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Nitric oxide synthase derangements and hypertension in kidney disease

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Abstract

Purpose of Review—Nitric oxide (NO) deficiency occurs by multiple mechanisms and contributes to the pathogenesis of progression of chronic kidney disease (CKD) and its cardiovascular complications. This article concentrates on recent developments on the regulation of the endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) in chronic kidney disease and on the importance of the NO synthases in kidney disease progression, particularly in diabetic nephropathy.

Recent Findings—The increased plasma ADMA seen in renal disease is generally predictive of severity of CKD progression and cardiovascular risk. However, some assumptions about the control of ADMA have been challenged; the primacy of the kidney as a metabolic organ for plasma ADMA regulation coming under scrutiny and the relative importance of the 2 isoforms of the ADMA-metabolizing enzymes dimethylarginine dimethylaminohydrolases (DDAHs) is being reevaluated. Alterations in nitric oxide synthases also contribute to CKD progression with the endothelial isoform playing a major role in diabetic nephropathy.

Summary—Improving our understanding of ADMA regulation is important since pharmacologic targeting of DDAH is underway. The major role of eNOS-derived NO in diabetic nephropathy should lead to novel therapies. The beneficial actions of dietary nitrate supplementation on blood pressure and kidney disease are of considerable clinical relevance.

Keywords

asymmetric dimethylarginine; dimethylarginine dimethylaminohydrolase; nitric oxide synthase

Introduction

In animal studies, experimentally induced chronic NO synthase (NOS) inhibition causes systemic and glomerular hypertension, glomerular ischemia, glomerulosclerosis, tubulointerstitial injury and proteinuria [1,2]. There is also considerable clinical and experimental evidence that nitric oxide (NO) deficiency develops as a result of chronic kidney disease (CKD) and that NO deficiency is linked to progression of renal dysfunction [1–4].

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NO acts locally, therefore the location of the NO deficiency dictates the pathology. Widespread vascular endothelial NO deficiency (=endothelial dysfunction) occurs throughout the course of CKD and contributes to the increased risk of adverse cardiovascular events [3]. In addition, animal studies implicate intrarenal NO deficiency in the pathogenesis of CKD progression [1]. In support of a key role for NO deficiency in the pathogenesis of renal disease, NO repletion by administration of nitrite (NO₂) or nitrate (NO₃) reduced BP and greatly retarded progression of CKD in 2 rat models of hypertension and kidney damage [5,6]. The high dietary content of NO₃ in vegetables and fruit may contribute to the cardiovascular protection afforded by these foods [7].

There are several potential mechanisms by which NO deficiency could occur in CKD and in this review we consider recent research on the endogenous NOS inhibitors which compete with the NOS substrate arginine, and on variations in specific NOS activity/ abundance in CKD.

Endogenous NOS inhibitors

Asymmetric dimethylarginine (ADMA) is an endogenous NOS inhibitor recognized as a major cardiovascular risk factor [8]. Even within the “normal” population, those with plasma ADMA in the upper range of normal are at increased risk for adverse cardiovascular events. Elevations in plasma ADMA are seen in many pathologies including renal disease and individuals in the upper range have the worst outcome [8]. As a note of caution, while a high plasma ADMA probably represents an elevation in intracellular ADMA, the relationship between circulating and tissue levels of ADMA is not straightforward and will likely vary for different tissues [8,9].

ADMA is a difficult molecule to measure and a number of HPLC, GC-MS and recently ELISA methods have been used, resulting in variability in the values considered “normal” and in those seen in renal disease [1,8,10,11–13]. Plasma ADMA can be very high in end stage kidney disease (ESKD) and is variable, even within a defined population, with the cardiovascular risk being exacerbated when plasma ADMA > 50% percentile [8,10]. In chronic kidney disease (CKD) plasma ADMA is very variable and reported as unchanged in some patients, increased mildly or markedly without correlation to the severity of CKD and increased in proportion to the severity of CKD [1,13]. In stage 1, non diabetic CKD and stage 1 IgA nephropathy patients, where GFR is normal, the plasma ADMA correlated with proteinuria [14,15] while in the MDRD population of predominantly non-diabetic stage 3–4 CKD patients, high plasma ADMA correlated with low GFR but not proteinuria [16]. In recent studies from Taiwan (~ 50% diabetic, stage 3–4 CKD) and Norway (predominantly nondiabetic, stage 1–5 CKD) an inverse relationship was reported between plasma ADMA and creatinine [17,18]. In general the highest plasma ADMA was associated with rapid CKD progression [15,19,20] and greater cardiovascular and all cause mortality [15,17,18], presumably due to NO deficiency in the endothelium and elsewhere. Nevertheless, Caplin recently reported a gain of function polymorphism in the DDAH1 gene, associated with reduced plasma ADMA in both normal subjects and individuals with CKD. Rather than being protective, however, the genotype conferring lower plasma ADMA was associated with a more rapid rate of CKD progression in 2 separate populations [21]. This serves as a reminder that there are many unknown factors determining CKD progression.

The loss of renal clearance in renal failure causes plasma ADMA to increase although relatively little ADMA is excreted unchanged in the urine, ~15–20% in normal man. While we should not ignore the importance of renal clearance, the increase in plasma ADMA in stage 1 CKD [1,14,15] implicates additional causes. Enzymatic degradation of ADMA by dimethylarginine dimethylaminohydrolase, DDAH1 and 2, provides the majority of ADMA

removal and DDAH are widely distributed and are most abundant in the kidney, also liver and vascular endothelium [11]. DDAH co-segregates with NO synthase (NOS), and DDAH1 is predominantly located in neuronal and epithelial tissues with neuronal (n)NOS and is abundant in kidney and liver. DDAH2 is found predominantly in the vasculature, in association with endothelial (e)NOS although there is overlap in the distribution of the isoforms [11]. The activity of DDAH clearly influences NO generation and cardiovascular function, via control of ADMA [1, 9–11,22,23].

It is widely held that renal and hepatic DDAH1 activity controls plasma ADMA while DDAH2 regulates vascular tissue ADMA and hence vascular tone, without influencing plasma ADMA [1,11] although recent gene manipulation studies have raised some questions. Global DDAH1 overexpression causes enhanced vascular and cardiac DDAH activity vs. wildtypes but without any increase in renal or hepatic DDAH activities [24]. Nevertheless, plasma ADMA and BP falls, implicating a role for increased cardiovascular DDAH activity in plasma ADMA control in these mice. Selective endothelial DDAH1 knockout also causes increased plasma ADMA and BP with falls in NO-dependent vascular responses [25]. Thus, vascular DDAH1 can take over as a prime controller of plasma ADMA and vascular tone.

The DDAH1 global homozygous knockout is embryonic lethal on the C57Bl6 background [23], while the heterozygote displays increased plasma ADMA and BP. A recent study reports that in the 129-Tg(Prm-cre)58Og/J mouse, homozygous DDAH1 knockouts are viable, with increased plasma and tissue ADMA and ~20 mmHg increase in BP but no other phenotype in the unstressed state [26]. What makes this mouse particularly interesting is that despite normal tissue levels and distribution of the DDAH2 enzyme, DDAH activity were *undetectable* in kidney, liver and lung of the DDAH1 null [26]. In other words DDAH2 does not have any ADMA degrading activity, at least in this mouse. This agrees with Pope and colleagues, who suggest that DDAH2 regulates endothelial NO production by non-ADMA mechanisms [27]. However, DDAH2 global overexpression does lower plasma ADMA and prevents vascular response to infused ADMA [28] and overexpression of DDAH 2 in cultured endothelial cells reduces ADMA and increases NO production [29]. Further, several DDAH1 and 2 polymorphisms have been described in man [22] including a strong association between DDAH2 polymorphisms and plasma ADMA in type 2 diabetics [30]. Thus, the relative importance of DDAH1 and 2 in the clearance of ADMA remains to be determined.

Some of the new information, discussed above, questions the dogma that plasma ADMA is primarily controlled by renal and hepatic uptake and metabolism. In addition to the transgenic mice studies [24,25] in which endothelial DDAH activity controls plasma ADMA, plasma ADMA *falls* in normal man after acute uninephrectomy for kidney donation [31] and a similar observation was reported in the rat [32] after total nephrectomy. There is no clear mechanism for the fall in plasma ADMA immediately post nephrectomy although the acute inflammatory response to the surgery may be involved [31]. Whatever the explanation it is clear that renal and hepatic DDAH activity are not the only controllers of plasma ADMA. Thus, while there is interest in exploring the therapeutic potential of boosting “in vivo” DDAH activity [22], the DDAH isoform and organ(s) to be targeted remain to be determined.

In addition to enzyme abundance the activity of the DDAH enzymes will play a role in regulation of ADMA levels. There is not a great deal known about post-translational regulation of DDAH activity but it is clear that oxidative stress inhibits DDAH transcription and activity [11] and that inflammation and oxidative stress are evident in CKD, even when only mild renal insufficiency is present [1,33]. Earlier in vitro work suggested that oxidative

stress is also associated with enhancement of ADMA synthesis via stimulation of protein methyltransferase 1, PRMT1 [34,35]. There has been a lull in research in this important area but the relationship between reactive oxygen species (ROS) and ADMA levels in CKD/ESKD merits further exploration. Of considerable potential therapeutic interest, dietary NO₃ supplementation in a hypertensive salt dependent model of CKD prevented oxidative stress and restored the elevated ADMA to normal [6].

With regard to oxidative stress in CKD and hypertension, we know that this results from an imbalance between oxygen radical production and removal, however, specific causes of oxidative stress are many and varied [35]. One source of ROS is via stimulation of NADPH-dependent superoxide generation due to increased angiotensin II activity in the damaged kidney. There are a variety of other oxidases that can also generate ROS (eg. Xanthine oxidase, cyclooxygenases, uncoupled NOS, etc), although the NADPH oxidase is thought to be the most important renal oxidase. In addition to excessive ROS generation oxidative stress can develop due to failure of the antioxidant systems such as the superoxide dismutases that convert superoxide to H₂O₂ or the downstream enzymes such as catalase or glutathione that get rid of the H₂O₂ [35]. In ESKD HDL falls and what is left loses its ability to inhibit oxidation of lipids and lipoproteins [36]; then there are the prooxidant “uremic toxins” [33]. The location of generation of ROS is also critical since ROS act locally and are scavenged or enzymatically removed very quickly. So superoxide arising in mitochondria will have a different impact to cytosolic or extracellular superoxide and ROS made in an infiltrating macrophage will have a different action to ROS generated in a vascular smooth muscle cell.

Therefore, there are probably many types/causes of oxidative stress that occur in CKD and hypertension which can have a wide variety of impacts. In addition to stimulation of ADMA levels by both increased PRMT1 and decreased DDAH activities [1,11,34,35], ROS have many other effects on NO bioavailability. For example superoxide reacts with and inactivates NO and also oxidises tetrahydrobiopterin which is essential for NOS activity and converts the NOS to superoxide generators [36], thus creating a vicious cycle. Also, oxidative stress leads to inflammation which leads to arginase induction which may also reduce arginine, and thence NO, availability [36,38,39].

Arginine availability

While there has been extensive study of aspects of ADMA metabolism in renal disease, there has been little recent investigation into arginine availability, which is surprising since the normal kidney synthesizes the majority of the arginine in the circulation [40,41]. The impact of renal disease on the ability of the kidney to produce arginine is controversial [39], although we recently reported that loss of the renal cortical arginosuccinate synthase and lyase enzymes occur early and persist through the course experimentally induced CKD [42]. This results in a marked fall in renal arginine release into the circulation, despite which plasma arginine remained at normal values [42]. Normal plasma arginine is routinely seen in renal failure patients and animals and we have suggested that the “normal” plasma arginine level camouflages an intracellular arginine deficiency and that plasma arginine levels are maintained because endothelial arginine uptake from plasma is impaired [1,39]. This is due both to the presence of circulating inhibitors of the arginine transporters and to reduction in transporter abundance/activity in blood vessels from rats with experimentally-induced CKD and age-dependent nephropathy (1,39,43,44). Further studies on other CKD models and in man would be helpful in evaluating the arginine status in renal disease.

NOS proteins

In addition to ADMA and arginine levels, the location, abundance and activity of the various NOS are critical in determining NO production. As well as producing de novo hypertension and CKD, chronic pharmacologic non-selective NOS inhibition promotes CKD progression in otherwise resistant strains such as the Wistar Furth and spontaneously hypertensive rat (SHR) [1,4]. The C57Bl6 mouse is also resistant to renal mass reduction- induced CKD and we recently reported that chronic, non-selective NOS inhibition greatly exacerbated CKD progression [45].

As discussed above the vascular endothelium develops NO deficiency in CKD. There are no eNOS selective NOS inhibitors but a number of recent studies in the eNOS knockout mouse have yielded novel insights, in particular with regard to diabetic nephropathy. The C57Bl6 mouse is resistant to the development of diabetic nephropathy but the eNOS knockout develops nephropathy in response to induction of type 1 diabetes with streptozotocin [46, 7]. Furthermore, crossing the eNOS knockout with the type 2 diabetic db/db mouse amplifies the renal injury [48,49] and both type 1 and type 2 mice develop the histological features of advanced human diabetic nephropathy. Recent work suggests that whereas both ACE inhibition (ACEI) and angiotensin receptor blockade (ARB) effectively prevented both glomerular and tubulointerstitial diabetic injury in wildtypes, the type 1 diabetic eNOS knockouts were much less responsive [50], suggesting that eNOS – derived NO is required for the full therapeutic response to angiotensin blockers. When the eNOS^{+/-} mouse is crossed with the Akita model of spontaneous type 1 diabetes there is also amplification of the diabetic nephropathy [51]. These mice resemble the pattern seen in diabetic man with putative “loss of function” eNOS polymorphisms [52–54]. A lack or deficiency of eNOS-derived NO within the kidney will provoke both glomerular and peritubular capillary injury leading to tubulointerstitial damage and exacerbating the damaging effects of hyperglycemia [55].

We have also reported that the renal cortical nNOS abundance and activity fall in multiple rat models of CKD [1]. In the renal ablation/ infarction model there is a linear relationship between loss of cortical nNOS and progression of glomerular damage. The Wistar Furth male and Sprague-Dawley female rat are both resistant to CKD and maintained renal cortical nNOS abundance after an insult to the kidney [1]. The CKD-resistant C57Bl6 mouse is rendered vulnerable to CKD by both nonselective NOS inhibition and “selective” nNOS inhibition [45].

These observations have focused us on the kidney cortex nNOS in the pathogenesis of CKD progression. We recently reported the existence of a splice variant of nNOS, the truncated nNOS β , in the rat kidney cortex [56]. This is a fully active enzyme which lacks the unique amino-terminus of the nNOS α and is located in the cytosol. Under normal conditions nNOS β is present in low abundance but as nNOS α abundance declines during CKD progression, the nNOS β increases in several rat models of CKD (56–58). This is unlikely to be a compensatory response since the increased nNOS β in the 5/6AI KC does not replace the nNOS α in location or function [1,57]. We also recently reported that the senescent Fisher 344/ Brown Norway cross, which develop very mild glomerular damage, exhibits increases in nNOS β before any falls in nNOS α [59]. We are currently investigating whether the increased nNOS β may play a causal role in the injury development.

Conclusions

There is strong evidence that increased ADMA both contributes to CKD progression and to the elevated cardiovascular risk. There are some indications that vascular DDAH activity

may play an important role in control of plasma ADMA and can perhaps compensate in some individuals when the kidney is damaged. The relative importance of the 2 DDAH isoforms in ADMA regulation remains uncertain and must be determined in order to optimize drug targeting.

Alterations in the NO synthases also influence CKD progression and eNOS deficiency is particularly involved in the pathogenesis of diabetic nephropathy. The use of dietary NO₃ supplements to restore normal NO and oxidant status provides a novel and simple therapeutic option for CKD.

Acknowledgments

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- Dietary nitrate supplementation may provide a novel and economic treatment for kidney and cardiovascular disease.
- While plasma ADMA predicts cardiovascular risk in CKD, the role of the kidney damage in the elevation and the variability of plasma ADMA remains unclear.
- The relative importance of the 2 DDAH isoforms in regulation of ADMA is controversial.
- NO deficiency due to loss of eNOS activity plays a key role in development of diabetic nephropathy.
- The possible pathogenic role of the nNOS β isoform in mediation of CKD progression is under investigation.