

Nitric oxide deficiency in chronic kidney disease

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Baylis C. Nitric oxide deficiency in chronic kidney disease. *Am J Physiol Renal Physiol* 294: F1–F9, 2008. First published October 10, 2007; doi:10.1152/ajprenal.00424.2007.—The overall production of nitric oxide (NO) is decreased in chronic kidney disease (CKD) which contributes to cardiovascular events and further progression of kidney damage. There are many likely causes of NO deficiency in CKD and the areas surveyed in this review are: 1. Limitations on substrate (L-Arginine) availability, probably due to impaired renal L-Arginine biosynthesis, decreased transport of L-Arginine into endothelial cells and possible competition between NOS and competing metabolic pathways, such as arginase. 2. Increased circulating levels of endogenous NO synthase (NOS) inhibitors, in particular asymmetric dimethylarginine (ADMA). Increased methylation of proteins and their subsequent breakdown to release free ADMA may contribute but the major culprit is probably reduced ADMA catabolism by the enzymes dimethylarginine dimethylaminohydrolases. 3. Reduced renal cortex abundance of the neuronal NOS (nNOS) α protein correlates with injury while increasing nNOS β abundance may provide a compensatory, protective response. Interventions that can restore NO production by targeting these various pathways are likely to reduce the cardiovascular complications of CKD as well as slowing the rate of progression.

arginase; asymmetric dimethylarginine; dimethylarginine dimethylaminohydrolase; L-arginine transporters; neuronal nitric oxide synthase

WHY SHOULD WE BE INTERESTED in the possibility that nitric oxide (NO) deficiency occurs in chronic kidney disease (CKD)? There is considerable evidence from animal studies that experimentally induced chronic NO synthase (NOS) inhibition causes systemic and glomerular hypertension, glomerular ischemia, glomerulosclerosis, tubulointerstitial injury, and proteinuria (141). There is also considerable evidence, reviewed in this article, to suggest that multiple pathways lead to NO deficiency as a result of CKD. It is therefore likely that CKD-induced NO deficiency contributes to progression of CKD and that is the rationale for investigation of this area.

Total NO production can be assessed using the stable oxidation products of NO ($\text{NO}_2 + \text{NO}_3 = \text{NO}_x$), although this is only valid under conditions of dietary NO_x control (12). The NO_x output is decreased in end-stage renal disease (ESRD) patients on both peritoneal dialysis (PD) and hemodialysis (HD) as well as in CKD patients (Fig. 1, *left*) (2–4). A similar finding was reported by Blum et al. (17) in CKD patients. Although U_{NO_xV} is a useful qualitative index of NO production, it cannot be used as a quantitative measure of bioactive NO (12). Using the more direct approach to measure the $^{15}\text{N}_2$ -labeled arginine-to-citrulline conversion, Wever et al. (136) also concluded that NO production was decreased in humans with CKD. Most evidence suggests decreased total NO production in humans with renal disease, although there is one report of increased arginine-to-citrulline conversion in humans with ESRD (68). This might reflect activation of inducible

NOS (iNOS) by dialysis in this particular population. In the absence of acute inflammatory events, decreased total NO production (measured by urinary NO_x output) has also been reported in different animal models of CKD, including renal mass reduction, chronic glomerulonephritis, chronic puromycin aminonucleoside (PAN) nephritis, and normal aging (3, 39, 41, 42, 116, 130, 132) (Fig. 1, *right*).

NO is made in many locations, and it is the local production that determines physiological actions. Endothelial dysfunction occurs in ESRD and CKD (characterized by blunted endothelial NO release) even early in the course of the disease (9, 22, 47, 67, 119). Since the vascular endothelium represents the largest organ in the body, decreased total NO production in renal disease likely reflects the endothelial dysfunction. Animal studies also implicate intrarenal NO deficiency in CKD (3, 39, 41, 42, 116, 130, 132), which would contribute only slightly to the falls in U_{NO_xV} , since the kidney is a minor source of NO generation (12). There may be reductions in NO generation from other locations as well, although this is unlikely to be universal since, for example, cerebellar NOS activity is unaffected in chronic glomerulonephritis or in aging (41, 132).

There are many ways in which NO deficiency could occur in renal disease (Fig. 2), and it is likely that multiple mechanisms are recruited as CKD develops. For example, L-arginine is the limiting substrate for NO synthesis and arginine deficiency could develop due to 1) decreased endogenous arginine production (synthesized mainly in the kidney) as well as decreased L-arginine dietary intake; 2) diversion of L-arginine through other metabolic pathways (e.g., via activation of arginase); 3) reductions in arginine transport into cells to the vicinity of the NOS; and 4) failure of renal tubular reclamation of

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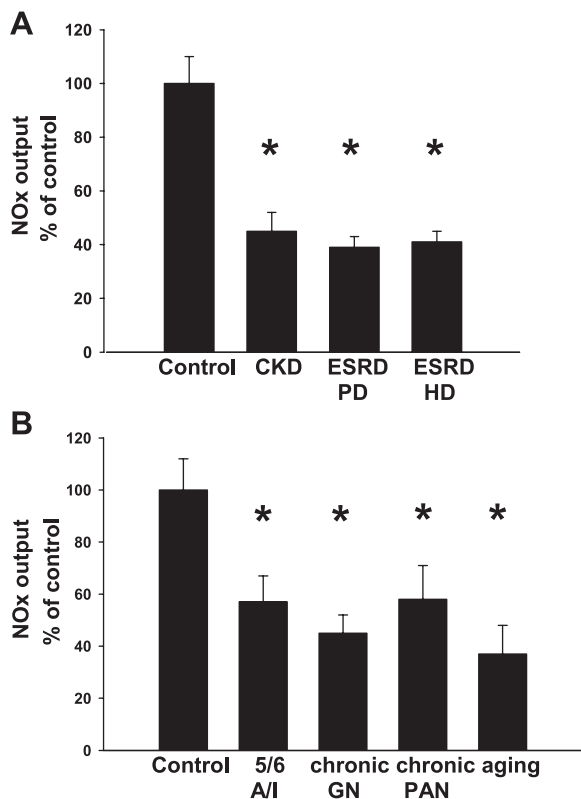


Fig. 1. A: nitrite+nitrate (NO_x) output, expressed as a percentage of the normal control value measured either as urinary excretion or output in dialysate, in subjects with normal renal function (control), in patients with chronic kidney disease (CKD), and in end-stage renal disease (ESRD) patients on either peritoneal dialysis (PD) or hemodialysis (HD). All subjects were on a constant similar low NO_x diet. *Significant difference vs. control. Data are from Refs. 105–107. B: NO_x output measured as urinary excretion in male Sprague-Dawley rats studied in control, 5% renal ablation/infarction (A/I), at both 11 wk after the start of the observation period, with chronic glomerulonephritis after 20 wk of observation, with chronic puromycin aminonucleoside nephrosis (PAN) after 15 wk of observation, and in rats at 22 mo of age (aging). All rats were maintained on a similar low-NO_x diet. *Significant difference vs. control. Data are from Refs. 39, 41, 42, 132.

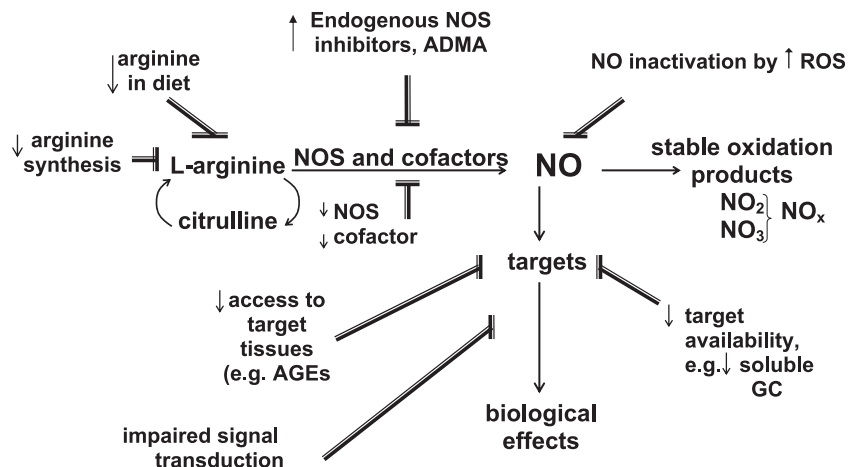
L-arginine. Other inhibitory influences include increases in the endogenous NOS inhibitors such as asymmetric dimethylarginine (ADMA) and decreases in activity of the NOS enzymes for many reasons, including reduction in protein abundance,

events that reduce inherent enzyme activity, and reduced availability of essential cofactors. Any of these situations would reduce the total NO generated, leading to a reduced NO_x output. In addition, a net NO deficiency occurs in the presence of oxidative stress, due both to inactivation by oxygen radicals as well as switching of NOS to become superoxide generators. There is clear evidence that oxidative stress occurs early in the course of CKD and is amplified as the disease progresses (51, 93). Finally, deposition of advanced glycosylated end products occurs in advanced renal disease (96), which decreases the access of NO to its target tissue and also contributes to net NO deficiency. Our work has focused on the proximal causes of NO deficiency in CKD, namely, substrate availability, ADMA levels, and NOS abundance/activity, and is the subject of this review.

Possible Mechanisms of Substrate Deficiency

L-Arginine synthesis and salvage. L-Arginine is classified as a “semiessential” amino acid, which means that in the healthy adult, endogenous arginine production is adequate for metabolic needs, but that under stress conditions dietary intake of L-arginine is also required (10, 13). As shown in Fig. 3, arginine is synthesized from L-citrulline (produced in the gut) in a two-step reaction involving the enzymes arginosuccinate synthase and lyase (ASS and ASL, respectively). There is abundant arginine production in the liver which is immediately utilized in the urea cycle and is not available for export. The L-arginine synthesized in the proximal tubules of the kidney cortex provides the major endogenous supply distributed throughout the body (137). In patients with advanced CKD or ESRD, where there is little normal functional renal mass, renal production of L-arginine may be compromised. Indeed, renal L-arginine synthesis is significantly reduced in CKD patients (121) and in rats with severe renal mass reduction (28). However, in milder, experimentally induced CKD caused by renal mass reduction, the combination of increased plasma citrulline concentration and proximal tubular hypertrophy can compensate and maintain renal L-arginine production. We do not yet know the relationship between the level of residual renal function and arginine biosynthetic capacity, nor do we know the impact of other forms of CKD on renal arginine production. Surprisingly, total arginine synthesis (measured from N15 citrulline-to-arginine conversion) is reportedly pre-

Fig. 2. Simplified scheme of the nitric oxide (NO) biosynthesis pathway showing a number of possible mechanisms that could cause a state of NO deficiency. NOS, nitric oxide synthase; ADMA, asymmetric dimethylarginine; ROS, reactive oxygen species; AGEs, advanced glycosylation end products; GC, guanlyl cyclase.



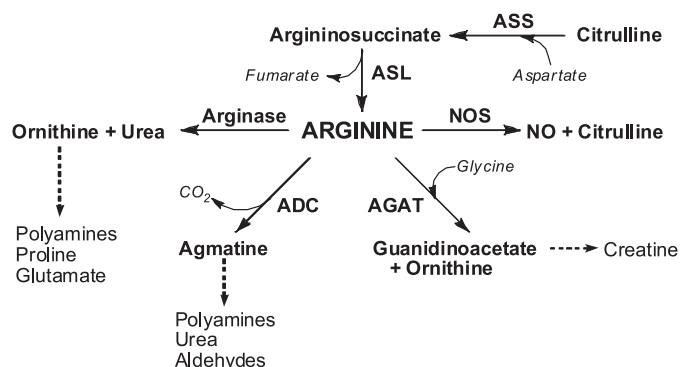


Fig. 3. Pathways of L-arginine metabolism. ASS and ASL, argininosuccinate synthase and lyase, respectively; ADC, arginine decarboxylase; AGAT, arginine:glycine amidinotransferase. Reproduced from Ref. 85 with permission.

served in ESRD patients (68), although this remains to be confirmed. If correct, this suggests compensatory increases in other tissues. The vascular endothelium has all the enzymes necessary for arginine synthesis, but there is no information on endothelial L-arginine production in CKD.

In addition to synthesis, the kidney also salvages almost all of the filtered L-arginine by reabsorption in the proximal tubule via a cationic amino acid antiporter that requires Na, the $b^{0,+}$ (24). ESRD patients lack this salvage mechanism and lose L-arginine during dialysis (138). The impact of different types of CKD and the severity of renal damage on arginine salvage is unknown.

Competing pathways for arginine metabolism. As shown in Fig. 3, there are four metabolic pathways that compete for arginine. The NOS and arginine decarboxylase (ADC)-agmatine pathways together account for only a small percentage of the total daily turnover of L-arginine (137). Creatine synthesis, to replace the quantity broken down and lost as creatinine each day, is significant but fairly constant, requiring an estimated ~15% of renal L-arginine production in humans (24). Arginase

is the most voracious consumer of L-arginine, leading to the production of urea, proline, and polyamines. The liver is the other site of major “de novo” arginine synthesis, but this never enters the circulation since it is immediately utilized by arginase I (cytosolic form) for urea production, in the process generating ornithine (24). Since ornithine is recycled to form citrulline and then arginine, hepatic arginase activity does not cause much depletion of the overall arginine supply. The kidney contains significant quantities of arginase II (mitochondrial form), while endothelial cells contain both isoforms (24, 74, 102) and there is evidence that renal and vascular arginases compete with NOS for the available L-arginine and can thus limit NO production. For example, endothelial cells overexpressing arginase I and II show reduced NO production (74). An increase in vascular arginase activity occurs in experimental hypertension, and arginase inhibition restores endothelial NO synthesis (35, 56, 102, 142) and protects the kidney from structural damage in the 5% renal mass ablation/infarction model of CKD (102). There is currently no information on whether arginase is activated in humans with CKD.

Arginine transport. Plasma L-arginine concentration is normal in CKD, although ototic acid increases, which is indicative of net L-arginine deficiency (100). If L-arginine transport into endothelial cells was impaired in CKD, this would reduce the rate of removal of L-arginine from plasma and camouflage a reduction in intracellular L-arginine availability. As shown in Fig. 4, when cultured endothelial cells are exposed to a 20% solution of uremic plasma, L-arginine transport is inhibited (139). Studies with artificial solutions containing high concentrations of urea suggest that urea contributes and that the effect is not osmotically mediated or acutely reversible with excess arginine and exhibits an “all-or-nothing” response, switching on when the concentration in the medium exceeds ~15 mmol/l or ~45 mg/100 ml (139). Furthermore, urea has to enter the endothelial cell to act, since in the presence of phloretin, a urea transport inhibitor, the inhibitory effect of urea on arginine transport was abolished (131). After 7 days of urea-induced

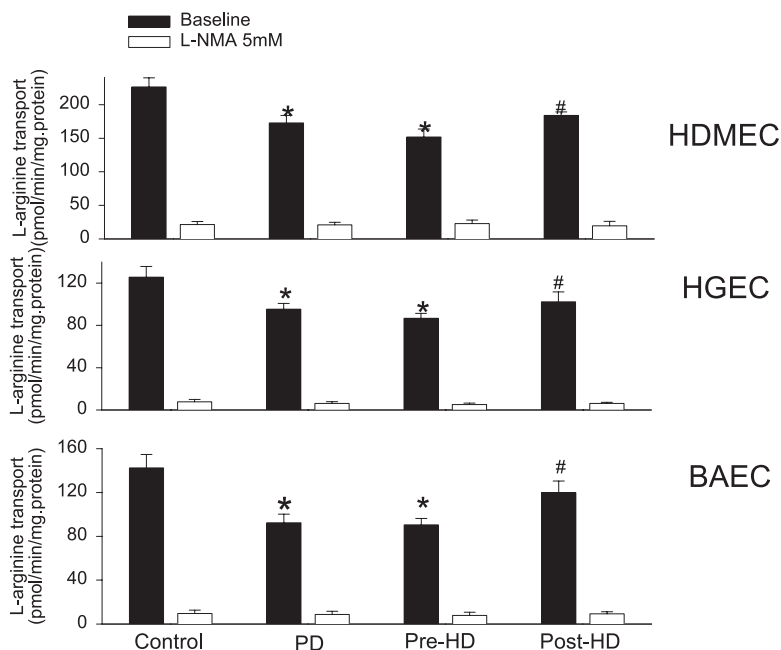


Fig. 4. L-Arginine transport in human dermal microvascular endothelial cells (HDMEC), human glomerular endothelial cells (HGEC), and bovine aortic endothelial cells (BAEC) after 6-h incubation with a 1:5 dilution of plasma from healthy subjects (control) or from patients undergoing peritoneal dialysis (PD) or hemodialysis (HD) immediately before (pre-HD) and immediately after (post-HD) dialysis. Measurements were made in the baseline state (solid histograms) and in the presence of 5 mM *N*-methyl-L-arginine (L-NMA; open histograms), a cationic amino acid transporter predominantly expressed in endothelial cells. *Significant difference ($P < 0.01$) compared with control. #Significant difference ($P < 0.05$) compared with both pre-HD and control. Reproduced from Ref. 139 with permission.

inhibition of L-arginine transport, we observed decreased endothelial NOS (eNOS) activity in cultured endothelial cells, in the absence of changes in eNOS protein abundance, suggesting that cumulative substrate deficiency might lead to limitation of NO production (139).

There must be other transport-inhibitory factors in uremia in addition to urea since a synthetic solution of 5 mmol/l urea has no impact on transport of L-arginine whereas a one-fifth dilution of human ESRD plasma (containing ~5 mmol/l urea) causes significant inhibition of L-arginine transport in cultured endothelial cells (139). The cationic amino acid transporter CAT1 is the primary, functionally significant endothelial cell L-arginine transporter (37), and both lysine and ornithine have a high affinity for CAT1 and could thus serve as competitive inhibitors; however, their concentration is either low or normal in uremia (16, 114, 121). Concentrations of the endogenous methylated arginines, both asymmetric and symmetric dimethylarginines (ADMA and SDMA, respectively) are increased in plasma in ESRD and CKD (6, 7, 22, 44, 58, 80, 78, 105, 106, 107, 129, 133) and are also transported by CAT (23, 30). Both methylated arginines have affinity for CAT1 (constitutive) and inducible CAT2 (30, 133), but *in vitro* we find that maximal uremic levels of ADMA (10 $\mu\text{mol/l}$) are too low to compete with L-arginine for membrane transport (139); thus the other L-arginine transport inhibitors remain unknown. CAT1 protein abundance is reduced in aortas and glomeruli from rats with CKD (109). Defects in endothelial L-arginine transport in renal failure patients could explain the poor responses to both acute and chronic L-arginine supplementation in the renal failure population (14, 31, 34, 143). It has been reported that increased L-arginine transport occurs into erythrocytes and platelets from ESRD patients, although whether this reflects ongoing acute inflammation or is routinely observed in the ESRD population remains to be determined (82, 83).

Endogenous NOS Inhibitors

Many studies have confirmed the original observation by Vallance and colleagues that ADMA, the competitive inhibitor of NOS, increases in the plasma of ESRD patients (6, 7, 22, 44, 58, 78, 80, 105, 107, 129, 133). There is considerable variation in baseline values of plasma ADMA (6, 7, 22, 44, 58, 59, 78, 80, 105, 107, 129, 133, 128), but most groups report plasma ADMA levels in excess of 2 $\mu\text{mol/l}$ in ESRD and that this concentration is sufficient to inhibit endothelial cell NO synthesis *in vitro* (138). We have also observed that when cultured endothelial cells were exposed to a 20% dilution of ESRD patient plasma, NOS activity was inhibited and this effect was reversible with L-arginine (138), suggesting competitive inhibition. Of note, this finding also implicates the presence of other, as yet unidentified NOS inhibitors in ESRD plasma since a 20% dilution of a synthetic solution containing ESRD level ADMA (~4–6 $\mu\text{mol/l}$) gives ADMA concentrations below the threshold able to inhibit eNOS activity (138).

Initially, it was assumed that the increased plasma ADMA in ESRD reflected loss of renal clearance (129); however, it is now known that very little ADMA is excreted unchanged in the urine and the majority is broken down by the enzymes dimethylarginine dimethylaminohydrolase 1 and 2 (DDAH1 and 2) (86, 124) (Fig. 5). DDAH is widely distributed and is most abundant in the kidney and also highly expressed in the liver

and vasculature (19, 87, 128). Control of ADMA levels by DDAH activity has significant influence on NO generation both in cultured endothelial cells (26) and *in vivo*, since mice that overexpress DDAH produce NO in excess and have low blood pressure (27). Two isoforms of DDAH cosegregate with NOS; DDAH1 is located in neuronal and epithelial tissues, cosegregates with NOS1, and is abundant in the kidney and liver, while DDAH2 is found predominantly in endothelium and is highly expressed in the vasculature (19, 87, 134). Mice heterozygous for DDAH1 knockout exhibit elevated plasma ADMA and endothelial dysfunction (71). Using a small interfering RNA approach in rats, Wilcox and colleagues (134) reported that DDAH1 knockdown increased plasma ADMA but had little impact on endothelium-dependent vascular relaxation, while the reverse was seen with DDAH2 silencing. However, DDAH2 overexpression in mice reduces plasma ADMA (50), so the relative roles of renal/hepatic DDAH1 and vascular endothelial DDAH2 in regulation of tissue and circulating ADMA remain to be determined.

ADMA levels in ESRD is of major importance since the high cardiovascular risk of this population is exacerbated when plasma ADMA is in the upper 50th percentile (22, 47, 144, 145, 146). Renal failure is a state of marked oxidative stress, and oxidative stress inhibits DDAH activity, causing ADMA levels to increase (20, 54). Oxidative stress is also associated with enhancement of ADMA synthesis via stimulation of protein methyltransferase 1 (21). Reduction in oxidative stress should improve DDAH activity and reduce ADMA levels, and some studies suggest that antioxidants do lower ADMA levels *in vitro* and *in vivo* (55, 65, 103). Despite this, we found that while vitamin E treatment reversed renal NADPH-dependent superoxide overproduction in the 5% ablation kidney model, the elevated plasma ADMA and depressed renal DDAH activity persisted (118). Thus the importance of renal DDAH activity in overall control of ADMA levels, as well as the impact of redox state, remains to be determined.

There are increases in cardiovascular events, inflammation, and oxidative stress as well as endothelial dysfunction in patients with CKD, even when only mild renal insufficiency is present (9, 47, 51, 67, 93, 119). In a small series, we reported that plasma ADMA levels are variable in CKD patients and not correlated to the severity of CKD (140), and similar findings were reported by Saran and colleagues (103). In contrast, Fleck et al. (44) report mild (30–40%) but consistent elevations in plasma ADMA in CKD patients that do not correlate with

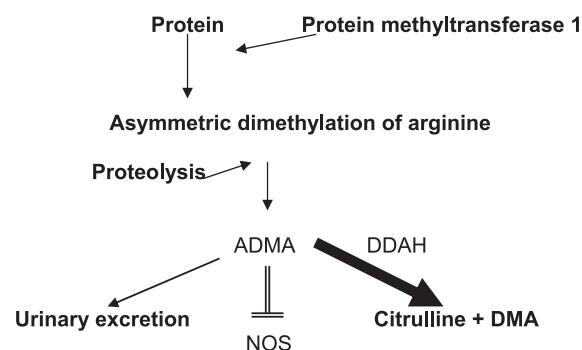


Fig. 5. Simplified scheme showing the main metabolic pathways for asymmetric dimethylarginine (ADMA). DDAH, dimethylarginine dimethylaminohydrolase; DMA, dimethylamine.

plasma creatinine. Kielstein and colleagues (59) observed marked (300–400%) consistent elevations in plasma ADMA from CKD stages 1–5, with no correlation to plasma creatinine. Two recent studies have reported that plasma ADMA varies inversely with the severity of CKD, in contrast to the studies cited above (46, 99), and Caglar et al. (25) observed that plasma ADMA in stage 1 CKD is predicted by the level of proteinuria rather than plasma creatinine (when analyzed by multivariate analysis) (25). This variability in the CKD population may reflect variations in the activity of DDAH.

The functional importance of DDAH activity in renal and vascular injury is beginning to be explored in animal studies. The DDAH2-overexpressing mouse is protected from vascular (aortic) and cardiac injury induced by chronic ADMA or ANG II administration (50). Overexpression of DDAH1 (delivered via an adenoviral vector) protects renal function and structure in the 5% renal ablation model in the rat (81). It seems likely that reduction of circulating/tissue ADMA via stimulation of DDAH activity may be a useful therapeutic option to retard progression of CKD and reduce cardiovascular events. Which DDAH isoform should be targeted and why the clinical literature shows such variability on the subject of circulating ADMA in the CKD population remain to be determined.

Renal *no* Production

In addition to the widespread cardiovascular NO deficiency, NO derived from the kidney is also impaired in CKD. There are several reports of reductions in NOS protein abundance in CKD produced by renal mass reduction (3, 42, 116, 130). In a model of 5% renal ablation/infarction (A/I) we found that the cortical neuronal NOS (NOS1) abundance fell linearly as glomerular damage increases above a threshold of ~20% (116). We have seen similar reductions in renal cortical nNOS abundance in other renal mass reduction models, accelerated 5% A/I (with high sodium and protein intake) (116), and in a model of pure 5% renal ablation (by polectomy) (118). Because a massive reduction of renal mass does not recapitulate the pathogenesis of CKD in humans, we have also investigated other models, i.e., chronic glomerular nephritis (132), chronic PAN (39), the aging Sprague-Dawley (SD) male (41), the Zucker obese inbred hyperglycemic rat (11a), and chronic allograft nephropathy (89), and in every case the renal cortical nNOS abundance is reduced (Fig. 6) while the eNOS abundance is highly variable. Furthermore, the *in vitro* NOS activity in the soluble fraction of the renal cortex (primary location of the nNOS) (42, 39, 116, 132) is reduced in parallel. These findings have focused our interest on the possible role of the renal cortical nNOS in progression of CKD. The macula densa is the site of greatest nNOS density in the renal cortex, although nNOS immunoreactivity has also been observed in proximal tubules, cortical collecting ducts, and perivascular nerves in the kidney cortex (8a). How this distribution is changed in CKD remains to be determined.

If the decline in renal nNOS causes progression of CKD, protection from progression should coexist with preserved renal nNOS. This prompted us to investigate the Wistar-Furth (WF), rat which is resistant to both mineralocorticoid- and renal ablation-induced CKD and hypertension (40, 42, 43, 125). We found elevated renal cortical nNOS abundance and preserved activity after 5% A/I in the WF vs. the SD rat, while

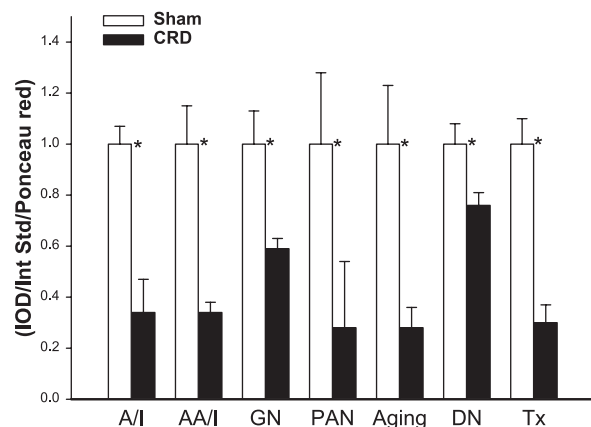


Fig. 6. Abundance of the neuronal NOS α , given as a percentage of the respective control given as integrated optical density (IOD) factored for an internal standard (cerebellar lysate) and for total protein loaded from Ponceau red. Measurements were made in homogenates of renal cortex from sham controls (open histograms) and experimental models of CKD (closed histograms) in male rats subjected to 11 wk A/I; accelerated A/I (A/II) = 3 wk-post A/I with high sodium and protein intake; 20 wk after induction of glomerulonephritis (GN); 15 wk after induction of puromycin aminonucleoside nephrosis (PAN); 22-mo-old, aging Sprague-Dawley rats; 10-mo-old lean and obese Zucker rats, a model of diabetic nephropathy (DN); and 22 wk after transplantation (TX) of a Fisher 344 rat kidney into a Lewis host. *Significant difference vs. controls. Data are from Refs. 11, 39, 41, 42, 89, 116, 132. Reproduced from Ref. 11 with permission.

addition of a low level of chronic NOS inhibition converts the WF into rapidly progressive CKD and severe hypertension (42). We have made similar observations in normotensive chronic PAN, in that SD rats develop severe injury, while WF rats have near normal kidney function and structure (39) and in the hypertensive DOCA/salt model (40). In each case, this recovery or failure to progress in the “protected” WF rat was associated with maintained renal nNOS abundance and activity. There is also sexual dimorphism in the susceptibility to progression of CKD, females being protected, and this sex difference is particularly marked in age-dependent kidney damage. In the old male SD rat, severe structural damage and loss of function is associated with declines in renal nNOS protein, while in the protected aging female SD there is preserved renal cortical nNOS protein (41). Finally, the C57BL6 mouse is highly resistant to development of renal mass reduction-induced injury (66), and we observed that both chronic nonselective NOS inhibition and selective nNOS inhibition greatly accelerate the development of the renal damage (88, 117). Taken together, these data suggest that a deficiency of renal cortical nNOS-derived NO may play a primary role in progression of CKD.

In the absence of a direct renal insult, the WF rat is also resistant to chronic, high-dose systemic NOS inhibition-induced CKD (38), despite evidence of significant reductions in total NO production. This suggests that “two hits” are necessary for development of progressive CKD; a primary insult to the kidney and a resulting loss of renal cortical nNOS derived-NO. Presumably, protected animals (WF rats, female SD rats, C57BL6 mice) can somehow preserve their renal nNOS-generating capacity after a primary kidney injury. The protection of the WF rat from CKD with high-dose NOS inhibition alone (38) may be associated with highly efficient autoregulation by the afferent arteriole. Acute high-dose NOS inhibition in-

creased systemic blood pressure in both SD and WF rats, but glomerular blood pressure was only elevated in the SD rat (38).

There are multiple splice variants of nNOS (5), and, in the rat kidney, in addition to the full-length ~160-kDa nNOS α protein, we find nNOS β , at both the transcript and protein level (110). The nNOS β transcript is an exon 2 deletion which encodes a ~140-kDa nNOS protein that is an active enzyme and which lacks the unique NH₂ terminus of the nNOS α (5). Since the amino terminus contains both the membrane associated PDZ domain and a site for protein-protein interactions, the nNOS β will always reside in the cytosol and will be unaffected by regulatory protein interactions. All our work described above (11a, 39, 41, 89, 116, 118, 132) was conducted using an amino-terminus antibody which therefore detects the nNOS α but not the nNOS β . We have preliminary (unpublished) data in the % ablation and % A/I models of CKD that, as nNOS α abundance falls with injury, there is an upregulation of the nNOS β variant. This might represent an attempt at compensation for the loss of nNOS α -derived NO and could be an advantage since the protein inhibitor of nNOS is upregulated in the rat kidney following % reduction of renal mass (110) but could not interact with (inhibit) nNOS β . However, considerably more research is required in this area before we can determine whether the activity of the nNOS splice variants actually controls the rate of progression of CKD.

Conclusions

Total NO production is decreased in renal disease due to impaired endothelial and renal NO production. Many mechanisms are likely to be responsible, including substrate limitation due to decreased renal synthesis of L-arginine, utilization by arginase, and decreased L-arginine delivery into cells; increased ADMA mainly because of reduced DDAH activity; and loss of renal NO production due to reduced renal nNOS α abundance/activity. Targeting these various causes of NO deficiency may provide novel therapeutic targets for slowing the progression of CKD and reducing cardiovascular disease in this population.

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