Potential Therapeutic Role of Phosphodiesterase Type 5 Inhibition in Hypertension and Chronic Kidney Disease

Kayleigh E. Brown, Neeraj Dhaun, Jane Goddard, David J. Webb

Chronic kidney disease (CKD) is common, affecting 13% of adults in the United States, with 8% having an estimated glomerular filtration rate (eGFR) of <60 mL/min per 1.73 m² in the survey from National Health and Nutrition Examination Survey (1999–2004).1 Prevalence is likely to increase further with an aging population and high levels of obesity, hypertension, and diabetes mellitus and has emerged as a major public health concern worldwide. Importantly, despite increased awareness and treatment of hypertension in patients with CKD, blood pressure (BP) control rates are low.2 The presence of CKD is an independent risk factor for the development of cardiovascular disease (CVD),3 showing a graded risk of cardiovascular events, hospitalizations, and all-cause mortality as eGFR declines, with a sharp rise in events as eGFR falls <45 mL/min per 1.73 m².4 The 1998 National Kidney Foundation Task Force on Cardiovascular Disease in Chronic Renal Disease report5 emphasized the high prevalence of CVD in CKD and revealed that cardiovascular mortality, defined as death caused by arrhythmias, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease, or pulmonary edema, is 10 to 30× higher in dialysis patients than in the general population. In fact, the risk of CVD in people with CKD far outweighs the risk of progression, with >70% of patients with CKD in a large retrospective study dying of cardiovascular events, whereas only 4% started renal replacement therapy.6,7 Hence, in this area of considerable unmet medical need, we require new therapies that will lower BP, slow the progression of CKD, and minimize cardiovascular risk. This will not only reduce the number of patients reaching end-stage renal disease and the requirement for dialysis but also alleviate the significant incremental burden of CVD with which worsening CKD is associated.

In recent years, considerable evidence has accrued to suggest that CKD is a state of relative renal and systemic NO deficiency caused by a combination of decreased renal and vascular NO production and increased NO bioinactivation.8 Because of the important protective role that NO plays in health in both the renal and the cardiovascular systems, it is likely that a deficit in NO will accelerate the progression of CKD and increase cardiovascular risk, making the NO pathway a promising therapeutic target.

Nitric Oxide

NO is synthesized through the oxidation of l-arginine to l-citrulline in a wide range of cell types, including vascular endothelial cells, platelets, macrophages, and neuronal cells. l-Arginine is predominantly synthesized endogenously and also has dietary origins. The conversion of l-arginine to l-citrulline takes place under the effect of NO synthase (NOS) of which there are 3 isoforms: neuronal NOS (nNOS/NOS1), endothelial NOS (eNOS/NOS3), and inducible NOS (iNOS/NOS2). The activity of these enzymes is modulated by a range of stimuli, including shear stress and neurotransmitters, and is also dependent on the availability of cofactors, such as tetrahydrobiopterin, that act as an electron donor during the catalytic cycle. The NOS enzymes are regulated by endogenous inhibitors, the arginine analogues asymmetrical dimethyl arginine (ADMA) and NG-monomethyl-l-arginine, that are degraded by dimethylarginine dimethylaminohydrolase (DDAH) enzymes to produce dimethylamine and l-citrulline.

NO diffuses locally to target cells to activate soluble guanylate cyclase (sGC) that in turn catalyzes the conversion of guanosine triphosphate to 3′,5′-cGMP (Figure). cGMP mediates the biological effects of NO via the regulation of intracellular kinases and calcium channels, before it is degraded by the phosphodiesterase (PDE) family of enzymes.9 The rapid sequestration of NO by hemoglobin, or its oxidation to nitrite and nitrate, means that the molecule has mainly autocrine and paracrine effects. In addition, some of the NO produced scavenges the superoxide anion (O2-) to produce peroxynitrite (ONOO-), a potent oxidant with a relatively long half-life.

Physiological Role of NO in the Cardiovascular System

Within the vasculature, endothelium-derived NO induces the relaxation of vascular smooth muscle cells to cause vasodilatation. NO is critical in the regulation of vascular tone, BP, and regional blood flow in man,10 maintaining dilator tone in the coronary and pulmonary vessels, as well as maintaining perfusion of vital organ systems, including the brain, heart, and kidneys.11 NO can also cause vasodilatation indirectly through the inhibition of vasoconstrictor influences. For example, NO...
Hypertension has been found to regulate the expression of endothelin-1 (ET-1) and platelet-derived growth factor in endothelial cells. Beyond its role in the control of vascular tone, NO has many other actions in the cardiovascular system. In conjunction with prostacyclin, it has antithrombotic properties, acting to inhibit ADP-induced platelet aggregation and adhesion to the vascular endothelium. NO has also been shown to inhibit leukocyte adhesion to the vascular endothelium to modulate inflammatory responses, as well as limiting vascular smooth muscle proliferation cell in vivo.

**Physiological Role of NO in the Kidney**

As a major site of endogenous l-arginine synthesis, the kidney generally has an excess of substrate for NO production. The various isoforms of NOS are widely distributed in the kidney. eNOS is expressed in renal vascular structures, particularly in the endothelium of glomerular capillaries—the afferent and the efferent arterioles—and the medullary vasa recta. Here, NO will modulate vascular tone thus affecting GFR. nNOS is localized primarily in the macula densa of the juxtaglomerular apparatus, where it will influence renin secretion. iNOS is located in mesangial cells, where it may affect GFR, and in the renal tubules, where its effects modulate renal salt and water handling (see online-only Data Supplement for further detail).

**Chronic Kidney Disease**

CKD is an umbrella term used to describe a spectrum of diseases characterized by progressive decline in renal function over time. It is defined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines as kidney damage present for ≥3 months, featuring structural or urinary abnormalities (such as proteinuria or hematuria of renal origin) or an eGFR <60 mL/min per 1.73 m². CKD is further classified into stages 1 to 5, by the level of eGFR, with higher stages reflecting lower GFRs. Any disease process involving nephron loss can contribute to CKD, such as diabetic nephropathy or polycystic kidney disease. As the number of functioning nephrons declines, the surviving nephrons attempt to compensate by hyperfiltration and increasing solute reabsorption. This ultimately leads to damage to the remaining nephrons and acceleration of disease progression. High capillary pressures cause podocyte damage, leading to proteinuria. The subsequent uptake of the filtered protein by tubular epithelial cells causes parenchymal damage and renal scarring. CKD is also associated with inflammation and oxidative stress leading to endothelial dysfunction, glomerular fibrosis, and mesangial expansion.

**Decreased Production of NO in CKD**

l-Arginine synthesis, a major substrate for renal NO generation, is reduced in CKD, most likely because of loss of functional renal tissue. Loss of renal mass also strongly correlates with reduced NOS protein expression and disease progression in animal models of CKD. Cortical nNOS abundance has been shown to fall with increasing glomerular damage in several animal models of renal mass reduction and CKD, whereas eNOS abundance is more highly variable. In health, the kidney acts to reabsorb filtered l-arginine in the proximal tubule via a cationic acid antiporter. In patients with end-stage renal disease, this mechanism is impaired and, additionally, these patients lose l-arginine through dialysis. However, in early CKD, plasma l-arginine levels may be preserved by a compensatory increase in l-arginine synthesis in the vascular endothelium. Uprogulation of the arginase enzymes in
the liver (arginase I) and kidney (arginase II) in CKD may also contribute to a decline in NO production because these enzymes compete with NOS for the substrate l-arginine. Arginase inhibition has been found to restore endothelial function and NO production in experimental hypertension models,35,36 as well as reduce glomerulosclerosis, and increase GFR and plasma arginine levels. It also decreased proteinuria in a 5/6 ablation model of CKD.37 In contrast, Mori et al38 found that there was no difference in arginase I and II protein abundance among CKD and control groups in a rat 5/6 nephrectomy model. Here, high urea concentrations in CKD may inhibit arginase activity, may attenuate l-arginine catabolism, and may mitigate the fall in l-arginine levels.

Vallance et al19 were the first to report the accumulation of the endogenous NOS inhibitor, ADMA, in patients with end-stage renal disease, with circulating concentrations of the inhibitor being sufficiently high to inhibit NO synthesis. A wealth of research has since been conducted into the contribution of ADMA to renal disease and it now seems that this endogenous NOS inhibitor is a key player in NO deficit, with levels being predictive of progression to dialysis and death in patients with CKD.40,41 Accumulation is thought to be because of impaired breakdown of ADMA by the enzymes DDAH 1 and 2. The conditions of oxidative stress inherent in CKD negatively influence the efficacy of DDAH, reducing the catabolism of ADMA.42 The effects of DDAH stimulation in a 5/6 nephrectomy model of CKD seem promising, with DDAH 1 overexpression being achieved through injection of a recombinant adenovirus vector (Adv) encoding the enzyme. Tubulointerstitial fibrosis and proteinuria were ameliorated in Adv-DDAH-1 rats in comparison with controls, demonstrating a potentially protective effect in maintaining DDAH activity in renal disease. However, a recent study in which a SNP (single nucleotide polymorphism) associated with overexpression of DDAH 1 reduced plasma ADMA levels but contributed to progression of CKD suggests that further work is needed on this approach.43

Bioinactivation of NO in CKD

CKD is a state of oxidative stress.44 High levels of reactive oxygen species and reduced antioxidant levels are frequently found in patients with CKD, particularly those undergoing renal replacement therapies.45 Under these conditions, a greater proportion of the NO synthesized by endothelial cells is converted to peroxynitrite through reaction with the free radical, superoxide, in turn reducing the availability of NO and inducing a state of endothelial dysfunction. Oberg et al46 found that plasma protein-associated carbonyl content and free F2-isoprostane levels, markers of oxidative stress and lipid peroxidation, respectively, were significantly raised in stage 3 to 5 patients with CKD in comparison with healthy subjects. These findings were accompanied by significantly raised levels of the inflammatory markers C-reactive protein and interleukin-6 and significantly lowered plasma protein-reduced thiol content (a measure of antioxidant capacity) in the patients with CKD. As would be expected, oxidative and inflammatory stress were correlated with the development of atherosclerosis and CVD in these subjects.46

Consequences of NO Deficiency in CKD

These mechanisms contribute to the relative NO deficiency found in CKD, leading to a state of both systemic and renal endothelial dysfunction. Many types of renal injury occur in conjunction with the chronic NOS inhibition rat model, including glomerulosclerosis, interstitial expansion, and microvascular lesions.47 Through intravenous infusion of [15N2]-arginine as a substrate for NOS, and measurement of in vivo arginine to citrulline conversion, Wever et al48 found that basal whole-body release of NO is reduced in patients with CKD. Therefore, the pathological burden of CKD is not confined to the kidney but involves the cardiovascular system as a whole. NO deficiency is thought to contribute to CVD in CKD through 2 modalities: atherosclerosis and arteriosclerosis. A low level of vascular NO favors the development of atherosclerotic plaques through encouraging macrophage adherence to the endothelium, migration to the intima, and subsequent smooth muscle cell proliferation and thrombosis.49 However, of the estimated 40% of end-stage renal disease deaths in the United States attributed to CVD, only 9% of these are attributable to vasculo-occlusive events, such as acute myocardial infarction and cerebrovascular accidents.50 Sudden cardiac death, arrhythmias, and congestive cardiac failure are more commonly cited as the cause of death in dialysis patients, and vulnerability to these conditions is thought to be a result of the presence of structural heart disease. Research is now focused on the contribution of arteriosclerosis (the stiffening of large arteries), a process to which NO deficiency contributes, in the development of the cardiovascular complications in CKD. Aortic arterial stiffness, and its failure to be reduced by antihypertensive treatment, has been shown to be a strong independent predictor of all-cause and cardiovascular mortality in CKD,51,52 causing a rise in systolic BP, increasing the workload on the heart, and in turn inducing structural damage. A rise in systolic BP also increases the likelihood of stroke and contributes to the progression of renal disease.53,54

Current Treatment of CKD

Treatment of CKD is complex and multimodal, targeting both BP and proteinuria, both of which act as strong independent predictors of disease progression.55 BP management in CKD is beneficial on 3 levels: in reducing cardiovascular risk, in reducing proteinuria, and in slowing the progression of intrinsic renal disease.55 Blocking the renin–angiotensin–aldosterone system, using angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, is the current gold standard treatment approach for hypertension and proteinuria in CKD.56 These agents act not only to lower BP but also through a BP-independent effect on proteinuria and intrarenal pathology.55,57 However, alternative pharmacological agents are emerging as future candidates. With compelling evidence to suggest that NO is critical to normal renal function, and that its dysfunction is implicated in CKD, restoring functionality in this pathway seems a logical therapeutic approach.

PDE Type 5 Inhibition

cGMP, an effector molecule of NO, is hydrolyzed by the cGMP-specific enzyme, cGMP-binding phosphodiesterase type 5 (PDE5). Through the inhibition of this enzyme
using PDE5 inhibitors, such as sildenafil, the available pool of cGMP can be increased and the signaling actions of NO prolonged. By stimulating vasodilatation within the corpora cavernosa during sexual stimulation, sildenafil can facilitate penile erection, and this has proven useful in the treatment of male erectile dysfunction.58 PDE5 inhibitors can also induce vascular smooth muscle relaxation in the pulmonary vascular bed and are indicated for the treatment of pulmonary arterial hypertension because of their efficacy in reducing pulmonary artery pressure and improving functional capacity.59

Inhibitors of PDE5 are also vasodilators in the systemic circulation, and regular administration may, therefore, constitute an effective antihypertensive therapy. Regular sildenafil monotherapy in untreated patients with hypertension, at a dose of 50 mg 3x daily for 16 days, has been shown to produce a significant fall in BP comparable with other antihypertensive drugs, reducing both daytime ambulatory systolic and diastolic BP by 6 mm Hg in comparison with control.60 Sildenafil thus constitutes an effective antihypertensive therapy, with only minor side effects, such as dyspepsia, headache, and myalgia, being noted in this study. Indeed, PDE5 inhibitors have an excellent safety record overall. However, sildenafil has a short duration of action, requiring repeated daily dosing to achieve a therapeutic plasma concentration. Current research is thus now turning to the assessment of the efficacy and safety of novel longer acting agents, such as tadalafil.61 An alternative therapeutic option involves using PDE5 inhibitors in combination with existing hypertension therapies. The simultaneous provision of exogenous NO from organic nitrates and the inhibition of cGMP breakdown with PDE5 inhibitors is currently contraindicated in ambulatory patients, as well as the adverse effect profile and potential of inducing hypotension and the possible development of nitrate tolerance. Investigating the effects of prolonged use in ambulatory patients, as well as the adverse effect profile and dosing requirements, is, therefore, a research priority.

Novel therapeutic indications for PDE5 inhibitors are emerging with the discovery that PDE5 is expressed in a number of tissues. Abundant PDE5 expression has been identified in the kidney and, therefore, it is proposed that, through its inhibition, the function of the NO-cGMP pathway in the kidney can be enhanced, relieving the NO deficit associated with CKD.67 In vivo, a wealth of research has been conducted into PDE5 inhibition, both in diabetic and nondiabetic models of CKD. A seminal study conducted by Rodríguez-Iturbe et al68 found that sildenafil treatment initiated immediately after 5/6 renal ablation in rats prevented the development of hypertension, reduced tubulointerstitial injury scores, and retarded the development of proteinuria in comparison with controls. However, the beneficial impact of sildenafil on proteinuria was lost if therapy began 4 weeks after surgery, suggesting that efficacy is reduced if pathological changes are already established.

The beneficial effects of PDE5 inhibitors in models of CKD seem to extend far beyond their BP-lowering capabilities, suggesting renoprotective properties. They have been shown to exert a potent antiproliferative effect, preventing mesangial cell proliferation and extracellular matrix expansion; outcomes that cannot be replicated through a comparable reduction in BP using hydralazine.69,70 Studies have also demonstrated the beneficial effects of PDE5 inhibition on renal cell apoptosis,68,71 oxidative stress,72 and inflammation73 in CKD models. Therefore, preclinical data support this approach.

To date, only 1 clinical trial of PDE5 inhibition in CKD has been published.74 In this study, 40 men with type-2 diabetes mellitus were treated for 1 month with either 50-mg sildenafil daily or matching placebo. The sildenafil-treated group had a 50% reduction in albuminuria from baseline, and the drug was well tolerated. Although providing a clinical precedent, this pilot study had several limitations. Albuminuria was a secondary end point, and levels were given without reference to GFR, thus limiting full appreciation of the impact of sildenafil on renal function. In addition, patients had only microalbuminuria, so translation to patients with heavy proteinuria is unclear. Subjects were not on existing angiotensin-converting enzyme inhibitor or angiotensin receptor blocker therapy, the gold standard treatments for diabetic nephropathy to which PDE5 inhibitors are likely to be added. In addition, the pharmacokinetic properties of sildenafil dictate that a single 50-mg dose of the drug may not provide the long duration (24 hours) activity likely required for clinical efficacy. To advance clinical understanding of these potential benefits, Pfizer are currently undertaking a randomized, placebo-controlled trial, investigating the impact of a long-acting PDE5 inhibitor on patients with diabetes mellitus and overt nephropathy (clinicaltrials.gov NCT01200394), the results of which are eagerly awaited.

**Other Therapeutic Approaches to Enhance NO Signaling**

1-Arginine Supplementation

NO deficiency in CKD could also be combated through increasing the available substrate for NOS. A degree of success has been reported in animal studies, with supplementation of 1-arginine ameliorating proteinuria and glomerular hypertension in remnant kidney models.75,76 However, these positive results have failed to translate into benefit in clinical trials.77,78 The lack of efficacy found in such studies has been attributed to intrinsic impairment in the NO synthesis apparatus. For example, commonly found features of CKD, such as elevation of ADMA levels or a deficiency in cofactors for eNOS (such as tetrahydrobiopterin), may negate the benefit of 1-arginine supplementation and lead to enhanced free radical generation.
Endothelin Antagonism

In vivo, ET-1 is a potent vasoconstrictor, and its upregulation is known to pathogenically influence endothelial dysfunction and atherosclerosis. Uprogulation of the ET system is thought to play a role in the renal and cardiovascular complications of CKD. Systemic administration of the selective endothelin-type A (ET_A) receptor antagonist, BQ-123, to hypertensive patients with CKD produced a substantial reduction in BP (10 mm Hg) and increased renal blood flow, supporting an upregulation of the ET system in CKD-associated hypertension. In addition, chronic treatment with the mixed ET_A/ET_B receptor antagonist, avosentan, and the selective ET_A receptor antagonist, atrasentan, in addition to standard angiotensin-converting enzyme inhibitor/angiotensin receptor blocker treatment, substantially decreased albumin excretion in patients with diabetic nephropathy. The positive effects of ET_A receptor antagonism in CKD are attributed to reduced ET-1–mediated effects and to restoration of the NO system, an effect thought to be mediated at least in part through endothelial cell ET_A receptor activation leading to an upregulation of eNOS. This effect may also be amplified through a possible synergistic relationship that exists between ET_A receptor antagonists and angiotensin-converting enzyme inhibitors.

NO Donors

An alternative to promoting endogenous NO synthesis would be to use donor NOs that liberate NO after biotransformation. They are currently used for the treatment of angina. However, their generally short duration of action, and the development of nitrate tolerance, may render them unsuitable for the long-term treatment of CKD. Discouraging results from animal models have arrested any progression to clinical trials. Molsidomine, a free NO donor, induced a deterioration in renal function in a nonimmune model of chronic glomerulonephritis, worsening creatinine clearance, increasing tubulointerstitial injury, and increasing renal malondialdehyde (a measure of lipid peroxidation). This highlights a mechanistic problem associated with using NO donors to treat renal disease, in that these compounds have the potential to act as pro-oxidants, with elevated levels of renal cortical superoxide anions in CKD acting as a stimulus for peroxynitrite formation in the presence of exogenous NO donors.

sGC Activators and Stimulators

Still in development for CKD are sGC agonists. In targeting sGC, the intracellular receptor and effector of NO, these agents act further down the NO–sGC–cGMP signaling pathway to mimic the effects of NO. Several sGC agonists, in the form of both stimulators and activators, have been developed. Riociguat (BAY 63–2521) is a sGC stimulator that acts to sensitize sGC to low levels of endogenous biowailable NO, whereas the sGC activator, cinaciguat (BAY 58–2667), acts to trigger directly sGC activity. Cinaciguat was administered to 5/6 nephrectomy rats for 18 weeks in a model of CKD and was found to significantly reduce BP, left ventricular hypertrophy, and arterial wall thickness, as well as significantly improving indices of renal function in treated rats in comparison with controls. This is supported by a further study in which BAY 41-2272 prevented extracellular matrix expansion and fibrosis in an anti–th1-induced model of chronic glomerulonephritis. BP reduction is credited as the major mediator of improvement in renal function in such studies. However, further research is warranted into possible renoprotective mechanisms. In preventing the degradation of cGMP, PDE5 inhibitors rely on a sufficient cGMP stimulus to amplify. However, sGC activators bypass this requirement and, therefore, may be advantageous in the NO-deficient state of CKD.

Conclusions

The rising prevalence of CKD, and its associated hypertension and proteinuria, represents a significant clinical challenge for renal and cardiovascular medicine. Relative deficiency of NO in the blood vessels has the potential to contribute to hypertension, arteriosclerosis, and atherosclerosis, whereas deficiency in the kidney contributes to a steeper decline in renal function and its associated adverse cardiovascular outcomes. Among the therapeutic candidates that may have the potential to improve NO functional activity in CKD, PDE5 inhibition seems a relatively safe and promising approach, with the success of animal models translating well into early clinical trials showing that these agents lower BP and may reduce proteinuria. Considerable work is still needed to better understand the position of PDE5 inhibitors and related agents, such as the potentially attractive sGC activators, in the treatment of hypertensive CKD.

Disclosures

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Running header: Phosphodiesterase type 5 (PDE5) inhibition in CKD
The physiological role of nitric oxide in the kidney

The renal vasculature

Endothelium-derived NO directly modulates renal vascular tone through its cGMP-dependent vasodilatory action, and is therefore critical in the control of total and regional renal blood flow [1]. Indeed, systemic infusion of L-NMMA, a non-selective inhibitor of all NOS isoforms, significantly reduces renal blood flow and GFR [2]. NO may also induce vasodilatation in the kidney through indirect mechanisms, with data suggesting that NO production in afferent arterioles acts to oppose angiotensin II (ANGII)-mediated vasoconstriction [3]. In addition, circulating levels of ANGII may be reduced through NO-induced modulation of the renin-angiotensin-aldosterone system [4]. However, the relationship between renin and NO is complex. Some groups have reported that NO stimulates renin release [5, 6], whereas others suggest an inhibitory relationship [7, 8]. It is likely that NO has a dual effect on renin, inhibiting renin generation when applied directly to juxtaglomerular granular cells but triggering renin production when the macula densa is stimulated [4].

The glomerulus

GFR is determined in part by the relationship between afferent and efferent arteriolar tone. Through its vasodilatory action on glomerular arterioles and capillaries, endothelium-derived NO has a role in regulating glomerular haemodynamics. The afferent arteriole is thought to be the preferential site of NO action [2], where it serves to increase GFR. However, it may also act on other components of the glomerulus that influence GFR, particularly mesangial cells and podocytes.

Mesangial cells are multifunctional. They display contractile properties that influence GFR, secrete pro-inflammatory mediators and extracellular matrix components, and are involved in the immune surveillance of the kidney via their phagocytic capabilities. Mesangial cell dysfunction has been implicated in the pathogenesis of CKD; with mesangial changes seen in glomerular pathology, including increased release of chemo-attractants for inflammatory cells, mesangial cell proliferation and excessive extracellular matrix production leading to mesangial expansion [9]. The role of NO in mesangial cell physiology is being elucidated.
through *in vitro* studies. Co-incubation of bovine aortic endothelial cells and rat glomerular mesangial cells has revealed that bradykinin-stimulated release of NO from endothelial cells triggers an increase in cGMP expression in mesangial cells, a rise sufficient to antagonise their contractile response to ANGII. NO therefore induces mesangial cell relaxation through the action of cGMP and should increase GFR. NO may act as a messenger for communication between the endothelium and mesangial cells in maintaining glomerular filtration. NO has also been shown to have anti-mitogenic properties, mediated at least in part through cGMP. It is able to induce transcriptional blockade through preventing the accumulation of the transcription factor EGR-1 and thereby inhibit mesangial cell growth. NO elaborated from cultured rat mesangial cells also appears to exert an anti-inflammatory and anti-fibrotic action, inhibiting production of inflammatory cytokines and extracellular matrix components such as collagen and fibronectin. As well as inducing a pro-fibrotic phenotype in mesangial cells, NO deficiency in CKD may also reduce GFR through increasing mesangial contractility.

Podocytes work in co-operation with mesangial cells and the glomerular basement membrane in maintaining glomerular filtration. They form a network of interdigitating foot processes and slit diaphragms that have an important role in determining the size and charge selectivity of the filtration barrier. Loss of these cells, therefore, results in a bare glomerular basement membrane that is highly susceptible to injury. Podocytes express soluble guanylate cyclase, meaning that NO can influence podocyte function, most likely inhibiting their contractile role. Impaired renal NO synthesis in hypercholesterolaemic rats has been found to lead to podocyte injury, low levels of NO being unable to counteract the high levels of oxidative stress associated with this condition. It is thus possible that an NO deficit in CKD could cause podocyte injury, this insult inciting further pathological repercussions in the glomerulus.

**The renal tubules**

One of the main roles of the kidney is the maintenance of the body’s water and electrolyte balance, and both *in vitro* and *in vivo* studies suggest that NO has important influence on this area of renal function. The overall effect of NO on sodium reabsorption is inhibitory, promoting salt and water excretion. NOS inhibition has been shown to reduce sodium and
water excretion in rats, inducing a salt-sensitive blood pressure phenotype \textsuperscript{17,18}. In addition, the administration of cGMP, SNAP (an NO donor) and L-arginine into the renal interstitial space of rats has been found to induce natriuresis without altering total renal blood flow or GFR \textsuperscript{19}. These studies strongly suggest that cGMP has a direct action on renal tubular sodium and fluid transport. It has been suggested that protein kinase G mediates cGMP-induced natriuresis, with SRC kinase acting as a downstream signalling molecule targeting renal epithelial cell basolateral membrane Na\textsuperscript{+}/K\textsuperscript{+} ATPase \textsuperscript{20}. NO has also been found to mediate the effects of other natriuretic peptides, such as ET-1 \textsuperscript{21,22}. NO therefore has a dual mechanism of action, promoting sodium and water excretion not only through altering vascular tone and preventing the right-shift of the pressure natriuresis curve caused by ANGII and chronic NOS inhibition \textsuperscript{36}, but also through a direct tubular action, particularly in the collecting duct \textsuperscript{23,24}.

Through the use of renal clearance techniques and in situ microperfusion of the proximal tubules, Wang et al demonstrated significantly increased urinary HCO\textsubscript{3}– excretion and significantly reduced rates of HCO\textsubscript{3}– and Na\textsuperscript{+} absorption in nNOS-knockout mice in comparison to wild type mice \textsuperscript{25}. This corresponded with significantly reduced arterial blood HCO\textsubscript{3}– concentration and pH, indicating a potentially significant role of nNOS in renal acid base balance. Further study by the group suggests that iNOS plays a similar role to nNOS in the proximal tubule, acting to upregulate Na\textsuperscript{+} and HCO\textsubscript{3}– absorption \textsuperscript{26}. No such role was found for eNOS.

With roles in controlling renal blood flow, glomerular haemodynamics, mesangial cell function and sodium balance, NO can be considered a vital component in the maintenance of normal renal functional integrity and the sum of its effects could significantly influence CKD progression.
References


