Online Supplement

Manuscript title: Potential therapeutic role of phosphodiesterase type 5 (PDE5) inhibition in hypertension and chronic kidney disease

Manuscript Number: HYPE201301774

Authors and affiliations:

Kayleigh Elizabeth Brown

*Neeraj Dhaun

*Jane Goddard

David J Webb

All: British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, QMRI, 47 Little France Crescent, Edinburgh, EH16 4TJ, Scotland, UK.

* Renal Unit, Royal Infirmary of Edinburgh

Correspondence:

Name: Prof David J Webb

Address: Room E3.22, Clinical Pharmacology Unit
Centre for Cardiovascular Science
University of Edinburgh
Queen's Medical Research Institute
47 Little France Crescent
Edinburgh EH16 4TJ
Scotland, UK

Fax: +44 870 134 0897

Telephone: +44 131 242 9215

Email: D.J.Webb@ed.ac.uk
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 other 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 \(^{17,18}\). 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 \(^{19}\). 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\(^+\)/K\(^+\) ATPase \(^{20}\). NO has also been found to mediate the effects of other natriuretic peptides, such as ET-1 \(^{21,22}\). 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 \(^{36}\), but also through a direct tubular action, particularly in the collecting duct \(^{23,24}\).

Through the use of renal clearance techniques and in situ microperfusion of the proximal tubules, Wang et al demonstrated significantly increased urinary HCO\(_3\)\(^-\) excretion and significantly reduced rates of HCO\(_3\)\(^-\) and Na\(^+\) absorption in nNOS-knockout mice in comparison to wild type mice \(^{25}\). This corresponded with significantly reduced arterial blood HCO\(_3\)\(^-\) 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\(^+\) and HCO\(_3\)\(^-\) absorption \(^{26}\). 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


