upregulated by hypoxia. NO has been shown to regulate the activity and/or expression of HIF degrading enzymes with no clear consensus on whether NO activates or blunts the HIF-degrading enzymatic activity. Very little information is available concerning whether HIF activates renoprotective mechanisms and profibrotic genes in the hypoxia-prone renal medulla during diabetes and/or whether NO regulates HIF levels under these conditions. The observations of Palm et al. should fuel future studies that focus on the interaction of NO and O\(_2\) availability, as well as the effects of NO on mitochondrial respiration and HIF-dependent responses, during the early stages of diabetes in the renal medulla. These processes associated with renal medullary hypoxia may interact in an additive or synergistic manner with the hemodynamic events that evoke glomerular hyperfiltration, ultimately contributing to the development of diabetic nephropathy.\(^49\)

**SUMMARY**

ACEIs (Angiotensin II converting enzyme inhibitors) and ARBs (Angiotensin II receptor blockers) are currently indicated for the treatment of hypertension, diabetic nephropathy, post-MI left ventricular dysfunction, and chronic heart failure, and their use has been associated with improved survival and considerable cardiovascular and renal benefits in high-risk patients. Therefore, future drugs against downstream targets of Ang II receptor signaling as a more specific drug target are expected for the prevention of Ang II–induced cardiovascular disease. Further elucidation of the functional regulation of these Ang II receptor interacting proteins including phosphorylation and dephosphorylation, transcriptional control via hypoxia inducible factors and finding out possible ligands could be useful for new drug discovery for ameliorating the enhanced tissue renin-angiotensin system.

**REFERENCES**


