Acta Physiol 2006, **187**, 433–446

REVIEW

Nitric oxide in the kidney: functions and regulation of synthesis

P. F. Mount[1,2] and D. A. Power[1,2]

1 The Austin Research Institute, Austin Hospital, Victoria, Australia

2 Department of Nephrology, Austin Hospital, Victoria, Australia

Received 28 December 2005, revision requested 30 January 2006, revision received 21 February 2006, accepted 1 March 2006

Correspondence: Dr P. Mount, Austin Research Institute, Kronheimer Building, Austin Hospital, Studley Road, Heidelberg 3084, Victoria, Australia.
E-mail: p.mount@ari.unimelb.edu.au

Abstract

In the kidney nitric oxide (NO) has numerous important functions including the regulation of renal haemodynamics, maintenance of medullary perfusion, mediation of pressure–natriuresis, blunting of tubuloglomerular feedback, inhibition of tubular sodium reabsorption and modulation of renal sympathetic neural activity. The net effect of NO in the kidney is to promote natriuresis and diuresis. Significantly, deficient renal NO synthesis has been implicated in the pathogenesis of hypertension. All three isoforms of nitric oxide synthase (NOS), namely neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3) are reported to contribute to NO synthesis in the kidney. The regulation of NO synthesis in the kidney by NOSs is complex and incompletely understood. Historically, many studies of NOS regulation in the kidney have emphasized the role of variations in gene transcription and translation. It is increasingly appreciated, however, that the constitutive NOS isoforms (nNOS and eNOS) are also subject to rapid regulation by post-translational mechanisms such as Ca^{2+} flux, serine/threonine phosphorylation and protein–protein interactions. Recent studies have emphasized the role of post-translational regulation of nNOS and eNOS in the regulation of NO synthesis in the kidney. In particular, a role for phosphorylation of nNOS and eNOS at both activating and inhibitory sites is emerging in the regulation of NO synthesis in the kidney. This review summarizes the roles of NO in renal physiology and discusses recent advances in the regulation of eNOS and nNOS in the kidney by post-translational mechanisms such as serine/threonine phosphorylation.

Keywords endothelial nitric oxide synthase, neuronal nitric oxide synthase, nitric oxide, phosphorylation.

In the kidney nitric oxide (NO) has numerous physiological roles including the regulation of renal and glomerular haemodynamics (Majid & Navar 2001), mediation of pressure natriuresis (Majid *et al.* 1993), maintenance of medullary perfusion (Mattson *et al.* 1992), blunting of tubuloglomerular feedback (TGF) (Wilcox 1998), inhibition of tubular sodium reabsorption (Ortiz & Garvin 2002) and modulation of renal sympathetic nerve activity (Eppel *et al.* 2003) (Fig. 1). The net effect of NO in the kidney is to promote natriuresis and diuresis (Lahera *et al.* 1991) and NO plays an important role in the renal adaptation to variations of dietary salt intake (Shultz & Tolins 1993). Significantly, deficient production of NO in response to increased dietary salt has been implicated in the pathogenesis of hypertension (Pallone & Mattson 2002). NO and the by-product L-citrulline are produced from molecular oxygen (O_2) and the amino acid L-arginine by nitric oxide synthase (NOS) of which there are three isoforms designated as neuronal NOS

 doi: 10.1111/j.1748-1716.2006.01582.x

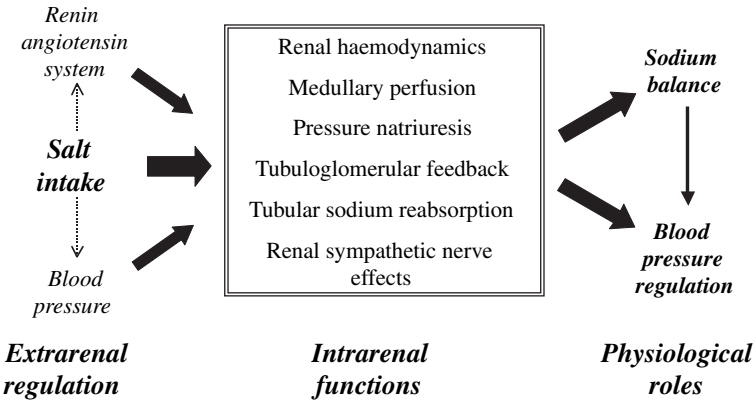

Figure 1 Overview of the roles of nitric oxide (NO) in renal physiology. The role of NO in the regulation of sodium homeostasis and blood pressure is emphasized. The various functions of NO within the kidney are discussed in this review. The extra-renal factors influencing NO synthesis within the kidney and how these regulate intra-renal NO activity remains incompletely understood.

(nNOS or NOS1), inducible NOS (iNOS or NOS2) and endothelial NOS (eNOS or NOS3) (Stuehr 1999). Whilst expression of all three isoforms of NOS is reported in the kidney, the mechanisms controlling NO synthesis in the kidney are incompletely understood. This review summarizes the distribution and regulation of NOS in the kidney and the roles of NO in renal physiology. In addition, recent advances in the understanding of NOS regulation by post-translational modifications such as phosphorylation are discussed.

Expression of nitric oxide synthase in the kidney

nNOS (NOS1)

Numerous studies have demonstrated a high level of nNOS protein expression in the macula densa (Fig. 2a) (Mundel *et al.* 1992, Wilcox *et al.* 1992, Bachmann *et al.* 1995, Tojo *et al.* 2000, Mount *et al.* 2005a), where NO is an important modifier of the TGF response (Wilcox 1998). In addition, a study by Bachmann *et al.* (1995) has identified nNOS in specialized neurones, known as the non-adrenergic, non-cholinergic nerves, within the renal arteries of the hilus, arcuate and interlobular arteries, and occasionally the pre-glomerular afferent arterioles. Detection of nNOS mRNA transcripts by polymerase chain reaction (PCR) on microdissected nephron segments has shown the highest level of nNOS RNA expression in the inner medullary collecting duct (IMCD) (Terada *et al.* 1992). This method also identified significant nNOS mRNA expression in the outer medullary collecting duct, the cortical collecting duct (CCD) and the inner medullary thin limb (Terada *et al.* 1992).

The reason for the failure of the earlier immunohistochemical studies to identify this high level of medullary nNOS expression is unclear, however, it may relate to

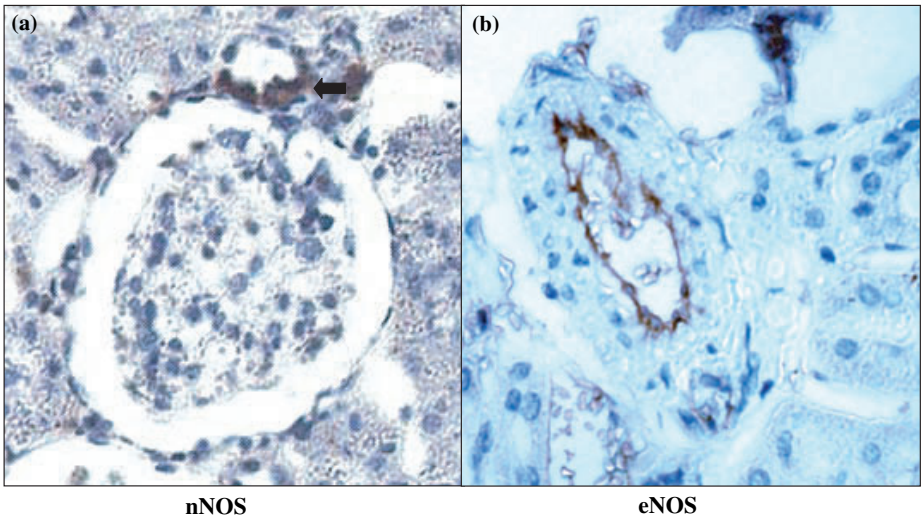

Figure 2 Expression of neuronal nitric oxide synthase (nNOS) (NOS1) and eNOS (NOS3) in the rat kidney. Paraffin embedded rat kidney sections were stained by immunohistochemistry with monoclonal antibodies specific for nNOS (NOS1) (a) and eNOS (NOS3) (b) (both antibodies from BD Transduction Laboratories, Lexington, KY, USA) as previously described (Mount *et al.* 2005a). (a) The arrow indicates prominent nNOS staining in the macula densa. (b) Staining for eNOS is noted in endothelial cells of an arteriole.

  doi: 10.1111/j.1748-1716.2006.01582.x

the specificity of the particular monoclonal antibody used in those studies. Western blot analysis confirms that the highest level of nNOS protein is in the medulla, and especially the inner medulla (Martin *et al.* 2002). In the collecting ducts, nNOS is found in the principal cells, which are responsible for sodium, potassium and water transport, with minimal nNOS in the acid-secreting intercalated cells (Wang *et al.* 1998). Finally, there is also evidence for low level expression of nNOS in the proximal tubule (Wang *et al.* 2000, Baines & Ho 2002) and the thick ascending limb (TAL) of Henle's loop (Wang *et al.* 2002), although the significance of these observations is unclear. The studies cited above have documented the distribution of nNOS in the rodent kidney. The distribution of nNOS in the human kidney was recently described with immunohistochemistry demonstrating tubular expression in most segments of the nephron including the macula densa, proximal tubule, TAL, distal tubule and collecting ducts (Jarry *et al.* 2003). This study found nNOS expression, both protein and mRNA, to be higher in the cortex than the medulla (Jarry *et al.* 2003).

Splice variants of nNOS

The human NOS1 gene spans over 200 kb on chromosome 12 (Kishimoto *et al.* 1992). The full-length transcript is encoded by 29 exons with translation initiating in exon 2, resulting in a 160 kDa protein designated as nNOSα (Fig. 3). The detection of residual NOS activity in nNOS-knockout mice generated by the deletion of exon 2 in embryonic stem cells led to the identification of two shorter splice variants of nNOS, nNOSβ (136 kDa) (Fig. 3) and nNOSγ (125 kDa) (Huang *et al.* 1993). Immunohistochemical data comparing wild type to nNOSα-knockout mice demonstrates that the nNOS expressed in the macula densa is nNOSα (Bachmann *et al.* 1998). Analysis by RT-PCR, however, shows that nNOSβ transcripts are predominant in the whole kidney and that this level of expression is the same in wild type and nNOSα-knockout animals (Bachmann *et al.* 1998, Oberbaumer *et al.* 1998). Thus it appears that, in the kidney, nNOSβ is expressed in tubular segments other than macula densa although the exact distribution needs further study (Kone 1999).

iNOS (NOS2)

There is controversy regarding the expression of iNOS in the kidney. Several investigators have been unable to detect iNOS protein expression in the normal rat (Bryant *et al.* 1998, Zhang *et al.* 2000, Stumm *et al.* 2002, Kosaka *et al.* 2003, Mount *et al.* 2005a), mouse (Park *et al.* 2003) or human (Jarry *et al.* 2003) kidney. In contrast, others such as Ni *et al.* have reported iNOS protein expression in the normal rat kidney (Ni & Vaziri 2001). As opposed to studies of iNOS protein expression, there is more consistent evidence for detectable expression of iNOS mRNA in the kidney (Mohaupt *et al.* 1994, Morrissey *et al.* 1994, Wu *et al.* 1999). In fact, Mohaupt *et al.* (1994) identified two different forms of iNOS mRNA in the normal rat kidney, which were equivalent to the murine macrophage form of iNOS (macNOS) and the rat vascular smooth muscle form of iNOS (vsmNOS). Interestingly, two studies have found that the site of highest basal expression of iNOS mRNA in the kidney is the medullary TAL (Mohaupt *et al.* 1994, Morrissey *et al.* 1994). Unlike eNOS, however, iNOS does not appear to contribute to NO mediated inhibition of sodium reabsorption in the TAL (Plato *et al.* 2000). There is consistent evidence that, in the kidney, iNOS expression, both protein and mRNA, is dramatically increased by pro-inflammatory stimuli such as ischaemia–

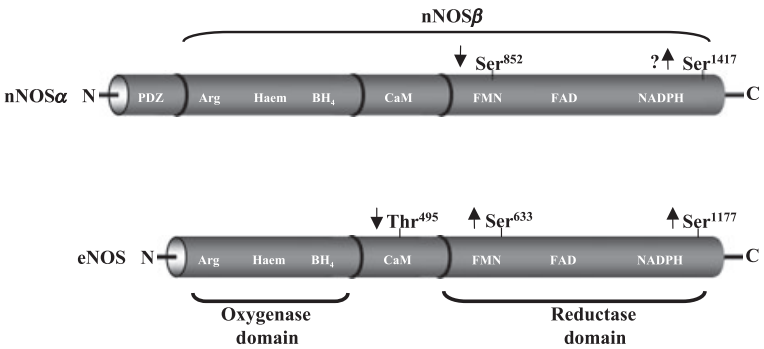

Figure 3 Neuronal nitric oxide synthase (nNOS) (NOS1) and endothelial NOS (eNOS) (NOS3) phosphorylation sites described in the kidney. All sites are numbered according to the human NOS sequences. The arrows indicate the effect of phosphorylation at each site on NOS activity. The difference between the nNOSα and nNOSβ splice variants is illustrated. Ser, serine; Thr, threonine; Arg, arginine; BH4, tetrahydrobiopterin; CaM, calmodulin; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate.

reperfusion (Kosaka *et al.* 2003, Park *et al.* 2003) and lipopolysaccharide (Mohaupt *et al.* 1994, Morrissey *et al.* 1994, Bryant *et al.* 1998, Zhang *et al.* 2000, Stumm *et al.* 2002, Kosaka *et al.* 2003).

eNOS (NOS3)

As expected, eNOS is strongly expressed in renal vascular endothelium (Fig. 2b), although tubular expression of eNOS also occurs. Immunohistochemical staining shows eNOS in renal arteries and arterioles, glomerular capillaries and medullary descending vasa recta (Bachmann *et al.* 1995). In contrast, eNOS expression is not seen in cortical capillaries or venous endothelium (Bachmann *et al.* 1995). Tubular expression of eNOS has been detected in the IMCD (Wu *et al.* 1999) the TAL of the loop of Henle (Plato *et al.* 2000) and the proximal convoluted tubule (Baines & Ho 2002). Western blot analysis shows equivalent expression in cortex and medulla (Martin *et al.* 2002).

Overview of NOS expression in the kidney

Expression of all three NOS isoforms has been reported in the kidney. The most consistently documented sites of constitutive NOS expression in the kidney are the expression of nNOS in the macula densa and the expression of eNOS in the renal vasculature (Fig. 2). In addition, expression of nNOS and eNOS in other sites in the kidney is well documented in at least some situations. Whether or not there is a significant level of iNOS protein expression in the normal kidney remains controversial. It is clear, however, that iNOS expression occurs in the kidney in pathological situations such as ischaemia–reperfusion injury (Kosaka *et al.* 2003, Park *et al.* 2003).

Overall, the unique topographical distribution of each of the NOS isoforms throughout the various segments of the nephron and other structures of the kidney confers a greater level of complexity to the regulation of NO synthesis in the kidney than is found in other organs. Knowledge of this topographical distribution of expression is, nonetheless, merely the first step in understanding how NO synthesis is regulated in the kidney. Presently, we are still a long way from a full understanding of the functions and the mechanisms of regulation of the individual NOS isoforms at each particular site within the kidney.

Functions of nitric oxide in the kidney

Regulation of renal haemodynamics

Given the importance of NO in vascular regulation, it is not surprising that NO is important in regulating renal haemodynamics. Studies using NOS inhibitors such as the L-arginine analogues nitro-L-arginine (NLA) and N^{ω}-nitro-L-arginine methyl ester (L-NAME) have shown that NO plays an important role in maintaining normal vascular tone in the kidney, which is known to have substantially lower vascular resistance than most other organs (Majid & Navar 2001). Inhibition of renal NO synthesis by intra-arterial infusion of NLA in anaesthetized dogs results in a 50% increase in renal vascular resistance and a 25% reduction in renal blood flow, with a similar reduction in perfusion to both cortex and medulla (Majid & Navar 1992). Despite the overall reduction in renal blood flow, inhibition of NO synthesis does not abolish the autoregulation response, although at any given level of blood pressure renal blood flow is reduced and renal vascular resistance is higher (Beirewaltes *et al.* 1992). In fact, evidence from the isolated perfused rat kidney model suggests that NO actually blunts autoregulation, possible due to its effect on TGF (Guan *et al.* 2003).

Mediation of pressure–natriuresis

The pressure–natriuresis response is central to the kidney's role in maintaining normal blood pressure and sodium balance (Guyton *et al.* 1972). This relationship describes the prompt increase in sodium excretion by the kidney (natriuresis) following physiological increases in blood pressure that returns blood pressure and total body sodium to normal. Development of hypertension, therefore, requires a right shift and/or a reduced slope of the pressure natriuresis curve. Importantly, NO synthesis appears to contribute to the normal pressure–natriuresis response (Majid *et al.* 1993). For example, inhibition of renal NO synthesis causes blunting and right-shift of the pressure–natriuresis curve, resulting in higher blood pressure (Salom *et al.* 1992). Moreover, excretion of urinary NO metabolites (NO_2^-) and (NO_3^-) increases with renal perfusion pressure (Suzuki *et al.* 1992, Majid *et al.* 1995). Finally, direct measures of renal cortical NO activity in the dog with an NO-sensitive microelectrode show that it increases steeply and linearly within the autoregulatory range (Majid *et al.* 1998). The mechanisms by which increased perfusion pressure increases renal NO synthesis are not well understood and need further investigation. One proposal has been that it results from increased endothelial cell shear stress (Majid *et al.* 1995). Exactly how increased renal NO synthesis causes the observed natriuresis is also not fully understood, although inhibition of tubular sodium transport appears to be important. In one study, pressure natriuresis depended on decreased tubular sodium reabsorption by way of an amiloride sensitive transport process, implicating the distal tubule

  doi: 10.1111/j.1748-1716.2006.01582.x

as the important site of NO action (Majid & Navar 1994).

Effects of nitric oxide on tubular sodium transport

The effect of NO on tubular sodium transport varies in different parts of the nephron. In most cases, however, the effect of NO is to inhibit sodium transport consistent with its known natriuretic and diuretic effects (Ortiz & Garvin 2002).

The effects of NO on salt and water transport in the proximal tubule, which is responsible for reabsorbing 50–60% of filtered sodium and water, are controversial. As reviewed in detail by Ortiz and Garvin, some studies suggest that NO inhibits proximal tubule transport, whilst others conclude that NO stimulates proximal tubule transport (Ortiz & Garvin 2002). Data from Wu *et al.* suggests that the explanation for these contradictory findings is that whilst NO has a direct inhibitory effect on proximal tubular sodium transport it is also required for the stimulatory effects on proximal tubular sodium transport that are mediated by renal sympathetic nerves (Wu & Johns 2002). The direct inhibitory effect of NO on proximal tubular sodium reabsorption seems to be due to decreased apical Na/H exchange (Roczniak & Burns 1996) and reduced Na/K-ATPase activity (Liang & Knox 1999).

The TAL of the loop of Henle reabsorbs 25–30% of filtered NaCl whilst being impermeable to water. Studies using the NO donor spermine NONOate and the NOS substrate L-arginine demonstrate that NO reduces NaCl absorption in the TAL (Plato *et al.* 1999). Expression of all three NOS isoforms has been reported in the TAL (Plato *et al.* 2000). Studies using NOS knockout mice are consistent with eNOS being the isoform responsible for the NO synthesis leading to inhibition of TAL NaCl resorption (Plato *et al.* 2000).

There is also evidence that NO inhibits sodium absorption in the CCD by inhibiting the amiloride sensitive sodium channel ENaC (Stoos *et al.* 1995). In addition, NO inhibits vasopressin stimulated osmotic water permeability in the CCD (Garcia *et al.* 1996a) by a mechanism involving a guanylate cyclase dependant increase in cGMP leading to a decrease in cytosolic cAMP (Garcia *et al.* 1996b). NO also inhibits H^+-ATPase activity in the acid secreting intercalated cells of the CCD (Tojo *et al.* 1994) and reduces urea transport in the IMCD (Zimpelmann *et al.* 2003). To date there are no studies of the effect of NO on transport in the distal tubule.

Taken together, the collective data is consistent with the NO having an overall inhibitory effect on tubular sodium reabsorption. This is considered to contribute to the diuretic and natriuretic effects of NO. Importantly, however, it remains unclear as to what extent the overall actions of NO in the kidney can be attributed to direct tubular effects, as opposed its other well-studied haemodynamic effects. In part, this is because many of the studies of the effect of NO on tubular sodium reabsorption have been performed in isolated tubule segments rather that the intact kidney. Resolving this issue should be an important priority for future studies.

Regulation of tubuloglomerular feedback

Tubuloglomerular feedback describes the afferent arteriolar vasoconstriction and resulting reduction in single nephron glomerular filtration rate that occurs with increased delivery of solute to the macula densa segment of the same nephron (Thomson *et al.* 1999). The effect of TGF is to minimize changes in GFR that would be predicted to result from acute changes in glomerular perfusion, such as may occur with fluctuations in blood pressure. Thus TGF tends to maintain the delivery of fluid and electrolytes to the macula densa (so-called distal delivery) fairly constant. Importantly, however, if the increase in distal delivery is maintained for longer than 15–30 min the TGF curve resets to the right (Thomson *et al.* 1999). This resetting has the effect of facilitating a natriuresis.

The signalling events that mediate the TGF response have been studied intensively. The sodium chloride co-transporter NKCC2 is expressed on the luminal surface of the macula densa and appears to be responsible for monitoring distal delivery, which is detected as a change in chloride concentration (Schlatter *et al.* 1989, Schnermann 1998). The details of the intracellular signalling that follows are controversial but the final result is an increased secretion of either adenosine (Osswald *et al.* 1991, Brown *et al.* 2001) or ATP (Nishiyama & Navar 2002) from the basolateral macula densa. Adenosine or ATP then binds to receptors on the smooth muscle cells of the afferent arteriole to mediate vasoconstriction.

The observation that nNOS is highly expressed in the macula densa was the first clue that NO may have a role in regulating the TGF response (Wilcox *et al.* 1992). A large number of studies using NOS inhibitors in the context of either micropuncture studies or studies using isolated juxtaglomerular preparations have established that the effect of NO is to blunt TGF (Wilcox 1998). This is consistent with NO being important in the rightward shift of the TGF response necessary for resetting and natriuresis when there is a sustained increase in delivery of salt to the distal nephron (Thomson *et al.* 1999). The mechanisms regulating NO production by nNOS in the macula densa have been of intense interest but are incompletely understood. It has been proposed that nNOS activity in the macula densa is regulated by intracellular [Ca^{2+}] mediated by NaCl transport by the

 doi: 10.1111/j.1748-1716.2006.01582.x

co-transporter NKCC2 (Bell *et al.* 1987). Direct measurements of macula densa intracellular [Ca^{2+}] following exposure to differing NaCl concentrations, however, have not supported this view (Salamonsson *et al.* 1991). Likewise, there is little observational data to support the hypothesis that nNOS activity in the macula densa is regulated by Na^+/H^+ antiporter mediated changes in intracellular pH (Yaqoob *et al.* 1996). The endogenously produced asymmetrical dimethylarginine (ADMA) competitively inhibits renal NOS activity and also inhibits uptake of L-arginine into macula densa cells via the specific cationic amino acid transporter y^+ (Tojo *et al.* 1997). However, whether ADMA has a physiological role in the regulation of nNOS in the macula densa is unclear. The enzyme N^G-N^G-dimethylarginine dimethylaminohydrolase (DDAH) inactivates ADMA by conversion to citrulline and has a distribution in the kidney that corresponds to NOS expression. DDAH has, therefore, been postulated as a possible regulator of renal NOS activity (Tojo *et al.* 2000). It has also been proposed that in some situations cellular uptake of L-arginine by the y+ transporter system is the rate limiting factor in macula densa NO synthesis (Welch & Wilcox 1997). Finally, it seems plausible that post-translational regulation of nNOS by mechanisms such as phosphorylation or protein–protein interactions will prove to be important in regulating macula densa NO synthesis, however, this hypothesis is yet to be tested.

Regulation of medullary blood flow

In addition to its well-known function in the counter-current exchange system of urinary concentration, the renal medullary circulation is increasingly recognized as playing an important role in the regulation of sodium balance and blood pressure (Cowley *et al.* 2003). NO is described as having a vital role in regulating medullary blood flow, thereby influencing both sodium balance and blood pressure regulation.

The role of NO in the renal medulla has been studied by directly infusing the NOS inhibitor L-NAME into the renal medulla. This reduces medullary blood flow by 40% with no change in cortical blood flow (Mattson *et al.* 1992). In contrast to the effects of L-NAME, infusion of the NO-dependant vasodilators bradykinin (Mattson & Cowley 1993) and acetylcholine (Mattson *et al.* 1992) into the renal medulla increases medullary blood flow. Importantly, when infusion of L-NAME into the renal medulla of rats is continued for 5 days this causes sodium retention and arterial hypertension (Mattson *et al.* 1994). When L-NAME is chronically infused intravenously at a low dose, medullary blood flow is reduced, whilst cortical blood flow is unchanged. Again the result is sodium retention and hypertension (Nakanishi *et al.* 1995). Reduced synthesis of NO in the renal medulla has also been implicated in the pathogenesis of hypertension in the Dahl salt sensitive rat, which is a genetic model of salt sensitive hypertension (Chen & Sanders 1991, Miyata & Cowley 1999).

Nitric oxide synthesis in the renal medulla is stimulated by the vasoconstrictors angiotensin II, noradrenaline and vasopressin (Cowley *et al.* 2003). For example, intravenous infusion of a subpressor dose of Ang II increases medullary NO production by nearly 150% (Zou *et al.* 1998). Similarly, subpressor amounts of noradrenaline increase medullary NO by 43% (Zou & Cowley 2000). Intravenous infusions of angiotensin II, noradrenaline and vasopressin at doses that are normally subpressor all cause hypertension in the presence of intramedullary infusion of L-NAME (Szentivanyi *et al.* 1999, 2000a,b). Taken together, these studies demonstrate that an important function of NO in the renal medulla is to counter-regulate the vasoconstrictive effects of these agents, so as to maintain medullary blood flow and protect against the development of hypertension.

Interactions between nitric oxide and renal sympathetic nerves

The renal sympathetic nervous system is important in the regulation of renal haemodynamics and also plays a role in sodium homeostasis. In different situations NO appears to either modulate or mediate the effects of the sympathetic nervous system in the kidney. Basal renal NO synthesis has been reported to blunt the vasoconstrictive effect of sympathetic nerve stimulation in the isolated perfused rat kidney (Reid & Rand 1992). As discussed above, NO is particularly important in the maintenance of renal medullary perfusion. Interestingly, basal medullary NO synthesis has also been found to contributes to the relative resistance of the renal medulla to the vasoconstrictive effect of renal sympathetic nerve activation (Eppel *et al.* 2003).

As discussed earlier, NO has been found to have an important role in mediating the increased proximal tubular reabsorption of sodium that occurs with renal sympathetic nerve stimulation (Wu & Johns 2002). Thus, in contrast to its overall natriuretic effects, this is a rare example of a situation in the kidney where NO is actually increasing renal sodium reabsorption.

Effects of dietary salt intake on renal nitric oxide synthesis

An important role for NO in the renal adaptation to dietary salt intake was first proposed by Shultz & Tolins (1993), who showed that high salt intake in rats for 2 weeks resulted in increased serum concentration and urinary excretion of the NO decomposition products NO_2^- and NO_3^- (NO_x). This study also found that

  doi: 10.1111/j.1748-1716.2006.01582.x

chronically salt-loaded rats had enhanced renal haemodynamic responses to NOS inhibition. This and other studies have demonstrated that increased renal NO production is seen following high dietary salt intake for 5–10 days (Tolins & Shultz 1994, Deng *et al.* 1995). In addition, Deng *et al.* (1994) found that the increased vascular response to NOS inhibition with L-NAME following high salt intake was not seen in the hindquarter, thus suggesting that the effect of salt loading on NO synthesis was a renal-specific phenomenon. There are no shorter-term studies to address the question of how quickly alterations in total renal NO synthesis occur following an increase in dietary salt intake. In a recent report, however, NO production by isolated TALs was increased after 1 day of high salt diet (Herrera *et al.* 2006). Furthermore, Atucha *et al.* (1994) has demonstrated that volume expansion with a 15-min infusion of intravenous saline results in a NO-dependant natriuresis and increase in papillary flow both during and immediately after the infusion. Overall, these studies are consistent with renal NO synthesis playing a role in both acute and chronic regulation of sodium balance.

The mechanisms mediating the increased renal NO production with high salt intake are incompletely understood. In general, increased NO production may be mediated by regulation of transcription and/or translation to increase total NOS protein, or at a post-translational level by increasing the amount of NO produced by preformed NOS. Post-translational mechanisms are generally more important in the responses to acute stimuli as they are able to regulate NO production more rapidly than changes in transcription or translation (Fig. 4). In contrast, regulation of total NOS abundance is considered to be of more importance in response to a sustained increase in demand (Fig. 4). Thus far, however, most studies of the effect of salt-loading on NOS in the kidney have been confined in their scope to the description of total NOS expression, which does not always correlate well with actual NO synthesis. Overall, in situations of acute regulation occurring over seconds or minutes the dissociation between total NOS expression and NO synthesis is unsurprising. Importantly, however, even in more chronic situations there are well-documented examples where measures of NO synthesis correlate poorly with total NOS expression (Welch & Wilcox 1997, Ortiz *et al.* 2003a, Herrera *et al.* 2006). This indicates that the role of post-translational mechanisms of NOS regulation is not confined exclusively to acute situations. In addition to both the regulation of total NOS expression and regulation by post-translational modifications, other mechanisms by which high dietary salt intake can regulate the actions of NO in the kidney include an increase in the sensitivity to NO signalling (Ortiz *et al.* 2003a) and alterations in renal oxidative stress (Bayorh *et al.* 2004).

The most consistent finding following an alteration in dietary salt intake is a change in nNOS expression in the macula densa. A number of studies have found that low salt diet increases macula densa nNOS expression, whereas high salt diet reduces macula densa nNOS expression (Bosse *et al.* 1995, Singh *et al.* 1996, Tojo *et al.* 2000, Mount *et al.* 2005a). In contrast, however, studies that measure the effect of dietary salt on actual NO production by the macula densa find that NO synthesis is increased by high salt and reduced by low salt (Wilcox & Welch 1996). The most convincing explanation for this observed paradox is derived from the observation that low salt diet reduces the availability and macula densa uptake of the NO precursor L-arginine (Welch & Wilcox 1997).

Studies examining the effect of dietary salt intake on renal NOS expression in sites other than the macula densa have produced variable results. In a study by Tojo *et al.* (2000) which compared the effects of high (6%) and low (0.03%) salt diets for 10 days on cortical expression of nNOS and eNOS, Western blot analysis

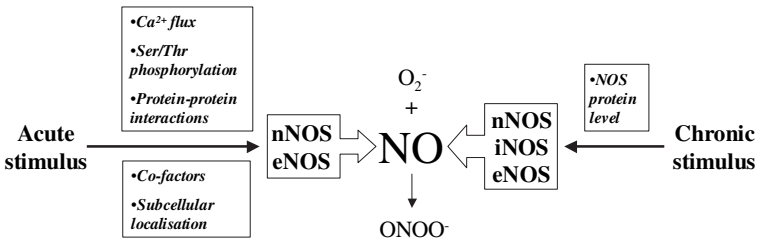

Figure 4 Possible mechanisms of regulation of nitric oxide (NO) synthesis in the kidney. The different types of regulation of NO synthesis proposed to be important in the kidney are shown. In general, post-translational mechanisms are important in response to acute stimuli requiring changes in renal NO synthesis within seconds or minutes. In contrast, regulation of total nitric oxide synthase (NOS) expression is important in response to more sustained changes in NO requirements occurring over hours or days. There is likely to be significant overlap in these two different types of NOS regulation in the kidney. The level of superoxide (O_2^-), which reacts with NO to form peroxynitrite ($ONOO^-$), also influences NO bioavailability in the kidney.

showed reduced expression of both nNOS and eNOS in the high salt group. By immunohistochemistry, the reduced nNOS expression occurred in the macula densa, whereas the reduced eNOS expression occurred in vascular endothelium. In a recent study, we also found that 2 weeks of high salt diet reduced both expression of eNOS in vascular endothelium and nNOS in the macula densa, whereas low salt diet had the opposite effect (Mount *et al.* 2005a). In contrast, we found that after 12-h nNOS expression was increased by high salt diet (8.95%) and reduced by low-salt diet (0%) (Mount *et al.* 2005a). Ni *et al.* have also noted a similar effect of dietary salt on renal NOS expression that varies with time (Ni & Vaziri 2001). In this study high salt (8%) diet for 3 weeks reduced expression of all three NOS isoforms in the renal cortex, and expression of nNOS and iNOS in the renal medulla (Ni & Vaziri 2001). The same study also found, however, that 48-h of high salt diet increased medullary expression of eNOS and iNOS (Ni & Vaziri 2001). An earlier study by Mattson & Higgins (1996) found, in contrast to the above findings, that 3 weeks of high salt increased expression of all three NOS isoforms in the medulla, whereas no changes in cortical NOS expression were seen. Roczniak *et al.* (1998) found that 3 days of high salt (4%) diet increased nNOS expression in the IMCDs, whereas nNOS expression in the cortex was reduced. In the same study, however, there were no changes in nNOS expression at either location when the high salt diet was continued for three weeks. Finally, a high salt diet has been reported to increase eNOS expression in glomerular endothelial cells by a pathway dependant on both mitogen-activated protein kinases (p38 MAPK and p42/44 MAPK) and TGF-β1 (Ying & Sanders 1998, 2002).

The mechanisms by which changes in dietary salt alter renal expression of NOS are not fully understood. Factors that have been implicated include angiotensin II (Tojo *et al.* 2000), renal perfusion pressure (Sato *et al.* 2004) and endothelin-1 (Herrera & Garvin 2005). Regarding angiotensin II, Tojo *et al.* (2000) observed increased renal eNOS expression with low salt diet that was prevented by the angiotensin II type 1 receptor antagonist losartan. Furthermore, this study found that a continuous subcutaneous infusion of angiotensin II increased renal eNOS expression. In the same study, angiotensin II did not appear to contribute to the increased macula densa expression of nNOS that was observed with a low salt diet (Tojo *et al.* 2000).

Renal perfusion pressure has also been implicated in the regulation of renal eNOS expression. Sato *et al.* (2004) observed that in mice deficient for the angiotensin type-1a receptor, 10 days of high salt diet increased both blood pressure and renal cortical eNOS expression, whereas a low salt diet had the opposite effect. In addition, these effects of dietary salt on blood pressure and eNOS expression were prevented by treatment with the vasodilator hydralazine. Interestingly, in this study no effect of dietary salt on blood pressure or renal eNOS expression was observed in wild type mice (Sato *et al.* 2004).

Endothelin-1 has been proposed to have a role in the regulation of eNOS expression in the TAL in response to high dietary salt. Herrera *et al.* found that 7 days of high salt diet increased eNOS expression in the TAL and that this was inhibited by the endothelin-1 receptor antagonist bosentan (Herrera & Garvin 2005). The mechanism appeared to be that a high salt diet increased outer medullary osmolality, which led to increased endothelin-1 release, which then stimulated TAL eNOS expression by activation of ET_B receptors.

Regulation of nitric oxide synthase

Overview

Both nNOS and eNOS are historically regarded as constitutive enzymes with low basal activity that are activated by stimuli that increase intracellular [Ca^{2+}], which then causes binding of calmodulin (Bredt & Snyder 1990). In recent years, however, it has become increasingly apparent that regulation of nNOS and eNOS is more complex and involves numerous levels of post-translational regulation including multi-site phosphorylation, protein-protein interactions and regulation of subcellular localization (Shaul 2002, Boo & Jo 2003, Kone *et al.* 2003) (Fig. 4). eNOS, for example, is known to have at least five regulatory phosphorylation sites and to be regulated by numerous kinases and phosphatases (Boo & Jo 2003). The eNOS phosphorylation sites that have been studied in the kidney are illustrated in Figure 3. nNOS is also reported to be regulated by both activating and inhibitory phosphorylation and numerous protein–protein interactions (Hayashi *et al.* 1999, Chen *et al.* 2000) (Fig. 3). Whilst the acute regulation of nNOS and eNOS is by post-translational mechanisms, chronic changes in NO synthesis, however, may be regulated by changes of total nNOS or eNOS expression (Fleming & Busse 2003) (Fig. 4). Another important factor influencing NO bioavailability in the kidney is the level of oxidative stress (Wilcox 2002). This is because increased oxidative stress is associated with high levels of superoxide anions (O_2^-), which rapidly reacts with NO to form the highly toxic peroxynitrite anion ($ONOO^-$) (Beckman & Koppenol 1996) (Fig. 4). Substrate and co-factor availability also have the potential to influence NO synthesis in the kidney. Finally, another described mechanism regulating the biological activity of NO in the kidney is regulation of the sensitivity to NO signalling beyond

  doi: 10.1111/j.1748-1716.2006.01582.x

NO production. For example, Ortiz *et al.* have demonstrated that seven to ten days of high salt diet did not alter TAL NO synthesis but did increase the sensitivity to NO of TAL NaCl reabsorption (Ortiz *et al.* 2003a).

In contrast to nNOS and eNOS, iNOS is characterized by Ca^{2+}-independence and high basal activity (Xie *et al.* 1992). This is because iNOS irreversibly binds CaM, even at low Ca^{2+} concentrations. In most situations iNOS is not constitutive and is predominantly regulated by control of transcription, which occurs in response to pro-inflammatory stimuli such as lipopolysaccharide and interferon (Lowenstein *et al.* 1993).

Recent insights into the regulation of nitric oxide synthase in the kidney

Whilst many of the descriptions of NOS regulation in the kidney are limited to descriptions of total NOS expression (Kone 2004), changes in NOS expression do not always correlate well measures of actual NO synthesis. For example, as discussed above, the effect of high salt and low salt diets on NO synthesis in the macula densa appears opposite to the effects on nNOS expression (Welch & Wilcox 1997). The potential for dissociation between NOS expression and NO synthesis in not surprising given that synthesis of NO by nNOS and eNOS is highly dependent on both adequate substrate and co-factor availability, as well as multiple types of post translational regulation (Fig. 4). Furthermore, changes in requirements for NO synthesis in the kidney are often too rapid for regulation of total NOS expression to play an important role. Thus far, studying the complex molecular events involved in the regulation of nNOS and eNOS in the kidney has proved relatively difficult. Nonetheless, as discussed below, several recent studies have indicated a role for post-translational regulation of nNOS and eNOS in the kidney by mechanisms such as serine/threonine phosphorylation.

In an interesting study, Oritz *et al.* analysed the molecular mechanisms by which luminal flow stimulates NO production by eNOS in isolated rat TALs of Henle's loop (Ortiz *et al.* 2004). Increasing luminal flow caused both translocation of eNOS to the apical membrane and increased phosphorylation at the activating site eNOS-Ser^{1177}. The increase in eNOS-Ser^{1177} phosphorylation was by a phosphatidylinositol 3-OH kinase (PI3-kinase) dependant pathway and required recruitment of heat shock protein 90. The effect of the increased NO synthesis was to inhibit reabsorption of sodium by the co-transporter NKCC2 (Ortiz *et al.* 2003b).

In the rat kidney, it has been shown that eNOS is basally phosphorylated at the activating sites eNOS-Ser^{1177} and eNOS-Ser^{633} as well as the inhibitory site eNOS-Thr^{495} (Mount *et al.* 2005a,b, Lee *et al.* 2005, Herrera *et al.* 2006). Interestingly, in comparison to cultured endothelial cells, in the kidney eNOS was found to have higher basal phosphorylation of the activating site eNOS-Ser^{1177} and lower phosphorylation of the inhibitory site eNOS-Thr^{495} (Mount *et al.* 2005a). Thus, the basal phosphorylation state of eNOS in the kidney appears to be an important contributor to the kidney's high basal NO synthesis and low vascular resistance. As yet, however, the kinases and phosphatases that determine eNOS phosphorylation in the kidney are unknown. Whilst high salt diet appears to increase renal NO synthesis, analysis of whole kidney homogenates after high salt diet did not demonstrate any change in phosphorylation of eNOS-Ser^{1177} and eNOS-Thr^{495} (Mount *et al.* 2005a). In contrast, Herrera *et al.* (2006) have recently demonstrated that high salt diet regulates phosphorylation of eNOS in the THAL and this regulation of eNOS phosphorylation is an important determinant of THAL NO synthesis. Specifically, this study found that dephosphorylation of the inhibitory site eNOS-Thr^{495} contributed to the increased THAL NO synthesis after 1 day of high salt diet, whereas after 7 days of high salt diet reduced phosphorylation of the activating sites eNOS-Ser^{1177} and eNOS-Ser^{633} explained why THAL NO synthesis was reduced despite an observed increase in total eNOS expression. In another interesting study, reduced inhibitory phosphorylation of eNOS-Thr^{495} has been observed in the renal medulla of diabetic rats, possibly explaining the increased medullary NOS activity that is observed with diabetic hyperglycaemia (Lee *et al.* 2005).

One important question is the extent to which the mechanisms regulating NO synthesis in the kidney are similar to those reported in other tissues. A recent study by Feliers *et al.* (2005) reported that stimulation of NO synthesis by vascular endothelial growth factor (VEGF) in glomerular endothelial cells was via activation of the PI3-kinase/Akt pathway leading to activating phosphorylation of eNOS-Ser^{1177}. This is the same mechanism by which VEGF stimulates NO synthesis in endothelial cells from other vascular beds (Brouet *et al.* 2001, Gelinas *et al.* 2002). It cannot be assumed, however, that the mechanisms identified for the regulation of NO synthesis in other systems will always apply to the kidney. For example, we have found that, in contrast to observations in the ischaemic heart (Chen *et al.* 1999), eNOS is not activated by phosphorylation of eNOS-Ser^{1177} during acute renal ischaemia (Mount *et al.* 2005b).

Relatively little is known about the molecular mechanisms regulating nNOS activity in the kidney. We have recently reported that basal phosphorylation of nNOS is detectable at the inhibitory site nNOS-Ser^{852} and the putative activating site nNOS-Ser^{1417} (Mount *et al.* 2005a) (these sites are numbered here according to the

human nNOS sequence and are equivalent to the nNOS-Ser^{847} and nNOS-Ser^{1412} sites in the rat nNOS sequence) (Fig. 3). The kinases involved in regulation of nNOS phosphorylation in the kidney are as yet unknown. Regarding the role of protein-protein interactions, Roczniak *et al.* (2000) have reported co-localization of nNOS with the protein inhibitor of nNOS (PIN) in the rat kidney and have proposed that increased expression of PIN contributes to reduced NO synthesis by nNOS in the $^5/_6$ nephrectomy model of chronic renal failure. nNOS is also reported to interact with post-synaptic density protein-93 (PSD-93) at the basolateral membrane of the macula densa (Tojo *et al.* 1999). Another interesting mechanism of nNOS regulation in the kidney appears to be availability of its substrate L-arginine. As discussed previously, this has been observed in the macula densa during low salt diet when cellular uptake of L-arginine by the y+ transporter system is reported to be the limiting factor in NO synthesis by nNOS (Welch & Wilcox 1997).

Conclusion

In the kidney, NO regulates sodium and water homeostasis via numerous mechanisms and impaired renal NO synthesis is implicated in the pathogenesis of hypertension. The particular topography and distribution of NOS isoforms within the kidney produce a complexity in the regulation of renal NO synthesis that is unique. Whilst our understanding of the mechanisms regulating NO synthesis by NOS in the kidney remains incomplete, it is increasingly appreciated that this regulation occurs at multiple levels including rapid mechanisms such Ca^{2+}/calmodulin, serine/threonine phosphorylation and protein–protein interactions and more long-term mechanisms such as regulation of NOS transcription and translation. Understanding the molecular mechanisms regulating NO synthesis in the kidney is predicted to provide insights into clinically important conditions such as hypertension and diabetic renal disease where dysregulation of renal NO synthesis is important.

Conflicts of interest

The authors have no conflicts of interest to declare in relation to this manuscript.

References

Atucha, N.M., Ramirez, A., Quesda, T. & Garcia-Estan, J. 1994. Effects of nitric oxide inhibition on the renal papillary blood flow response to saline-induced volume expansion in the rat. *Clin Sci (Lond)* **86**, 405–408.

Bachmann, S., Bosse, H.M. & Mundel, P. 1995. Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. *Am J Physiol Renal Physiol* **268**, F885–F898.

Bachmann, S., Oberhaumer, I., Bostanjoglo, M. & Schulke, M. 1998. NOS1 mRNA variants in the kidney: cell specific expression of alternative exons. *J Am Soc Nephrol* **9**, 303a.

Baines, A. & Ho, P. 2002. Glucose stimulates O2 consumption, NOS, and Na/H exchange in diabetic rat proximal tubules. *Am J Physiol Renal Physiol* **283**, F286–F293.

Bayorh, M.A., Ganafa, A.A., Socci, R.R., Silvestrov, N. & Abukhalaf, I.K. 2004. The role of oxidative stress in salt-induced hypertension. *Am J Hypertens* **17**, 31–36.

Beckman, J. & Koppenol, W. 1996. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* **271**, C1424–C1437.

Beirewaltes, W., Sigmon, D. & Carretero, O.A. 1992. Endothelium modulates renal blood flow but not autoregulation. *Am J Physiol Renal Physiol* **262**, F943–F949.

Bell, P., Franco, M. & Navar, L. 1987. Calcium as a mediator of tubuloglomerular feedback. *Annu Rev Phsiol* **49**, 275–293.

Boo, Y.C. & Jo, H. 2003. Flow-dependent regulation of endothelial nitric oxide synthase: role of protein kinases. *Am J Physiol Cell Physiol* **285**, C499–C508.

Bosse, H.M., Bohm, R., Resch, S. & Bachmann, S. 1995. Parallel regulation of constitutive NO synthase and renin at JGA of rat kidney under various stimuli. *Am J Physiol* **269**, F793–F805.

Bredt, D.S. & Snyder, S. 1990. Isolation of nitric oxide synthatase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci U S A* **87**, 682–685.

Brouet, A., Sonveaux, P., Dessy, C., Balligand, J.L. & Feron, O. 2001. Hsp90 ensures the transition from the early Ca^{2+}-dependent to the late phosphorylation-dependent activation of the endothelial nitric-oxide synthase in vascular endothelial growth factor-exposed endothelial cells. *J Biol Chem* **276**, 32663–32669.

Brown, R., Ollerstam, A., Johansson, B., Skott, O., Gebre-Medhin, S., Fredholm, B. & Persson, A.E. 2001. Abolished tubuloglomerular feedback and increased plasma renin in adenosine A1 receptor-deficient mice. *Am J Physiol Regul Integr Comp Physiol* **281**, R1362–R1367.

Bryant, C.E., Perretti, M. & Flower, R.J. 1998. Suppression by dexamethasone of inducible nitric oxide synthase protein expression in vivo: a possible role for lipocortin 1. *Biochem Pharmacol* **55**, 279–285.

Chen, P.Y. & Sanders, P.W. 1991. L-arginine abrogates salt-sensitive hypertension in Dahl/Rapp rats. *J Clin Invest* **88**, 1559–1567.

Chen, Z.-P., Mitchelhill, K.I., Michell, B.J., Stapleton, D., Rodriguez-Crespo, I., Witters, L.A., Power, D., Oritz de Montellano, P.R. & Kemp, B.E. 1999. AMP-activated protein kinase phosphorylation of endothelial NO synthase. *FEBS Letters* **443**, 285–289.

Chen, Z.-P., McConell, G.K., Michell, B.J., Snow, R.J., Canny, B.J. & Kemp, B.E. 2000. AMPK signalling in contracting human skeletal muscle: acetyl-CoA carboxylase and NO

  doi: 10.1111/j.1748-1716.2006.01582.x

synthase phosphorylation. *Am J Physiol Endocrinol Metab* **279**, E1202–E1206.

Cowley, A.W. Jr, Mori, T., Mattson, D. & Zou, A.-P. 2003. Role of renal NO production in the regulation of medullary blood flow. *Am J Physiol Regul Integr Comp Physiol* **284**, R1355–R1369.

Deng, X., Welch, W.J. & Wilcox, C.S. 1994. Renal vasoconstriction during inhibition of NO synthase: effects of dietary salt. *Kidney Int* **46**, 639–646.

Deng, X., Welch, W.J. & Wilcox, C.S. 1995. Renal vasodilation with L-arginine: effects of dietary salt. *Hypertension* **26**, 256–262.

Eppel, G.A., Denton, K.M., Malpas, S.C. & Evans, R.G. 2003. Nitric oxide in responses of regional kidney perfusion to renal nerve stimulation and renal ischaemia. *Pflugers Arch* **447**, 205–213.

Feliers, D., Chen, X., Akis, N., Choudhury, G.G., Madaio, M. & Kasinath, B.S. 2005. VEGF regulation of endothelial nitric oxide synthase in glomerular endothelial cells. *Kidney Int* **68**, 1648–1659.

Fleming, I. & Busse, R. 2003. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Am J Physiol Regul Integr Comp Physiol* **284**, R1–R12.

Garcia, N.H., Pomposiello, S.I. & Garvin, J.L. 1996a. Nitric oxide inhibits ADH-stimulated osmotic water permeability in cortical collecting ducts. *Am J Physiol* **270**, F206–F210.

Garcia, N.H., Stoos, B.A., Carretero, O.A. & Garvin, J.L. 1996b. Mechanism of the nitric oxide-induced blockade of collecting duct water permeability. *Hypertension* **27**, 679–683.

Gelinas, D.S., Bernatchez, P.N., Rollin, S., Bazan, N.G. & Sirois, M.G. 2002. Immediate and delayed VEGF-mediated NO synthesis in endothelial cells: role of PI3K, PKC and PLC pathways. *Br J Pharmacol* **137**, 1021–1030.

Guan, Z., Willgoss, D.A., Matthias, A., Manley, S.W., Crozier, S., Gobe, G. & Endre, Z.H. 2003. Facilitation of renal autoregulation by angiotensin II is mediated through modulation of nitric oxide. *Acta Physiol Scand* **179**, 189–201.

Guyton, A.C., Coleman, T.G., Cowley, A.V. Jr, Scheel, K.W., Manning, R.D. Jr & Norman, R.A. Jr 1972. Arterial pressure regulation. Overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med* **52**, 584–594.

Hayashi, Y., Nishio, M., Naito, Y., Yokokura, H., Nimura, Y., Hidaka, H. & Watanabe, Y. 1999. Regulation of neuronal nitric-oxide synthase by calmodulin kinases. *J Biol Chem* **274**, 20597–20602.

Herrera, M. & Garvin, J.L. 2005. A high-salt diet stimulates thick ascending limb eNOS expression by raising medullary osmolality and increasing release of endothelin-1 10.1152/ajprenal.00209.2004. *Am J Physiol Renal Physiol* **288**, F58–F64.

Herrera, M., Silva, G. & Garvin, J.L. 2006. A high-salt diet dissociates NO synthase-3 expression and NO production by the thick ascending limb. *Hypertension* **47**, 95–101.

Huang, P.L., Dawson, T.M., Bredt, D.S., Snyder, S.H. & Fishman, M.C. 1993. Targeted disruption of the neuronal nitric oxide synthase gene. *Cell* **75**, 1273–1286.

Jarry, A., Renaudin, K., Denis, M.G., Robard, M., Buffin-Meyer, B., Karam, G., Buzelin, F., Paris, H., Laboisse, C.L. & Vallette, G. 2003. Expression of NOS1 and soluble guanylyl cyclase by human kidney epithelial cells: morphological evidence for an autocrine/paracrine action of nitric oxide. *Kidney Int* **64**, 170–180.

Kishimoto, J., Spurr, N., Liao, M., Lizhi, L., Emson, P. & Xu, W. 1992. Localization of brain nitric oxide synthase (NOS) to human chromosome 12. *Genomics* **14**, 802–804.

Kone, B.C. 1999. Localization and regulation of nitric oxide synthase isoforms in the kidney. *Semin Nephrol* **19**, 230–241.

Kone, B.C. 2004. Nitric oxide synthesis in the kidney: isoforms, biosynthesis, and functions in health. *Semin Nephrol* **24**, 299–315.

Kone, B.C., Kuncewicz, T., Zhang, W. & Yu, Z.Y. 2003. Protein interactions with nitric oxide synthases: controlling the right time, the right place, and the right amount of nitric oxide. *Am J Physiol Renal Physiol* **285**, F178–F190.

Kosaka, H., Yoneyama, H., Zhang, L., Fujii, S., Yamamoto, A. & Igarashi, J. 2003. Induction of LOX-1 and iNOS expressions by ischemia-reperfusion of rat kidney and the opposing effect of L-arginine. *FASEB J* **17**, 636–643.

Lahera, V., Salom, M.G., Miranda-Guardiola, F., Moncada, S. & Romero, J.C. 1991. Effects of NG-nitro-L-arginine methyl ester on renal function and blood pressure. *Am J Physiol* **261**, F1033–F1037.

Lee, D.L., Sasser, J.M., Hobbs, J.L., Boriskie, A., Pollock, D.M., Carmines, P.K. & Pollock, J.S. 2005. Posttranslational regulation of NO synthase activity in the renal medulla of diabetic rats. *Am J Physiol Renal Physiol* **288**, F82–F90.

Liang, M. & Knox, F.G. 1999. Nitric oxide reduces the molecular activity of Na+,K+-ATPase in opossum kidney cells. *Kidney Int* **56**, 627–634.

Lowenstein, C., Alley, E., Raval, P. *et al.* 1993. Macrophage nitric oxide synthase gene: Two upstream regions mediate induction by interferon gamma and lipopolysaccharide. *Proc. Natl. Acad. Sci. USA* **90**, 9730–9734.

Majid, D.S.A. & Navar, L.G. 1992. Suppression of blood flow autoregulation plateau during nitric oxide blockade in canine kidney. *Am J Physiol Renal Physiol* **262**, F40–F46.

Majid, D.S. & Navar, L.G. 1994. Blockade of distal nephron sodium transport attenuates pressure natriuresis in dogs. *Hypertension* **23**, 1040–1045.

Majid, D.S. & Navar, L.G. 2001. Nitric oxide in the control of renal hemodynamics and excretory function. *Am J Hypertens* **14**, 74S–82S.

Majid, D.S.A., Williams, A. & Navar, L.G. 1993. Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anaesthetized dogs. *Am J Physiol Renal Physiol*, **262**, F718–F722.

Majid, D.S.A., Godfrey, M., Grisham, M.B. & Navar, L.G. 1995. Relation between pressure natriuresis and urinary excretion of nitrate/nitrite in anesthetized dogs. *Hypertension* **25**, 860–865.

Majid, D.S., Omoro, S.A., Chin, S.Y. & Navar, L.G. 1998. Intrarenal nitric oxide activity and pressure natriuresis in anesthetized dogs. *Hypertension* **32**, 266–272.

Martin, P.-Y., Bianchi, M., Roger, F., Niksic, L. & Feraille, E. 2002. Arginine vasopressin modulates expression of neuronal NOS in rat renal medulla. *Am J Physiol Renal Physiol* **283**, F559–F568.

Mattson, D.L. & Cowley, A.W. Jr 1993. Kinin actions on renal papillary blood flow and sodium excretion. *Hypertension* **21**, 961–965.

Mattson, D.L. & Higgins, D.J. 1996. Influence of dietary sodium intake on renal medullary nitric oxide synthase. *Hypertension* **27**, 688–692.

Mattson, D.L., Roman, R.J. & Cowley, A.W. Jr 1992. Role of nitric oxide in renal papillary blood flow and sodium excretion. *Hypertension* **19**, 766–769.

Mattson, D.L., Lu, S., Nakanishi, K., Papanek, P.E. & Cowley, A.W. Jr 1994. Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol* **266**, H1918–H1926.

Miyata, N. & Cowley, A.W. Jr 1999. Renal intramedullary infusion of L-arginine prevents reduction of medullary blood flow and hypertension in Dahl salt-sensitive rats. *Hypertension* **33**, 446–450.

Mohaupt, M.G., Elzie, J.L., Ahn, K.Y., Clapp, W.L., Wilcox, C.S. & Kone, B.C. 1994. Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. *Kidney Int* **46**, 653–665.

Morrissey, J., McCracken, R., Kaneto, H. *et al.* 1994. Location of inducible nitric oxide synthase mRNA in the normal kidney. *Kidney Int* **45**, 998–1005.

Mount, P.F., Fraser, S.A., Watanabe, Y., Lane, N., Katsis, F., Chen, Z.P., Kemp, B.E. & Power, D.A. 2005a. Phosphorylation of neuronal and endothelial nitric oxide synthase in the kidney with high and low salt diets. *Nephron Physiol* **102**, p36–p50.

Mount, P.F., Hill, R.E., Fraser, S.A., Levidiotis, V., Katsis, F., Kemp, B.E. & Power, D.A. 2005b. Acute renal ischemia rapidly activates the energy sensor AMPK but does not increase phosphorylation of eNOS-Ser1177. *Am J Physiol Renal Physiol* **289**, F1103–F1115.

Mundel, P., Bachmann, S., Bader, M., Fischer, A., Kummer, W., Mayer, B. & Kriz, W. 1992. Expression of nitric oxide synthase in kidney macula densa cells. *Kidney International* **42**, 1017–1019.

Nakanishi, K., Mattson, D.L. & Cowley, A.W. Jr 1995. Role of renal medullary blood flow in the development of L-NAME hypertension in rats. *Am J Physiol* **268**, R317–R323.

Ni, Z. & Vaziri, N.D. 2001. Effect of salt loading on nitric oxide synthase expression in normotensive rats. *Am J Hypertens* **14**, 155–163.

Nishiyama, A. & Navar, L.G. 2002. ATP mediates tubuloglomerular feedback. *Am J Physiol Regul Integr Comp Physiol* **283**, R273–R275; discussion R278–R279.

Oberbaumer, I., Moser, D. & Bachmann, S. 1998. Nitric oxide synthase 1 mRNA: tissue-specific variants from rat with alternative first exons. *Biol Chem* **379**, 913–919.

Ortiz, P.A. & Garvin, J.L. 2002. Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physiol* **282**, F777–F784.

Ortiz, P., Stoos, B.A., Hong, N.J., Boesch, D.M., Plato, C.F. & Garvin, J.L. 2003a. High-salt diet increases sensitivity to NO and eNOS expression but not NO production in THALs. *Hypertension* **41**, 682–687.

Ortiz, P.A., Hong, N.J., Wang, D. & Garvin, J.L. 2003b. Gene Transfer of eNOS to the Thick Ascending Limb of eNOS-KO Mice Restores the Effects of L-Arginine on NaCl Absorption. *Hypertension* **42**, 674–679.

Ortiz, P.A., Hong, N.J. & Garvin, J.L. 2004. Luminal flow induces eNOS activation and translocation in the rat thick ascending limb. II. Role of PI3-kinase and Hsp90. *Am J Physiol Renal Physiol* **287**, F281–F288.

Osswald, H., Muhlbauer, B. & Schenk, F. 1991. Adenosine mediates tubuloglomerular feedback response: an element of metabolic control of kidney function. *Kidney Int Suppl* **32**, S128–S131.

Pallone, T.L. & Mattson, D.L. 2002. Role of nitric oxide in regulation of the renal medulla in normal and hypertensive kidneys. *Curr Opin Nephrol Hypertens* **11**, 93–98.

Park, K.M., Byun, J.Y., Kramers, C., Kim, J.I., Huang, P.L. & Bonventre, J.V. 2003. Inducible nitric-oxide synthase is an important contributor to prolonged protective effects of ischemic preconditioning in the mouse kidney. *J Biol Chem* **278**, 27256–27266.

Plato, C.F., Stoos, B.A., Wang, D. & Garvin, J.L. 1999. Endogenous nitric oxide inhibits chloride transport in the thick ascending limb. *Am J Physiol* **276**, F159–F163.

Plato, C., Shesely, E. & Garvin, J.L. 2000. eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. *Hypertension* **35**, 319–323.

Reid, J.J. & Rand, M.J. 1992. Renal vasoconstriction is modulated by nitric oxide. *Clin Exp Pharmacol Physiol* **19**, 376–379.

Roczniak, A. & Burns, K.D. 1996. Nitric oxide stimulates guanylate cyclase and regulates sodium transport in rabbit proximal tubule. *Am J Physiol* **270**, F106–F115.

Roczniak, A., Zimpelmann, J. & Burns, K.D. 1998. Effect of dietary salt on neuronal nitric oxide synthase in the inner medullary collecting duct. *Am J Physiol Renal Physiol* **275**, F46–F54.

Roczniak, A., Levine, D.Z. & Burns, K.D. 2000. Localization of protein inhibitor of neuronal nitric oxide synthase in rat kidney. *Am J Physiol Renal Physiol* **278**, F702–F707.

Salamonsson, M., Gonzalez, E., Westerlund, P. & Persson, A. 1991. Intracellular cytosolic free calcium concentration in the macula densa and inascending limb cells at different luminal concentrations of sodium chloride and with added furosemide. *Acta Physiol Scand* **142**, 283–290.

Salom, M., Lahera, V., Miranda-Guaridola, F. & Romero, J. 1992. Blockade of pressure natriuresis induced by inhibition of renal synthesis of nitric oxide in dogs. *Am J Physiol Renal Physiol* **262**, F718–F722.

Sato, K., Kihara, M., Hashimoto, T., Matsushita, K., Koide, Y.-I., Tamura, K., Hirawa, N., Toya, Y., Fukamizu, A. & Umemura, S. 2004. Alterations in renal endothelial nitric oxide synthase expression by salt diet in angiotensin type-1a receptor gene knockout mice 10.1097/01.ASN.0000130922.75125.B8. *J Am Soc Nephrol* **15**, 1756–1763.

Schlatter, E., Salomonsson, M., Persson, A.E. & Greger, R. 1989. Macula densa cells sense luminal NaCl concentration

  doi: 10.1111/j.1748-1716.2006.01582.x

via furosemide sensitive Na+2Cl-K+ cotransport. *Pflugers Arch* **414**, 286–290.

Schnermann, J. 1998. Juxtaglomerular cell complex in the regulation of renal salt excretion. *Am J Physiol* **274**, R263–R279.

Shaul, P.W. 2002. Regulation of endothelial nitric oxide synthase: Location, Location, Location. *Annu Rev Physiol* **64**, 749–774.

Shultz, P.J. & Tolins, J.P. 1993. Adaptation to increased dietary salt intake in the rat. Role of endogenous nitric oxide. *J Clin Invest* **91**, 642–650.

Singh, I., Grams, M., Wang, W.H., Yang, T., Killen, P., Smart, A., Schnermann, J. & Briggs, J.P. 1996. Coordinate regulation of renal expression of nitric oxide synthase, renin, and angiotensinogen mRNA by dietary salt. *Am J Physiol* **270**, F1027–F1037.

Stoos, B.A., Garcia, N.H. & Garvin, J.L. 1995. Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. *J Am Soc Nephrol* **6**, 89–94.

Stuehr, D.J. 1999. Mammalian nitric oxide synthases. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1411**, 217–230.

Stumm, M.M., D'Orazio, D., Sumanovski, L.T., Martin, P.Y., Reichen, J. & Sieber, C.C. 2002. Endothelial, but not the inducible, nitric oxide synthase is detectable in normal and portal hypertensive rats. *Liver* **22**, 441–450.

Suzuki, H., Ikenaga, H., Hishikawa, K., Nakaki, T., Kato, R. & Saruta, T. 1992. Increases in NO2-/NO3- excretion in the urine as an indicator of the release of endothelium-derived relaxing factor during elevation of blood pressure. *Clin Sci (Lond)* **82**, 631–634.

Szentivanyi, M. Jr, Maeda, C.Y. & Cowley, A.W. Jr 1999. Local renal medullary L-NAME infusion enhances the effect of long-term angiotensin II treatment. *Hypertension* **33**, 440–445.

Szentivanyi, M. Jr, Park, F., Maeda, C.Y. & Cowley, A.W. Jr 2000a. Nitric oxide in the renal medulla protects from vasopressin-induced hypertension. *Hypertension* **35**, 740–745.

Szentivanyi, M. Jr, Zou, A.P., Maeda, C.Y., Mattson, D.L. & Cowley, A.W. Jr 2000b. Increase in renal medullary nitric oxide synthase activity protects from norepinephrine-induced hypertension. *Hypertension* **35**, 418–423.

Terada, Y., Tomita, K., Nonoguchi, H. & Marumo, F. 1992. Polymerase chain reaction localization of constitutive nitric oxide synthase and soluble guanylate cyclase messenger RNAs in microdissected rat nephron segments. *J Clin Invest* **90**, 659–665.

Thomson, S.C., Bachmann, S., Bostanjoglo, M., Ecelbarger, C.A., Peterson, O.W., Schwartz, D., Bao, D. & Blantz, R.C. 1999. Temporal adjustment of the juxtaglomerular apparatus during sustained inhibition of proximal reabsorption. *J Clin Invest* **104**, 1149–1158.

Tojo, A., Guzman, N.J., Garg, L.C., Tisher, C.C. & Madsen, K.M. 1994. Nitric oxide inhibits bafilomycin-sensitive H(+)-ATPase activity in rat cortical collecting duct. *Am J Physiol* **267**, F509–F515.

Tojo, A., Welch, W.J., Bremer, V., Kimoto, M., Kimura, K., Omata, M., Ogawa, T., Vallance, P. & Wilcox, C.S. 1997. Colocalization of demethylating and NOS and functional effects of methylarginines in rat kidney. *Kidney Int* **52**, 1593–1601.

Tojo, A., Bredt, D.S. & Wilcox, C.S. 1999. Distribution of postsynaptic density proteins in rat kidney: relationship to neuronal nitric oxide synthase. *Kidney Int* **55**, 1384–1394.

Tojo, A., Kimoto, M. & Wilcox, C.S. 2000. Renal expression of constitutive NOS and DDAH: separate effect of salt intake and angiotensin. *Kidney Int* **58**, 2075–2083.

Tolins, J. & Shultz, P. 1994. Endogenous nitric oxide synthesis determines sensitivity to the pressor effect of salt. *Kidney Int* **46**, 230–236.

Wang, X., Lu, M., Gao, Y., Papapetropoulos, A., Sessa, W.C. & Wang, W. 1998. Neuronal nitric oxide synthase is expressed in principal cell of collecting duct. *Am J Physiol Renal Physiol* **275**, F395–F399.

Wang, T., Inglis, F.M. & Kalb, R.G. 2000. Defective fluid and HCO3- absorption in proximal tubule of neuronal nitric oxide synthase-knockout mice. *Am J Physiol Renal Physiol* **279**, F518–F524.

Wang, H., Carretero, O.A. & Garvin, J.L. 2002. Nitric oxide produced by THAL nitric oxide synthase inhibits TGF. *Hypertension.* **39**, 662–666.

Welch, W.J. & Wilcox, C.S. 1997. Macula densa arginine delivery and uptake in the rat regulates glomerular capillary pressure. Effects of salt intake. *J Clin Invest* **100**, 2235–2242.

Wilcox, C.S. 1998. Role of macula densa NOS in tubuloglomerular feedback. *Curr Opin Nephrol Hypertens* **7**, 443–449.

Wilcox, C.S. 2002. Reactive oxygen species: roles in blood pressure and kidney function. *Curr Hypertens Rep* **4**, 160–166.

Wilcox, C.S. & Welch, W.J. 1996. TGF and nitric oxide: effects of salt intake and salt-sensitive hypertension. *Kidney Int Suppl* **55**, S9–S13.

Wilcox, C.S., Welch, W.J., Murad, F., Gross, S.S., Taylor, G., Levi, R. & Schmidt, H.H.H.W. 1992. Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc Natl Acad Sci U S A* **89**, 11993–11997.

Wu, X.C. & Johns, E.J. 2002. Nitric oxide modulation of neurally induced proximal tubular fluid reabsorption in the rat 10.1161/hy0302.105681. *Hypertension* **39**, 790–793.

Wu, F., Park, F., Cowley, A.W. Jr & Mattson, D.L. 1999. Quantification of nitric oxide synthase activity in microdissected segments of the rat kidney. *Am J Physiol Renal Physiol* **276**, F874–F881.

Xie, Q., Cho, H., Calaycay, J. *et al.* 1992. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. *Science* **256**, 225–228.

Yaqoob, M., Edelstein, C., Wieder, E., Alkhanaizi, A., Gengaro, P., Nemenoff, R. & Schrier, R. 1996. Nitric oxide kinetics during hypoxia in proximal tubules: effects of acidosis and glycine. *Kidney Int* **49**, 1314–1319.

Ying, W.-Z. & Sanders, P.W. 1998. Dietary salt enhances glomerular endothelial nitric oxide synthase through TGF-beta 1. *Am J Physiol Renal Physiol* **275**, F18–F24.

Ying, W.-Z. & Sanders, P.W. 2002. Dietary salt intake activates MAP kinases in the rat kidney. *FASEB J* **16**, 1683–1704.

 doi: 10.1111/j.1748-1716.2006.01582.x

Zhang, C., Walker, L.M. & Mayeux, P.R. 2000. Role of nitric oxide in lipopolysaccharide-induced oxidant stress in the rat kidney. *Biochem Pharmacol* **59**, 203–209.

Zimpelmann, J., Li, N. & Burns, K.D. 2003. Nitric oxide inhibits superoxide-stimulated urea permeability in the rat inner medullary collecting duct. *Am J Physiol Renal Physiol* **285**, F1160–F1167.

Zou, A.P. & Cowley, A.W. Jr 2000. Alpha(2)-adrenergic receptor-mediated increase in NO production buffers renal medullary vasoconstriction. *Am J Physiol Regul Integr Comp Physiol* **279**, R769–R777.

Zou, A.P., Wu, F. & Cowley, A.W. Jr 1998. Protective effect of angiotensin II-induced increase in nitric oxide in the renal medullary circulation. *Hypertension* **31**, 271–276.

  doi: 10.1111/j.1748-1716.2006.01582.x